Fibrostenotic Inflammatory Bowel Disease

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Editor
Florian Rieder
Department of Gastroenterology
Hepatology and Nutrition
Digestive Diseases and Surgery Institute
Cleveland Clinic Foundation
Cleveland, OH
USA

ISBN 978-3-319-90577-8 ISBN 978-3-319-90578-5 (eBook)
https://doi.org/10.1007/978-3-319-90578-5

Library of Congress Control Number: 2018950482

This Springer imprint is published by Springer Nature, under the registered company Springer International Publishing AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Foreword

As clinicians, we encounter fibrosis frequently in practice, nowhere more challenging than in the management of inflammatory bowel disease (IBD). Fibrosis, the final common pathway of many diseases, causes strictures and obstruction. These conditions cause our patients to have serious clinical problems, significantly and chronically affecting their health and quality of life. Additionally, fibrosis in the form of adhesions causes scarring that complicates the surgical and endoscopic procedures that are needed to manage their disease.

For many decades, researchers have studied the initiating inflammatory causes of IBD. Surprisingly, the concept of investigating the fibrosis caused by this inflammation has been a relatively recent development. Indeed, there is a paucity of available data in this young, but critically important field. This book presents an innovative and comprehensive review of our current understanding of fibrosis. In addition, key international experts discuss the future of the field.

After an insightful introduction by Dr. Rieder, setting the stage for the remainder of the work, the early chapters explore the epidemiology, genetics, and biology of fibrosis. The next section reviews the clinical consequences of fibrosis, as well as exploring developments in detection and imaging. The final chapters provide an in-depth review of current pharmacological and interventional management of IBD-related fibrostenotic disease. Especially interesting to clinicians will be the chapters about endoscopic and surgical management of the clinical consequences of fibrosis, written by experienced international leaders in the field.

This is a fascinating and timely book. It will be enjoyed by scientists and clinicians who manage this challenging condition and lead to an improved understanding of both the biology and management of fibrosis, helping our current and future patients.

Conor P. Delaney
Chairman, Digestive Disease and Surgery Institute, Cleveland Clinic
Cleveland, OH, USA

Victor W. Fazio Endowed Professor of Colorectal Surgery, Cleveland Clinic
Cleveland, OH, USA

Foreword

Inflammation is a fundamental response essential to health and disease. Because of this biological duality inflammation exerts both beneficial and harmful effects. These effects are highly context-dependent and differ with the multiple components of the inflammatory process, including the host, age, cause, time, organ, and tissue. Each of these components is intrinsically variable, adding to the complexity of the inflammation and its ultimate outcome. The perfect inflammatory response is quick, highly regulated, and totally effective, eliminating the offense agent and restoring the affected site to a state of pre-inflammation anatomical and functional normality. This ideal series of events seldom occurs and the exact opposite is actually the most common reality. In clinical practice the majority of inflammatory diseases that currently affect humanity are chronic and relapsing, are accompanied by complex and aberrant immune responses, and inflict various degrees of lasting anatomical and structural damage. One of the most serious consequences is the formation of scar tissue—*fibrosis*—in the affected organ, which can then be translated into altered function and clinical symptoms. Because the most common diseases of modern era consist of chronic inflammation, fibrosis is now considered as a major universal problem with similarities as well as peculiarities that depend on which organ is being compromised by it.

The simple fact that only recently fibrosis has been recognized as a major problem in itself implies that until now attention has been almost exclusively devoted to its cause, i.e., the underlying inflammatory process, rather than the ensuing fibrotic response. Although this is justifiable in rational but simplistic terms, the factual realization that the available anti-inflammatory therapies have limited or no appreciable antifibrotic effects has alerted the research community to focus on organ fibrosis and its mechanisms as the only way to discover brand new and truly effective antifibrotic strategies. Progress in this line of discovery has taken multiple and diverse paths depending on the frequency and clinical severity of the fibrotic response in different organs. For instance, liver fibrosis has been receiving substantial attention for a much longer time than fibrosis in other organs, where it may result in just as much morbidity and clinical suffering. The best example of the latter

situation is inflammatory bowel disease (IBD)-driven intestinal fibrosis, the subject of this much needed and timely publication.

In the various sections of this book the individual pathogenic and clinical components of IBD-associated fibrosis are sequentially introduced and dissected allowing the reader to gain a comprehensive overview on the mechanisms, consequences, and possible solutions to fibrostenotic IBD.

Introductory chapters discuss the poorly appreciated history of intestinal fibrosis in IBD, the still widely accepted notion that gut fibrosis develops inexorably, and the acceptance that there is nothing much to do about it. This reflects a defeating mentality that is about to change based on advances in the understanding of the biology of fibrosis and where, when, and how to intervene, as discussed in subsequent chapters. The fact that intestinal fibrosis develops not only in Crohn's disease, where its most florid clinical manifestations are more easily detected, but in all forms of chronic intestinal inflammation, including ulcerative colitis and diverticulitis, receives needed attention.

Where and when fibrostenosis emerges in the evolution of IBD, how it evolves, and what genetic, epigenetic, and environmental factors may contribute to it also receive proper consideration and discussion. Importantly, how fibrostenotic IBD may eventually evolve independently of inflammation in the late stages of disease is also discussed, as this unique aspect of the biology of intestinal fibrosis has crucial pathogenic and therapeutic implications.

Some of the scientific biological bases of intestinal fibrosis are included to inform the reader about the numerous and intertwined cellular components of fibrogenesis. It is now clear that these are not restricted to the response of classical mesenchymal cells such as fibroblasts, myofibroblasts, and muscle cells, but also the response of the extracellular matrix and nonimmune cells, such as epithelial and endothelial cells, that transform into collagen-producing cells in response to persistent inflammatory pressure. Particularly innovative is the inclusion of fat cells as possible contributors to fibrostenotic IBD, as it may be the case for the creeping fat classically observed in long-standing Crohn's disease. Part of the biological bases of intestinal fibrosis also includes a discussion of animal models, still relatively underutilized but holding a great potential for unraveling the intimate molecular events responsible for fibrogenesis in specific situations.

A major unmet need in clinical practice is the early detection of fibrosis in IBD, which would obviously be of paramount importance and extremely valuable to allow early clinical interventions aimed at preventing or limiting scar tissue formation and narrowing of the intestinal lumen. To this end, clinical, cellular, and serologic biomarkers of intestinal fibrosis are elaborated upon, and their potential value and limitations are objectively assessed. Complementing these clinically valuable areas is a discussion on imaging of intestinal fibrosis, a particularly challenging area under active investigation with the help of numerous novel imaging techniques. The challenge here resides in the practically inseparable existence of the immune inflammatory response and the temporally concomitant fibrogenic response, both of which differ in relative proportions but not in qualitative (yes or no) terms.

The chapters on management of fibrostenotic IBD cover all necessary clinical, endoscopic, and surgical aspects. They offer a valuable state-of-the-art update that lets the reader have a comprehensive and objective understanding of what can be practically done in daily clinical practice to alleviate the suffering resulting from the various forms of intestinal architectural modifications in different clinical settings.

The concluding chapters of this book examine the current medical approaches to fibrostenotic IBD and look at what has been and is being done in fibrotic conditions of other organs that could be applied to combat fibrogenesis in the intestine. What can be expected from neutralizing factors known to be involved in fibrogenesis, such as TGF-β1, IL-4 or IL-13, or blocking signaling pathways and integrin receptors, inhibiting certain enzymes or components of the extracellular matrix, is presented in a comprehensive fashion and realistic expectations proposed.

In summary, this is a timely and unique publication that truly represents a first of its kind in the area of intestinal fibrosis. It is hoped that the broad and well-integrated components of this book will entice a new generation of basic, translational, and clinical investigators to make intestinal fibrosis the focus of their study because only cohesive information and novel approaches can offer real solutions to the challenges posed by fibrostenotic IBD.

Claudio Fiocchi
Department of Pathobiology
Lerner Research Institute, Cleveland Clinic
Cleveland, OH, USA

Department of Gastroenterology and Hepatology
Digestive Disease and Surgery Institute, Cleveland Clinic
Cleveland, OH, USA

Contents

Chapter 1
Fibrostenotic Inflammatory Bowel Disease: A Cinderella Story

Florian Rieder

Abstract Intestinal fibrosis in inflammatory bowel disease (IBD) leading to stricture formation, intestinal obstruction and need for surgical intervention remains one of the largest unresolved clinical challenges in IBD. Despite the emergence of novel anti-inflammatory drugs the incidence of stricture formation and surgery remained largely unchanged. Challenges in testing anti-fibrotic compounds have so far prevented progress in this area, but recent development put clinical trials for anti-fibrotic compounds into reach.

Keywords Stricture · Crohn's · Surgery · Anti-fibrotic · Obstruction

Intestinal fibrosis remains one of the largest unresolved problems in the field of inflammatory bowel diseases (IBD). It is estimated that more the half of the patients with Crohn's disease (CD) develop fibrostenosing complications over their lifetime leading to intestinal obstruction and need for resection [1]. This importance is emphasized by the belief that strictures precede internal penetrating disease, which is based on the observation that isolated stricturing disease is common, but internal penetrating disease is associated with strictures in >85% of cases and located upstream of strictures in the area of pre-stenotic dilation [1, 2]. More than 80% of patients with Crohn's disease undergo surgery at least once during their lifetime with strictures being a major indication [3]. In addition, accumulating evidence suggests that fibrosis may be a clinically relevant factor in Ulcerative colitis, where it can be found in 100% of colectomy specimen [4] and could potentially lead to clinical symptoms such as urgency or diarrhea.

Fibrosis is considered the final pathological outcome of the majority of chronic inflammatory diseases [5] and consequences in other organs, such as liver, lung, kidney and pancreas are well documented. There is a robust understanding of its

F. Rieder
Department of Gastroenterology, Hepatology and Nutrition, Digestive Diseases and Surgery Institute, Cleveland Clinic Foundation, Cleveland, OH, USA
e-mail: riederf@ccf.org

F. Rieder (ed.), *Fibrostenotic Inflammatory Bowel Disease*,
https://doi.org/10.1007/978-3-319-90578-5_1

pathophysiology, leading to excessive accumulation of extracellular matrix (ECM). This led to approval of now two drugs for the use in idiopathic pulmonary fibrosis [6, 7].

Fibrosis in IBD, to the contrary, to date has not yet been substantially explored. No specific anti-fibrotic therapy is available. Disease progression to complications may be slightly delayed by immunomodulatory or biologic therapy in IBD [8], but this does not lead to a robust reduction in the need for intestinal surgery [9]. Endoscopic therapy as well as bowel resection are still the major therapeutic modalities for CD patients with clinically symptomatic fibrostenosis [10]. Strikingly, fibrosis has been reported to be reversible and may not represent a unidirectional process from tissue damage, abnormal repair over excessive ECM accumulation to clinical symptoms and surgery. When patients undergo strictureplasty for established strictures in CD the surgical recurrence rate was 39% for jejunoileal strictures. The reason for re-surgery, however, was at site of the original strictureplasty in only 3% of the cases [11]. When examining the site of the original strictureplasty, a regression of fibrosis was noted, which is consistent with cross sectional imaging studies following strictureplasties [12]. This model is now used prospectively for strictureplasties over the ileocecal valve [13].

Multiple mechanisms may be exploited for a therapeutic intervention that are multifactorial and dynamic, meaning dependent on the quality, quantity and timing of the inflammatory process [1]. Genetic and epigenetic factors may play a role, as do cytokines and numerous growth factors [1]. The gut is unique in its exposure to environmental factors and we now begin to understand that those, including the microbiota, smoking or dietary components, could also drive fibrogenesis [14]. It is apparent that tissue damage, once established, may progress in the absence of inflammation [1, 15]. This is highly relevant as it may explain, why conventional anti-inflammatory therapies do not seem to be able to prevent fibrostenosis. Understanding mechanisms mediating this process, such as cell to matrix interactions or tissue mechanoproperties could offer future therapies. The chief pro-fibrotic cell type, the mesenchymal cell, can arise from a variety of sources in IBD, including cellular transformation, proliferation of local fibroblasts, intestinal stellate cells or circulating precursors, so called fibrocytes [1]. Preventing the accumulation of mesenchymal cells, rather than controlling their activation could be used to prevent or treat fibrosis.

Despite the large clinical problem and possible reversibility of fibrosis, as well as known mechanisms of fibrosis the progress of developing novel anti-fibrotic drugs in IBD has been slow. This could be explained by multiple obstacles: we are missing accurate biomarkers to predict, which patients develop fibrostenosis or what the fibrotic burden of each individual patient is at any given time [16]. The current phenotype classifications are only grouping patients, based on their clinical symptoms, therefore missing clinically silent fibrostenosis. Biomarker studies are based on patient populations using solely clinical classifications and hence are bound to be inaccurate. We are currently lacking imaging tools to quantify fibrosis or separate fibrosis from inflammation [17]. Major progress is occurring, including validation of radiologic endpoints for clinical trials. The time from disease diagnosis to

stricture detection is long and hence a clinical trial would need to be large, long and hence very expensive, an investment that pharmaceutical companies were shying back from to date.

How do we make progress in the area of fibrostenosing IBD: we need to continue to discover novel mechanisms of fibrogenesis. Learning from other intestinal diseases with wound healing abnormalities, such as intraabdominal adhesions or fistulizing disease, or from fibrotic disease of other organs will fuel progress. International interest groups are forming to develop and validate biomarkers and clinical trial endpoints, with the goal to use those in proof of concept clinical trials and make the field of intestinal fibrogenesis a 'Cinderella-story'. This book is intended to discuss all the above-mentioned concepts in depth and provide the reader with the necessary tools to understand obstacles and promises in the area of stricture formation in IBD.

In summary, intestinal stricture formation due to fibrosis remains one of largest unresolved obstacles in IBD. Current therapy is insufficient and no specific anti-fibrotic approach is available. Significant progress is made to overcome the challenges to develop novel anti-fibrotic bringing anti-fibrotic therapies within reach.

Financial Support This work was supported by grants from the National Institutes of Health [T32DK083251, P30DK097948 Pilot Feasibility Study, K08DK110415] and the European Crohn's and Colitis Foundation to F.R.

Conflicts of Interest F.R. Consulting: UCB, Celgene, Samsung, Roche, Pliant, Thetis, Boehringer-Ingelheim, Helmsley; AdBoards: AbbVie, UCB, Receptos, RedX, Celgene; Speakers Bureau: AbbVie.

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Chapter 2
Epidemiology and Natural History of Fibrostenosing Inflammatory Bowel Disease

Wee Khoon Ng and Siew C. Ng

Abstract Crohn's disease (CD) and ulcerative colitis (UC) are chronic, relapsing inflammatory gastrointestinal diseases. CD frequently results in transmural inflammation and is more commonly associated with stricturing diseases compared with UC. Inflammation in UC is usually only limited to the mucosa and stricture formation in the colon occurs rarely. Strictures in IBD can be secondary to either inflammation or fibrosis and it is important to determine the aetiology to provide definitive treatment. Once strictures occur, they can be challenging to manage. Despite the advent of multiple new therapeutic agents for IBD, there has been no significant impact on the incidence and morbidity of strictures.

Keywords Crohn's disease · Ulcerative colitis · Inflammatory bowel disease · Strictures · Inflammation · Fibrosis · Fibrostenosing

2.1 Introduction

Inflammatory bowel disease (IBD) consists of Crohn's disease (CD) and ulcerative colitis (UC). CD is characterized by transmural inflammation which frequently leads to the formation of strictures. The risk of developing strictures has been shown to progress over time. Strictures often develop a fibrotic component that is refractory to medical therapy resulting in the need for endoscopic or surgical intervention. This chapter highlights the incidence, prevalence and natural history of stricturing CD and UC and discusses potential pitfalls in depicting the true incidence and their natural history and recommendations on how to overcome them.

W. K. Ng
Department of Gastroenterology and Hepatology, Tan Tock Seng Hospital, Singapore, Singapore
e-mail: wee_khoon_ng@ttsh.com.sg

S. C. Ng (✉)
Department of Medicine and Therapeutics, Institute of Digestive Disease, State Key Laboratory of Digestive Diseases, LKS Institute of Health Science, Chinese University of Hong Kong, Hong Kong, China
e-mail: siewchienng@cuhk.edu.hk

F. Rieder (ed.), *Fibrostenotic Inflammatory Bowel Disease*,
https://doi.org/10.1007/978-3-319-90578-5_2

2.2 Epidemiology of Fibrostenosing Inflammatory Bowel Disease

The incidence of inflammatory bowel disease (IBD) is the highest in the West with reported annual rates as high as 29.3 per 100,000 persons for CD and 24.3 per 100,000 persons for UC [1, 2]. The incidence of IBD is increasing with time in many regions around the world, especially in newly urbanized regions. Certain highly urbanized regions in the West have started to demonstrate a plateauing effect in the incidence of IBD [3]. Asia has a lower incidence and prevalence of IBD, however the incidence is rising rapidly in parallel with urbanization [4]. East Asia has the highest IBD incidence in the region, especially in China, Korea and Japan, with highest reported incidence rate of 3.4 per 100,000 persons [5].

IBD is a chronic disease that commonly affect young individuals with low mortality rates. The stable or increasing incidence of IBD accompanied by better healthcare delivery have resulted in an exponential increase in the global prevalence of IBD. This epidemiological phenomenon known as compounding prevalence, will result in a rapid increase in the global IBD prevalence [3]. Approximately 0.7% of the population in Canada has IBD, equating to more than one in every 150 Canadians, which is twice as common as multiple sclerosis or Parkinson's disease [6].

2.2.1 Crohn's Disease

Intestinal fibrosis is common in patients with CD, and clinically significant stricturing disease affects at least 30% of patients 10 years after diagnosis [7, 8]. In a population based cohort study, the cumulative probability of stricturing CD after long term follow up was 4.8% at 90 days, 7.2% at 1 year, 12.4% at 5 years, 15.2% at 10 years and 21.6% at 20 years [9]. A retrospective study from several European countries reported that 48.2% of patients with CD presented with a stricturing behaviour [10]. A prospective population-based study from eight Asian regions (China, Hong Kong, Indonesia, Macau, Malaysia, Singapore, Sri Lanka and Thailand) reported that 14–33% of patients had stricturing disease, with similar frequency to the CD patients in Australia [5]. Stricturing disease has also been observed in 39.9% of CD patients in Japan [11], 33.6% in Taiwan [12] and 20.1% in Korea [13].

2.2.2 Ulcerative Colitis

Fibrostenosis is less common in patients with UC when compared with patients with CD. However, reports on the prevalence of strictures in UC are generally limited. Most studies on the prevalence of strictures in UC included both benign and malignant strictures. A retrospective study from New York reported the prevalence

of strictures (detected radiologically, endoscopically or surgically) amongst UC patients as 5.1% [14]. Colonic stricture in UC should always raise a concern for malignancy, however the majority (71–100%) of strictures detected in UC are benign [14–16]. The prevalence of benign strictures in UC varies widely from 1 to 11.2% [14, 15, 17–20]. The disparity in the reported prevalence of strictures is likely attributed to the heterogeneous definitions used across different studies. This will be discussed further in this chapter.

2.3 Natural History of Fibrostenosing Inflammatory Bowel Disease

2.3.1 Risk and Prognostic Factors Associated with Fibrostenosis in Crohn's Disease

Several genetic mutations have been associated with the development of fibrostenosing disease in CD. Nucleotide oligomerization domain 2 (*NOD2*) variants is one of the most important mutations in Caucasian population and is an independent predictive factor for ileal disease (OR = 1.9), stenosis (OR = 1.82) and penetrating disease (OR = 1.25). NOD2 is also the strongest risk factor associated with a complicated CD disease course (OR = 2.96) [10]. Patients with biallelic *NOD2* or caspase-recruitment domain 15 (*CARD15*) mutations have a ten times higher risk of developing strictures, when compared to patients carrying only a single mutation [21–23]. Janus-associated kinase 2 (*JAK2*) has also been associated with the development of bowel stenosis in patients with CD. Genetic predispositions vary in different ethnicities [4, 24]. For instance, the presence of tumour necrosis factor superfamily 15 (*TNFSF15*) and serological marker anti-*Saccharomyces cerevisiae* (ASCA) IgA are associated with stenosis or penetrating phenotype in Asian patients with CD [25].

Clinical factors associated with intestinal strictures include the age of diagnosis of less than 40 years, perianal disease and the need for steroids during the first flare [9]. In addition, smoking is an important reversible risk factor for complicated disease course and progression from CD to initial stricture formation. A history of appendicectomy and the presence of antimicrobial antibodies have also been reported to be associated with stricture formation [21, 26]. Endoscopic feature of small bowel deep mucosal ulcerations is predictive of developing strictures in CD [26].

2.3.2 Clinical Manifestations and Disease Progression of Fibrostenosis in Crohn's Disease

CD can potentially affect any segment of the gastrointestinal tract, with a predominant involvement of the ileum and colon. In CD population in the West, ileal, colonic and ileocolonic involvement are commonly found in equal frequencies. In contrast

to the West whereby ileal disease is more common, Ileocolonic disease appears to be the most common CD phenotype in East Asian CD population, ranging from 50.5 to 71% [27–30]. The locations of CD involvement usually remain relatively stable, with only 10–15% demonstrating a change in disease location 10 years after diagnosis [7, 8, 31].

The inflammatory form of CD usually predominates in the initial years of disease with a subsequent development of penetrating or stricturing disease. The disease course generally follows a sequence of flares and remissions, with 20% of subjects having a chronic, active, continuous course. The initial disease location may determine the time and type of complication. Complications with abscesses, fistulae and stricture formation are more common if there is small bowel involvement. On the contrary, colonic involvement tends to remain inflammatory in nature and uncomplicated for many years. There seems to be no direct relationship between symptoms and disease progression, as most strictures and fistulae are subclinical and may have little or no symptoms for many years [7]. Small bowel disease usually remains latent for many years, whereas colonic disease tends to presents early [1, 32]. Of importance, half of the CD population adopts a progressive and aggressive course with high rates of complications, relapse, admissions and surgery. Fortunately, the other half remains minimally progressive and adopts a milder disease course [33–35].

Progression of the disease may take weeks to years and may be slowed or halted with medical therapy. Current medical therapy with immunosuppressive drugs mostly relieve inflammatory symptoms, but have limited effects on fibrostenosis disease [36–42], with 64% of patients with strictures requiring surgery within 1 year of diagnosis [43]. It is therefore important to identify high risk patients who will be susceptible to complications, for aggressive initial treatment before severe irreversible fibrosis sets in.

2.3.3 Risk and Prognostic Factors Associated with Fibrostenosis in Ulcerative Colitis

Fibrostenosis is less common in UC compared to CD. Factors associated with fibrostenosis in UC include a longer disease duration and more severe colitis with larger ulcers and deep ulcerations [44–47]. Strictures detected within 10 years of disease onset are usually benign. However, the risk of malignant strictures increases thereafter with a longer duration of UC [14, 15]. This is also in keeping with the observation that colonic malignancies are usually only detected in patients with more than 10 years history of UC. It has been reported in a series [14] that the following factors have been observed to be associated with a higher risk of malignant strictures:

1. The appearance of strictures late in the disease course (61% probability of malignancy in strictures diagnosed after 20 years of UC, 0% probability of those diagnosed before 10 years)

2. The location of stricture is proximal to the splenic flexure (86% probability of malignancy in strictures proximal to the splenic flexure, 47% probability in the sigmoid colon and 10% probability in the rectum)
3. Symptomatic large bowel obstruction (100% probability of malignancy, 14% probability in the absence of obstruction or constipation)

It is important to note that the percentages above served only as a guide, because in this series of 1156 UC patients, only 59 patients had developed strictures.

2.3.4 Clinical Manifestations and Disease Progression of Fibrostenosis in Ulcerative Colitis

UC classically involves the rectum and extends proximally in a continuous manner with about 30–35% presenting with proctitis, 30–45% left sided colitis and 20–25% pancolitis [1, 32]. Backwash ileitis is more commonly seen in patients with pancolitis. The diagnosis of UC may be revised to CD in 5–10% of the adult patients [1, 32]. Mucosal inflammation is usually diffuse and superficial, but deep ulcerations may occur in patients with more severe UC. Disease activity tends to decrease over time, with one third of the patients exhibiting persistently active disease [48].

Fibrostenosing disease in UC is related to the severity of inflammation, with strictures more commonly observed in patients with extensive colitis (17%), compared to those with left sided colitis (11%). There is a significant variation in the severity of fibrostenosis in UC. Reported stricture lengths may vary from as short as 2–3 cm, to as long as 30 cm [15], with an average stricture lumen diameter of 1.1 cm [18]. With chronic inflammation, it will result in marked shortening [49, 50] and rigidity of the colon, with eventual formation of clinically significant colonic strictures.

2.4 Pitfalls in Depicting Incidence and Natural History of Fibrostenosis Disease

Information on the incidence and natural history of any disease is dependent on the availability of good quality reports. Insufficient data or biased reports may significantly affect the accuracy of the data especially for uncommon illnesses. In addition, most fibrostenotic diseases are subclinical during their initial years. This has resulted in significant underestimation of the true prevalence of fibrostenotic disease in IBD patients, because only clinically apparent cases are reported. Differing definition of strictures between clinicians, radiologists and pathologists may affect the rate of the detection of fibrostenosis, resulting in a disparate incidence. To circumvent these limitations, improved clinician awareness of fibrostenotic diseases in IBD, especially in UC, which was once thought to be uncommonly associated with

strictures, will be an integral first step to timely identification. Standardized definition of strictures together with routine screening protocols for stricturing diseases will be helpful in early detection, especially for CD patients, who tend to have a higher incidence of strictures.

In summary, fibrostenosing IBD is most commonly seen in CD patients, with risks increasing with the duration of inflammation. Fibrostenosis commonly remains subclinical for many years before diagnosis, which results in significant under reporting of its prevalence. Increase clinician awareness and screening will be integral to early detection.

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Chapter 3
Genetic Influences on the Development of Fibrosis in Inflammatory Bowel Disease

Bram Verstockt, Sare Verstockt, and Isabelle Cleynen

Abstract Intestinal fibrosis is a common complication in inflammatory bowel disease. These fibrotic processes develop in genetically susceptible individuals, influenced by an interplay with environmental, immunological and disease-related factors. A deeper understanding of the genetic factors driving fibrogenesis might help to unravel the pathogenesis, and ultimately lead to development of new, anti-fibrotic therapies. Here we review the genetic factors that have been associated with the development of fibrosis in patients with both Crohn's disease and ulcerative colitis, as well as their potential pathophysiological mechanism(s).

Keywords Stricturing disease · Fibrosis · Crohn's disease · IBD · Genetics · NOD2

3.1 Introduction

The study of the genetic architecture of inflammatory bowel disease (IBD), with Crohn's disease (CD) and ulcerative colitis (UC) as its main entities, has made great progress in the past decade. Genome-wide association studies and meta-analyses have identified a total of 242 IBD risk loci [1]. Although many patients with CD or UC undergo surgery during the course of their disease, with stricture formation being the most common indication for major intestinal surgery—especially in CD, a genomic basis that fully explains this disease heterogeneity has not yet been revealed [2, 3].

B. Verstockt
Translational Research in Gastrointestinal Disorders (TARGID), Department of Chronic Diseases, Metabolism and Ageing, KU Leuven, Leuven, Belgium

Department of Gastroenterology and Hepatology, University Hospitals Leuven, Leuven, Belgium
e-mail: bram.verstockt@kuleuven.be

S. Verstockt · I. Cleynen (✉)
Laboratory for Complex Genetics, Department of Human Genetics, KU Leuven, Leuven, Belgium
e-mail: sare.verstockt@kuleuven.be; isabelle.cleynen@kuleuven.be

F. Rieder (ed.), *Fibrostenotic Inflammatory Bowel Disease*,
https://doi.org/10.1007/978-3-319-90578-5_3

The development of fibrosis in IBD is likely influenced by various genetic, environmental, immunological and disease-related factors [4–7]. So far, the relative contribution of each component in the pathogenesis is not clear. This chapter aims to clarify the genetic contribution in developing fibrosis in patients with IBD.

3.2 Genetics and Fibrosis in Crohn's Disease

Published literature on the genetic background of fibrotic CD is broad and very often reports conflicting data. Identified variants are involved in different biological processes, suggesting that these processes contribute to the pathogenesis of fibrostenosis (Fig. 3.1). Below we provide an overview of individual variants and genes that have been associated with fibrotic disease in CD, and organized them according to the biological process they are involved in (Table 3.1). For each gene, we describe its general function, list the variants associated with fibrotic CD, and how they could be involved in the pathogenesis of fibrosis.

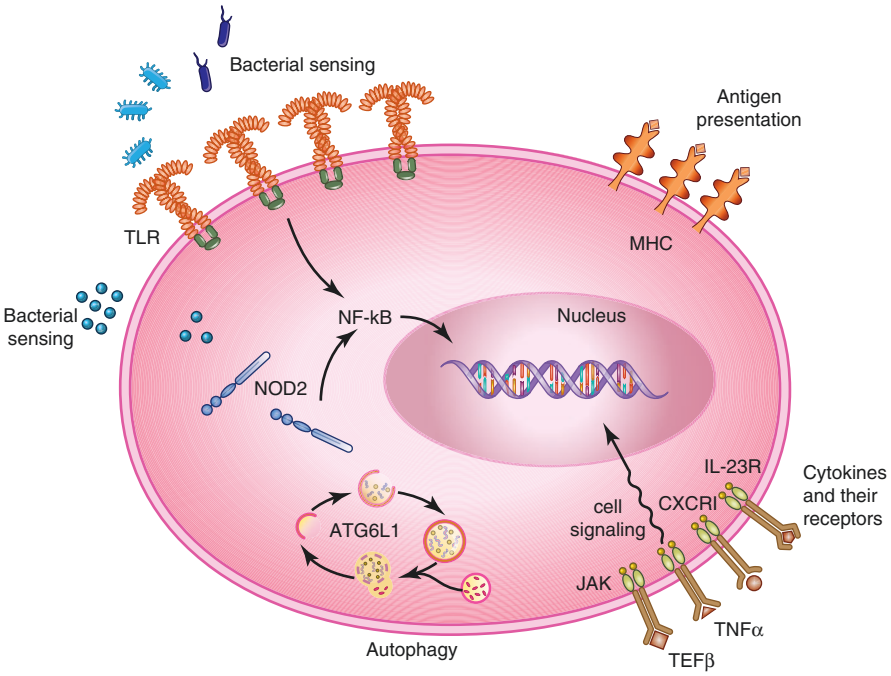

Fig. 3.1 Biological processes affected by the variants associated with fibrostenotic CD

Table 3.1 Key gene polymorphisms and their significance in intestinal fibrosis in CD

Pathophysiological process	Gene or region	Polymorphism	Association	Studied population	Sample size[a]	Reference
Bacterial sensing	NOD2	rs2066844, R702W rs2066845, G908R rs2066847, Leu1007fsinC	Discussed separately in Table 3.2			
Autophagy	ATG16L1	rs2241880, T300A	Ileal disease location	Australian	669–154	Fowler et al. [25]
			Fibrostenotic disease[b]			
Antigen presentation	MHC	rs77005575	Disease behaviour[c]	Caucasian	19,713	Cleynen et al. [26]
Cytokines and their receptors	IL-23R	rs1004819	Ileal disease location[d]	German	833	Glas et al. [27]
			Fibrostenotic disease[b,d]			
	CX3CR1	rs3732379, V249I/rs3732378, T280 M	Ileocolonic disease location	German	206	Brand et al. [28]
			Fibrostenotic disease[b]			
		rs3732379, V249I	Fibrostenotic disease[b]	Caucasian	239	Sabate et al. [24]
	TGF-β	rs1800471, R25P	Fibrostenotic disease[e]	Australian	235–112	Hume et al. [29]
Epithelial barrier	MAGI1	rs11924265	Fibrostenotic disease[c]	Spanish	1090–1296	Alonso et al. [30]
Cell signalling	JAK2	rs10758669	Ileal disease location	Caucasian	1528	Cleynen et al. [17]
			Fibrostenotic disease[c]			
Matrix metalloproteinases	MMP-3	−1613 5T6T	Colonic disease location	Dutch	134	Meijer et al. [31]
			Fibrostenotic disease[b]			

(continued)

Table 3.1 (continued)

Pathophysiological process	Gene or region	Polymorphism	Association	Studied population	Sample size[a]	Reference
Other processes	FUT2	rs601338	Fibrostenotic disease[c]	Belgian	647	Forni et al. [32]
	Close to IL-12B	rs1363670	Fibrostenotic disease[c]	Belgian	875	Henckaerts et al. [33]
	MIS18BP1	*rs35223850*	Fibrostenotic disease[b]	Belgian	403	Holvoet et al. [34]

Adapted from Verstockt et al. Genetic Influences on the Development of Fibrosis in Crohn's Disease [3]
If a significant association between the given variant and disease location is found in the reference, this is mentioned in the table
[a]Number of included CD patients in primary cohort—number of included CD patients in replication cohort (if applicable)
[b]Not corrected for disease location
[c]Corrected for disease location
[d]Not significant after Bonferroni-correction
[e]No longer significant after multivariate analysis taking into account disease location

3.2.1 Bacterial Sensing

3.2.1.1 Nucleotide-Binding Oligomerization Domain-Containing Protein 2, NOD2

The *NOD2* gene, located in the IBD1 locus on chromosome 16q12, is the most studied gene in relation to fibrostenotic disease in CD. *NOD2* encodes CARD15, a member of the Apaf-1/NOD1 family of CARD (caspase recruitment domain containing protein) proteins [35, 36]. NOD2/CARD15 is mainly expressed by monocytes and macrophages, where it acts as a cytosolic sensor for bacterial products. It is involved in apoptosis and activates NF-κB in response to lipopolysaccharide (LPS), binding its leucine-rich repeating region (LRR) [11, 19]. Moreover, through its CARD-domain, CARD15 is able to induce interleukin1-beta (IL-1β) processing and release [37]. Importantly, *NOD2* is also expressed in Paneth cells in the terminal ileum [38].

In the early 2000's, three *NOD2* variants, including two amino acid substitutions (R702W in exon 4, and G908R in exon 8) and one frameshift mutation (Leu1007fsinC in exon 11), were found to be associated with CD susceptibility [16, 35, 39–41]. Several other *NOD2* SNPs were later added to this list, although the first three still represent the strongest association signals. Many genotype-phenotype studies were then performed to find their role in defining specific CD subtypes (CD disease location and/or behaviour). While practically all studies agree on an association between *NOD2* and ileal disease location (Table 3.2), none of the *NOD2* SNPs was uniformly found as an independent risk factor for developing fibrostenotic disease [6, 8–24, 26, 38, 42–55]. Some studies however did show associations between at least one of the three *NOD2* variants and fibrostenotic disease [19–21, 24], often independent of an association with small bowel disease [11, 14, 17, 22, 23] (Table 3.2).

The lack of uniformity seems mainly based on the small sample sizes in the different studies (Table 3.2). In a Northern-French population of 205 CD patients, *NOD2* R702W (rs2066844) was found a strong predictor of fibrostenotic disease, independently of ileal disease location [8], but no other group could confirm this association. An association of *NOD2* G908R (rs2066845) and fibrostenotic disease was first reported in a Spanish CD cohort ($n = 204$), although fibrostenotic disease was mainly dependent on location of disease in the terminal ileum [9]. Later, a meta-analysis including a total of 8833 CD patients reported G908R as being associated with fibrostenotic disease (pooled RR = 1.90) [10]. It is important to highlight however, that only 12 of the included 49 studies in this meta-analysis had enough data to analyse individual *NOD2* variants, and most included studies did not differentiate between G908R homo- and heterozygotes. Of the three *NOD2* variants, the Leu1007fsinsC frameshift mutation (rs2066847) shows the strongest association with fibrostenotic disease, but again it is unclear whether this is dependent on ileal disease involvement [11–14]. Seiderer et al. calculated a positive predictive value (PPV) of 80% and a negative predictive value (NPV) of 75% for the diagnosis of small bowel stenosis in clinically symptomatic patients with a Leu1007fsinC variant. Furthermore, they noticed 62% of their patients being

Table 3.2 Overview of original studies showing an association between NOD2 and fibrotic CD

Polymorphism	Association	Studied population	Sample size	Reference
rs2066844 R702W	Fibrostenotic Disease[a]	French	205	Heresbach et al. [8]
rs2066845 G908R	Fibrostenotic Disease[b]	Spanish	204	Mendoza et al. [9]
	Fibrostenotic Disease[c]	Meta-analysis	8833	Adler et al. [10]
rs2066847 Leu1007fsinC	Ileal disease location	North-American	201	Abreu et al. [11]
	Fibrostenotic disease[c]			
	Ileal disease location	Italian	133	Vavassori et al. [12]
	Fibrostenotic disease[b]			
	Fibrostenotic Disease[b]	German	97	Radlmayer et al. [13]
	Ileal disease location	Italian	316	Annese et al. [14]
	Fibrostenotic disease[c]			
	Fibrostenotic Disease[b]	German	80	Seiderer et al. [15]
	Ileal disease location	German	303	Seiderer et al. [16]
	Fibrostenotic disease[b]			
	Ileal disease location	Caucasian	1528	Cleynen et al. [17]
	Fibrostenotic disease[a]			
	Ileal disease location	German	550	Schnitzler et al. [18]
	Fibrostenotic disease[b]			
All SNPs combined	Ileal disease location	British	244	Ahmad et al. [19]
	Fibrostenotic disease[d]			
	Ileal disease location	Finnish	271	Heliö et al. [20]
	Fibrostenotic disease[b]			
	Ileal disease location	Hungarian	527	Lakatos et al. [21]
	Fibrostenotic disease[b]			
	Ileal disease location	North-American	201	Abreu et al. [11]
	Fibrostenotic Disease[a]			
	Colonic disease location	Caucasian	453	Lesage et al. [22]
	Fibrostenotic Disease[a]			
	Ileal disease location	North-American	275	Brant et al. [23]
	Fibrostenotic disease[a]			
	Ileal disease location	Italian	316	Annese et al. [14]
	Fibrostenotic disease[a]			
	Ileal disease location	Caucasian	1528	Cleynen et al. [17]
	Fibrostenotic disease[a]			
	Ileal disease location	Spanish	239	Sabate et al. [24]
	Fibrostenotic disease[b]			

Adapted from Verstockt et al. Genetic Influences on the Development of Fibrosis in Crohn's Disease [3]

If a significant association between the given variant and disease location is found in the reference, this is mentioned in the table

[a]Corrected for disease location

[b]Not corrected for disease location

[c]Unclear if corrected for disease location

[d]No longer significant after multivariate analysis taking into account disease location

Leu1007fsinC homo- or heterozygous needed surgery, whereas the need for surgical intervention in patients without this variant was remarkably low [15]. A subanalysis of another cohort with 19 patients, all Leu1007fsinC homozygous, identified a high-risk population, characterized by for instance long-segment stenosis, frequent need for surgery and high risk for re-stenosis afterwards [16]. The same group confirmed these findings later on in a prospective study [15], after which the European IBDchip project reported comparable results in a retrospective study (n = 38) [17], as did Schnitzler et al. [18]. Besides studying the association of individual *NOD2* SNPs with a fibrostenotic CD phenotype, often the *NOD2* SNPs are considered together. The pooled relative risk (RR) of stricturing disease with the presence of any *NOD2* variant allele was 1.33 in the meta-analysis by Adler et al. [10]. Furthermore, Lesage et al. clearly described the 'gene dosage effect' of *NOD2* SNPs: patients carrying two SNPs have a higher incidence of stenosis compared to patients with one or two wild-type alleles [22], which was afterwards confirmed by others [10, 23, 55]. There are also several studies that could not find an association between *NOD2* variants and fibrostenotic disease: Louis et al. found that only disease location and number of flares per year are significantly different between different CD phenotypes, and that ileal disease location was associated with a stricturing disease pattern [51]. Although *NOD2* variants were associated with CD susceptibility in a Brazilian population, Baptista et al. could not find a genotype-phenotype correlation [43]. The biggest study thus far looking into genotype-phenotype associations in IBD to date, also did not find an association between *NOD2* and fibrotic disease, when considering disease location. They conclude that while disease location is in part genetically determined, it is considered an intrinsic aspect of a patient's clinical disease, and the major driver to changes in disease behaviour over time [26]. Because of the strong correlation between *NOD2* variants and ileal disease location, we assume that the observed association between fibrostenosis and *NOD2* relies on a confounded association due to disease location.

How could the *NOD2* variants be pathophysiologically linked to the development of fibrosis? They might induce fibrostenotic disease by shifting T lymphocytes towards Transforming Growth Factor beta (TGF-β) cytokine production, and by increasing collagen deposition by smooth muscle cells and fibroblasts in the intestine [11]. Functional data are primarily available for Leu1007fsinC: Leu1007fsinC leads to a truncated CARD15 protein, resulting in an altered activation of NF-κB following bacterial triggers [41]. It was previously thought that Leu1007fsinC was associated with an impaired IL-1β production and dendritic cell function, resulting in a dysregulation of the antibacterial host defence, increased intestinal permeability and impaired regulation of innate and adaptive immunity in the intestinal tract [15]. However, Maeda et al. later reported Leu1007fsinC is associated with enhanced NF-κB activation and IL-1β secretion in mice [37]. Additional mechanisms such as diminished mucosal alpha-defensin expression might also be involved [15]. It is possible that the other two variants also alter the structure of the LRR domain, resulting in abnormalities in bacterial recognition [46].

3.2.1.2 Toll-Like Receptors, TLRs

TLRs are transmembrane domain proteins with a tripartite structure: they contain an extracellular domain (including LRRs) responsible for ligand recognition, a single transmembrane spanning region, and a globular cytoplasmic Toll/IL-1 receptor (TIR) signalling domain. Currently, ten TLRs are described in humans [56]. They are expressed in myeloid cells and play a major role both in detecting microbes and in initiating innate immune responses. *TLR4*, expressed in the Golgi apparatus of intestinal epithelial cells, interacts with LPS, contributing to the perpetuation of inflammatory epithelial cell injury via Tumour Necrosis Factor Alpha (TNF-α)-induced alterations of enterocyte turnover in an (auto)paracrine matter [21].

Rs4986790 (Asp299Gly) located within *TLR4* has been shown to be a susceptibility variant for CD [57], although this could not be confirmed in another study by Lakatos et al. (possibly because the variant allele is more present in their control population compared to the study by Franchimont et al.) [21]. Neither of the two studies found an association with CD sub-phenotype. This variant is associated with decreased responsiveness to endotoxins in humans [58, 59]. Although there is no genetic evidence for a role for *TLR4* in the pathogenesis of fibrostenotic disease in CD, Rieder et al. suggested the first direct link between innate immunity to bacteria (via TLRs) and fibrosis in humans [60]. Furthermore, in other diseases like systemic sclerosis and liver fibrosis, *TLR4* is thought to have a pathophysiological contribution [61, 62].

3.2.2 Autophagy: Autophagy-Related 16-like 1, ATG16L1

The *ATG16L1* gene, member of a large family of genes involved in autophagocytosis, is located on chromosome 2q37. ATG16L1 is essential in the targeting and destruction of pathogen-derived proteins in the innate immune response [63, 64]. Autophagy is also important for degrading cytoplasmic components, sequestered within vesicles, by the lysosome [38].

The *ATG16L1* T300A variant (rs2241880) is an important susceptibility variant for CD [63, 65, 66]. This same variant has also been associated with ileal disease location, independent of *NOD2* genotype or disease duration; the study did not mention an association with stricturing disease [64]. Later, Fowler et al. reported a significant association between fibrostenotic disease, the GG risk genotype and ileal disease, independent of *NOD2* (although the number of *NOD2* variants in their Australian CD population might be too small) [25]. However, the European IBDchip Project could not confirm this association between *ATG16L1* T300A and fibrostenotic disease [17].

The T300A amino acid substitution is a highly-conserved residue that is located in the WD-repeat domain of ATG16L1, and may therefore affect interactions of the protein with other components of the autophagosome [64]. This variant plays an important role in pathogen clearance [67], resulting in imbalanced cytokine

production [68]. Moreover, presence of this *ATG16L1* risk allele seems associated with a reduced ability to generate a specific type of macrophages (Mφind, phenotypically closely resembling the anti-inflammatory $CD206^+$ M2-macrophages), also implying an impaired anti-inflammatory functioning [69]. The resulting inflammatory signals could eventually stimulate mesenchymal cells to make enormous amounts of collagen and other fibrogenic molecules [70]. Moreover, the *ATG16L1* T300A variant enhances NOD2-driven cytokine production in an autophagy independent manner [68, 71]. A link between NOD2 and ATG16L1 in the activation of autophagy could be relevant for intestinal fibrogenesis: it is possible that *NOD2* and/or *ATG16L1* variants jointly can alter the responsiveness of immune cells to bacterial components, thereby amplifying inflammatory signals leading to fibrosis [70].

Overall, based on the current genetic association data, there is currently no true genetic link between *ATG16L1* and fibrostenosis. Similar to *NOD2*, the described associations might be driven by the confounding role of ileal disease location. This does not preclude a role for ATG16L1 or the autophagic process in general in the pathogenesis of fibrostenosis.

3.2.3 Antigen Presentation: Major Histocompatibility Complex (MHC)

The MHC region encodes many immunological proteins, including the antigen-presenting classical human leukocyte antigen (HLA) molecules. Genome-wide association studies of IBD have shown strong evidence of association to genes belonging to the MHC complex [72]. Because of the complexity of the region, many researchers avoid including this region into their analysis. One study by Ahmad et al. studied 340 SNPs in 24 genes from the HLA region in relation with fibrotic CD, but did not find any associations [19]. The IIBDGC genotype-phenotype study found a genome-wide significant association with rs77005575 located in the MHC region and disease behaviour, independent of disease location [26]. None of the included classical HLA alleles were independently associated with disease behaviour in the same study.

3.2.4 Cytokines and Their Receptors

3.2.4.1 Interleukin-23 Receptor, IL-23R

IL-23R is located on chromosome 1p31, and encodes a subunit of the receptor for the pro-inflammatory cytokine interleukin-23 [73]. IL-23R is highly expressed on the cell membrane of memory T cells and other immune cells, such as natural killer cells, monocytes and dendritic cells, which identify foreign substances to defend the

body against infection. It is involved in the mediation of pro-inflammatory activities by the production of interleukin 17 via the activation of Th17 lymphocytes [38].

After Duerr et al. described *IL-23R* as a susceptibility gene for CD [73], Glas et al. published a genotype-phenotype correlation for the rs1004819 SNP within *IL-23R*. They found an increased incidence of ileal involvement and fibrostenotic disease in TT homozygous carriers compared to CC wildtype carriers, but this association did not withstand correction for multiple testing [27]. Another SNP within *IL-23R*, rs116630177, reached a statistically suggestive significance level in a nested case-control study focussing on the early development of fibrostenotic CD [34]. There is no evidence of an association of the main CD-associated SNP in *IL-23R*, rs11209026 [73], with intestinal fibrosis.

3.2.4.2 Fractalkine Receptor 1, CX3CR1

CX3CR1 (previously termed V28) is a leukocyte chemotactic and adhesion receptor that binds fractalkine (CX3CL1 or neurotactin, expressed in epithelial and endothelial cells), a CX3C chemokine that exhibits properties of both traditional chemokines and adhesion molecules [28]. CX3CR1 is expressed on natural killer cells, monocytes, $CD8^+$ and some $CD4^+$ T cells. By binding fractalkine, it regulates the migration of a subpopulation of $CD8^+$ intraepithelial lymphocytes into the intestinal lamina propria, and their interaction with intestinal epithelial cells [28]. After stimulation by bacteria (or bacterial degradation products), CX3CR1-expressing cells rapidly adhere to the inflamed vascular endothelium and may play a role as a vascular gateway for cytotoxic effector cells [24].

After two strongly correlated (D' = 0.99) *CX3CR1* polymorphisms (V249I, rs3732379; and T280 M, rs3732378) were identified in HIV-positive patients [74], Brand et al. investigated these SNPs in the context of CD. They observed an association between both SNPs and fibrostenotic disease (without Bonferroni correction), but this was not independent of ileocolonic disease location [28]. Later, Sabate et al. again noticed a trend towards fibrostenotic behaviour in V249I carriers (not statistically significant after Bonferroni correction), especially in smokers, independent of *NOD2* Leu1007fsinC carriage and ileal involvement [24]. Although the two SNPs are strongly correlated [74], Sabate et al. did not see a similar trend for T280M [24].

Several findings point towards CX3CR1 as a critical component in maintaining homeostasis of lamina propria macrophages, master regulators of inflammation and fibrosis [75]. Importantly, specifically for the described variants, it was shown in vitro that peripheral blood mononuclear cells (PBMCs) from individuals with wildtype *CX3CR1* genotype adhere more potently to membrane-bound fractalkine than do PBMCs from homozygous V249I-T280M donors [28, 76]. Despite the limited data about an association between *CX3CR1* and fibrostenotic disease, these functional data could point towards a true role for the CX3CR1/fractalkine axis in fibrosis in CD.

3.2.4.3 Transforming Growth Factor Beta (TGF-β)

TGF-β is encoded by a gene on chromosome 19q13. It is a regulatory protein that plays a key role in inflammatory, fibrotic and immunological events in the intestinal mucosa [29, 77]. Enhanced expression of TGF-β and its receptors seems to be involved in the pathogenesis of CD, and might contribute to fibrosis [78, 79]. After some SNPs (including C509T) in the *TGF-β1*-gene were described to lead to variations in the production of TGF-β serum levels in women [80, 81], some groups looked in vain for an association with susceptibility to CD [29, 79, 82]. However, Hume et al. observed a significant association between the AA genotype of a SNP in codon 25 in the *TGF-β1* gene and a fibrostenotic phenotype. CD patients homozygous for the profibrotic A allele also tended to have a shorter time to intestinal resection [29].

3.2.4.4 Angiotensinogen

Angiotensinogen, mapped to chromosome 1q42, is meant to function locally as a cytokine in several organ systems, participating in the regulation of inflammation and fibrosis. After being cleaved by renin into angiotensin I and processed to angiotensin II, it may increase the production of TGF-β1 [29].

Hume et al. studied the association of a gain of function SNP located 6 bp from the transcription site of the angiotensinogen gene with CD and CD phenotype [83]. They reported a positive association for the A allele and CD, although without any genotype-phenotype association at the univariate or multivariate level [29].

3.2.4.5 Tumour Necrosis Factor Alpha (TNFα)

As TNFα plays a pivotal role in the pathophysiology of IBD, confirmed by the efficacy of anti-TNF drugs such as infliximab and adalimumab [84], Meijer et al. investigated the association between a SNP (*G308A*) in TNFα and fibrostenotic disease [31]. In line with other reports [85, 86], they could not find an association between this SNP and fibrostenotic CD [31].

3.2.5 Epithelial Barrier: Membrane Associated Guanylate Kinase, WW and PDZ Domain Containing 1, MAGI1

MAGI1 is located on chromosome 3p14 and encodes the membrane associated guanylate kinase WW and PDZ domain-containing protein 1 [30]. This protein plays an important role in the tight junction of intestinal epithelial cells through interaction with JAM4, a junctional adhesion transmembrane molecule. Disruption of this

epithelial barrier can have dramatic effect on the mucosal integrity, which has been shown to contribute to the development of CD [30].

Alonso et al. recently published an interesting association between fibrostenotic CD and rs11924265, located in a 46.5 kb haplotype block inside a *MAGI1* intron. They validated this association in an independent replication cohort [30]. Previously, other groups have shown a significant increase in intestinal permeability in patients with stricturing disease [87]. Rs11924265 might induce an alteration in the MAGI1 protein function, contributing to an exaggerated immune response, and to the subsequent transmural inflammation of the gastrointestinal tract [30].

3.2.6 Cell Signalling: Janus Kinase 2 (JAK2)

JAK2, located on chromosome 9, encodes for an intracellular tyrosine kinase that transduces cytokine-mediated signals via the JAK-STAT pathway [17, 59]. The large, retrospective, multicentre IBDchip study found that rs10758669 (C allele), within the *JAK2* gene, is associated with an increased risk for ileal involvement and stenosing disease behaviour. One mechanism by which JAK2 contributes to this fibrostenotic disease could be by altering intestinal permeability [17]. Indeed, Prager et al. previously demonstrated that patients carrying the rs10758669 C risk allele significantly more often had an increased permeability compared with patients without the C allele [88].

3.2.7 Matrix Metalloproteinases (MMPs) and Tissue Inhibitors of MMPs (TIMPs)

MMPs, Zn-activated endoproteinases, are subdivided into four groups, depending on their structure and substrate specificity: collagenases, gelatinases, stromelysins, and membrane-type MMPs [31, 89, 90]. They mediate degradation of essentially all components of the extracellular matrix and can cleave a wide range of molecules such as soluble factors, membrane receptors, adhesion factors, signalling molecules, cytoskeleton proteins and proteins inside the nucleus. Additionally, MMPs also have non-catalytic functions: they act as intracellular transcription factors or as cell ligands, hereby activating (inflammatory) signalling pathways [91]. The enzymatic activity of these potentially harmful proteinases is tightly controlled and counterbalanced by endogenous inhibitors such as alpha 2 macroglobulin and specific tissue inhibitors of MMPs, the so-called TIMPs. TIMPs are produced by the same cell types that produce MMPs, primarily in cells resembling macrophages and fibroblasts [90, 92].

The last decade many different SNPs in these genes were described, related to processes like foetal development [93], primary sclerosing cholangitis [94], and coronary atherosclerosis [95]. Meijer et al. also studied their role in relation to CD

susceptibility and CD phenotype. They found that the 5T5T genotype (an additional thymidine insertion at −1613 of the *MMP-3* promoter) at the *MMP-3* locus was associated with fibrostenotic CD [31]. Expression data furthermore showed increased MMP-3 levels in stenotic and prestenotic resected CD ileum, pointing to an MMP-3 (stromelysin-1) mediated altered clinical course of CD patients [92]. These findings might explain the high recurrence rate of intestinal strictures, as tissue turnover is present in non-resected pre-stenotic CD ileum in which the anastomosis is made [92]. Conflicting evidence exists regarding the consequences of the 5T5T genotype: some groups reported upregulation of MMP-3 expression [96, 97] whereas others reported a downregulation [98]. In the study by Meijer et al., patients stratified according to *MMP-3* genotype had similar MMP-3 total activity [31].

3.2.8 Other Processes

In 2009, Henckaerts et al. examined the influence of some CD-associated susceptibility loci on changes in disease behaviour. They found that homozygosity for the rs1363670 G-allele in a gene encoding a hypothetical protein near the *IL-12B* gene, located on chromosome 5, was independently associated with stricturing disease behaviour, especially in patients with ileal involvement [33, 59]. So far, the pathophysiological consequences of this SNP, leading to a non-coding transcript variant, are not fully understood [59].

Because inherited risk factors (factor V Leiden, methylenetetrahydrofolate reductase (*MTHFR)* C677T) have been reported to be associated with fibrosis in other chronic inflammatory diseases, Novacek et al. performed a retrospective study in CD patients aiming to identify these risk factors in fibrostenotic CD. They concluded that the *MTHFR* 6777TT variant, factor V Leiden and the prothrombin G20210A variant are not associated with fibrostenosis in CD [99].

FUT2, located on chromosome 19 [59], encodes the Secretor enzyme alpha(1,2)-fucosyltransferase (Lewis blood group system) which allows expression of ABO antigens on the gastrointestinal mucosa and in bodily secretions (secretor phenotype) [32]. After a nonsense allele in *FUT2*, rs601338 (W143X), was identified as a susceptibility variant for CD [100, 101], Forni et al. found non-secretors to be at slightly higher risk of a stricturing/penetrating behaviour (OR 1.51, $p = 0.046$). Additionally, their analysis revealed patients with blood group O are less likely to develop a stricturing disease (OR 0.70, $p = 0.038$) [32]. Although it is known that FUT2 expression affects the composition of the gut microbiota [102], the pathophysiological link between this specific SNP and fibrostenotic disease has not been unravelled yet. Theoretically, an altered microbial environment might induce more severe inflammation, leading to a more aggressive phenotype.

Finally, a SNP (rs35223850) in *MIS18BP1*, located on chromosome 14 and encoding a protein which binds the SP1 transcription factor, has been found in a carefully phenotyped cohort to be associated with early development of fibrostenotic complications in CD [34].

3.3 The Combined Action of the Known Susceptibility Variants

Crohn's disease is a complex genetic disease, where several small-effect risk variants combined could influence disease onset. Combining the many individually weak signals into a genetic risk score might be a more powerful approach to study the genetic association with subphenotypes, or to predict disease onset or behaviour [26, 103]. Such a genetic risk score was calculated in the IIBDGC genotype-phenotype study, and tested for association with several disease subphenotypes. A strong association with disease behaviour was found ($p = 9.23 \times 10^{-18}$), indicating that the known susceptibility loci combined can be a useful measurement of CD subtypes, but still do not have enough predictive ability to distinguish between the different subtypes [26].

3.4 Genetics and Fibrosis in Paediatric CD

Both very-early-onset (<6 years) and later-onset (6–16 years) patients with CD can present with a fibrostenotic phenotype [104]. Currently, not much is known about the genotype-phenotype association in paediatric CD. Russell et al. studied *NOD2* variants in the Scottish early-onset CD population (aged <16 years), and noticed a relatively small contribution to CD susceptibility, but a major impact on phenotype. Presence of stricturing disease behaviour at diagnosis showed a trend toward an increase in carriers of *NOD2* variant alleles, which became significant by 2 years of follow up [54]. The association of *NOD2* variants and fibrostenotic paediatric CD was previously already reported by two other groups [105, 106]. Importantly, all three studies also report an association between *NOD2* variants and ileal disease location, which may therefore confound the association with fibrostenotic disease.

In contrast with a study in adult CD [29], Liberek et al. could not find any significant correlation between 4 common SNPs in *TGFβ* and any specific clinical parameter [107].

In 2014, Strisciuglio et al. performed a genotype-phenotype correlation study, focussing on autophagy gene variants. They observed a trend towards switching to a fibrostenotic disease in children homozygous for the *ATG16L1* T300A risk allele. They did not find an association between *NOD2* variants and stricturing CD, but observed an association between *NOD2* variants and ileal disease location [108].

Although data in children are currently limited, it is possible that the association with NOD2 and ATG16L1 is driven by a similar confounding by ileal disease location as in adult-onset cases. This would need to be considered in future studies in paediatric CD.

3.5 Genetics and Fibrosis in Ulcerative Colitis

Intestinal fibrosis in UC is a relatively new described entity, occurring in about 5% of UC patients. The common belief that extracellular matrix deposition was restricted to the mucosal and submucosal layers of the large bowels in UC, has recently been questioned [109, 110]. Ippolito et al. showed an upregulated expression of *RhoA*, important in the fibrogenic differentiation of intestinal smooth muscle cells, in the muscular layers of the colon in UC patients [109]. Despite clinically significant implications [111], lack of investigations explain why genetic associations with intestinal fibrosis in UC have not yet been reported.

3.6 Genetics and Fibrosis Around the World

Although the incidence of IBD is rising in developing countries [112, 113], epidemiological data on the clinical phenotype of disease, and genotype-phenotype association studies, in non-European populations are limited. Similar as for Caucasian populations, several smaller genotype-phenotype studies have been performed in non-Caucasian populations [43, 114–119]. These usually study the same variants as those considered in Caucasian populations (*NOD2, IL23R*…), but the only association found was between the *IL23-R* variant rs1004819 and stricturing and penetrating disease in a Korean patient population [118]. It is possibly not surprising that *NOD2* variants are not found to be associated with disease (subtypes) in different populations, as *NOD2* variants have been seen with different frequencies in geographically diverse populations. Whereas the prevalence of CD patients who carry at least one *NOD2* susceptibility variant varies from 27–50% in most Caucasian European populations, observed frequencies are much lower (15–21%) in Scandinavian countries [120, 121], which are generally characterized by more homogenous study populations. Caucasian populations, relatively far from Europe, but with European ancestry with hardly no racial mixing, like the United States, Canada and Australia, have *NOD2* variant frequencies comparable with those found across the rest of Europe [120]. In Asians (Japanese, Chinese and Korean), Arabs, Africans and African Americans, the *NOD2* variants are rare or even absent [38, 114, 122].

Recently, the first trans-ancestry association study of IBD was published by the IIBDGC [122]. They collected subphenotype data on 1991 patients with CD from East Asia, India and Iran and compared these data with available clinical phenotypes for 19,290 Europeans [7]. They showed some demographic differences, with for example more stricturing behaviour and perianal and less inflammatory CD in the non-European population compared to the European population, in line with previously reported prospectively collected clinical findings in incident cases of

IBD in non-Europeans [113]. It will be interesting to see if these differences are explained by genetic factors that differ between populations, or rather by environmental factors (including different health care systems), ascertainment bias, or a combination of these. The trans-ancestry association study showed that although for most of the IBD risk loci, the direction and magnitude of effect are consistent in European and non-European cohorts, genetic heterogeneity was seen between divergent populations at several established risk loci, driven by differences in allele frequency (*NOD2*), effect size (*TNFSF15* and *ATG16L1*), or a combination of both (*IL23-R* and *IRGM*). A large trans-ancestry genotype-phenotype study is under way, undoubtedly shedding light on possible genetic heterogeneity of disease subphenotypes in different populations.

3.7 Clinical Implications of the Found Associations

Based on current evidence, it is too early to adjust treatment in IBD according to genetic profiles to personalize treatment [26]. *NOD2* is by far the most studied genetic predictor for fibrostenotic disease in CD. Although many studies suggest an important role for *NOD2* variants in developing fibrostenotic CD, the low sensitivity of a single *NOD2* variant for predicting fibrostenotic disease does not justify *NOD2* genotyping in all patients [44]. It has been suggested that targeted early-intensive therapy for high-risk patients with two *NOD2* mutations might be beneficial, if proven by prospective trials [10], but so far there is no adequate scientific evidence for a top-down medical therapy based solely on *NOD2* variants. Importantly, based on the IIBDGC study including over 19,000 CD patients, it was found that none of the *NOD2* variants are associated with fibrostenotic disease after correcting for disease location. Disease location thus seems to be the major driver to changes in disease behaviour over time [26], although important influences of environmental factors (e.g. smoking) and therapeutic strategies (early top down versus step up) cannot be excluded. Preferential involvement of the terminal ileum could be explained by *NOD2* variants abrogating normal Paneth cell behaviour, as Paneth cells express NOD2/CARD15 throughout the small intestine, with maximal expression in the terminal ileum [46, 123].

3.8 Conclusions and Future Directions

Several genotype-phenotype studies have been performed to find which genetic variants play a role in defining disease location and behaviour, but hardly any variant was uniformly found as independent risk factor for developing fibrostenotic disease. Different reasons can be put forward. A first one is related to power of the individual studies. Many studies indeed included relatively small patient numbers (Table 3.1), and sub-analyses make the sample sizes even smaller. It should also be

noted that various studies may include patient groups from either population-based registries and/or from secondary or tertiary referral centres. This has a direct influence on the proportion of patients with more severe disease as opposed to inflammatory disease, which in turn could lead to over- or under-representation of certain genetic associations. An example are the Scandinavian registries which are population-based, and where indeed a lower proportion of stenosing and penetrating CD is seen [7]. *NOD2* frequencies in these populations are also lower (see above) [121], but this could be linked to the population-based character of the study population. Third, most susceptibility variants are not the pathophysiological causal ones, but are in LD with the true causal variant(s) at that locus, which might have more qualitative or quantitative effects and explain the association with a certain clinical features. Fourth, many studies apply different definitions for stenosing disease or use a limited number of variables given in the Vienna Classification [52]. This of course is an important bias in genetic association studies which rely heavily on the robustness of the phenotypical information. In addition, patients with only subclinical fibrosis without any (sub)obstructive complaints may incorrectly be classified in the unaffected, rather than the affected subgroup which may lead to false or inconclusive findings. Extensive and consistent phenotypical data collections are key to identify novel, and potential causal, SNPs associated with fibrostricturing disease.

Another reason could be the dramatic change in disease behaviour over the course of the disease, implying disease behaviour of CD cannot be analysed without considering the duration of disease [51, 124]. Also, because of the importance of disease location in driving changes of disease behaviour over time [26], disease location should always be considered when analysing risk factors for stenosing disease. In the case of for example *NOD2*, there is a strong correlation of *NOD2* and ileal disease location [125], which might induce a false, confounded association between *NOD2* variants and fibrostenotic disease in those cases where disease location is not considered in the analysis. Finally, disease behaviour is influenced by environmental factors [126], which can be dramatically different in the different studies. Examples include smoking and NSAIDs use, but also specific treatments may hide patients at risk to develop certain subtypes of disease. Any disease behaviour and severity analysis should be interpreted with caution, when there is no access to medication use and response to medications, especially for patients in the biologics era.

Among the 163 genome-wide significant IBD susceptibility loci as identified in the study by Jostins et al. [127], genetic variants in immune system components (*NOD2, IL23R, IL-12B, JAK2*, *FUT2*) and autophagy (*ATG16L1,* leucine-rich repeat kinase 2 (*LRRK2*)) could (jointly) contribute to the activation of mesenchymal cells and pathogenesis of fibrosis [127–129]. Although these susceptibility genes might pathophysiologically contribute to fibrostenotic processes, not all have been found to be associated with stricturing CD. For example, the *LRRK2* CD-associated M2397 allele inhibits Nuclear Factor of Activated T cells (NFAT) [130], which is known to control fibroblast plasticity in the heart [131]. LRRK2 might thus also be involved in fibrosis in the gut, although so far this has not been

reported. The development of fibrosis is preceded by a period of initial inflammation, and not all patients with CD express a fibrostenotic phenotype [124, 132, 133]. This highlights the possible difference between loci predisposing to overall disease (CD or UC), and loci predisposing to clinical phenotypes or disease course [127, 129, 134, 135]. It is thus important to consider the idea of different genes driving susceptibility on the one hand, and disease behaviour on the other. The IIBDGC study for the first time does this on a large scale, but hardly finds any genome-wide significant loci for disease behaviour independent from disease location, except from rs77005575 (MHC) [26].

Despite the lack of validated genotype-phenotype associations in large genome-wide studies, reported SNPs identified in smaller cohorts (as described earlier) contributed to unravelling fibrostenotic CD pathogenesis. The different biological processes that might be suggested based on genetics findings are summarized in Fig. 3.1. We feel that genetics alone will not be able to predict the development of fibrostenotic complication in IBD, largely owing to the large environmental component in disease pathogenesis and its interaction with the genetic background of the individual. We therefore want to advocate that future studies need to be integrated with transcriptomics and clinical, serological, and microbial characteristics. The key predictors found in all these different fields might lead to an integrated, clinically relevant multi-omics biomarker panel, guiding diagnosis and therapeutic decisions in fibrostenotic disease [1].

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Chapter 4
Epigenetic Regulation of Intestinal Fibrosis

Chao Li and John F. Kuemmerle

Abstract Genome-wide association studies have identified over 200 risk loci associated with Inflammatory Bowel Diseases (IBD), Crohn's disease and Ulcerative colitis. These genetic factors, however, account for only a small proportion of genetic inheritability of disease. Our understanding of the pathogenesis of IBD has evolved and currently is thought to occur through the interaction between the host genome and their intestinal microbiome and metabolome with the innate and adaptive immune responses. Genetic risk alone, however, predicts only 25% of disease indicating that other factors including the intestinal environment can shape the epigenome and also independently confer heritable risk to patients. Epigenetic modifications regulate gene expression and protein production and play critical roles in shaping the intestinal immune response, mucosal homeostasis, and the wound-healing process. Analysis of the genetic risk in patients with Crohn's disease combined with epigenetic marks reveals regulatory mechanisms that affect gene expression and disease phenotype. This chapter will focus on what is known about the alteration in the epigenome in Crohn's disease and the mechanisms by which epigenetic risk factors determine development of fibrosis in Crohn's disease. Studies of the epigenome have highlighted new therapeutic targets for therapeutic intervention of the development and progression of fibrosis.

Keywords Fibrosis · Epigenetics · Inflammatory bowel diseases · Mesenchymal cells · Crohn's disease

C. Li
Department of Medicine, Virginia Commonwealth University, Richmond, VA, USA
e-mail: chao.li@vcuhealth.org

J. F. Kuemmerle (✉)
Department of Medicine, Virginia Commonwealth University, Richmond, VA, USA

Department of Physiology and Biophysics, VCU Program in Enteric Neuromuscular Sciences, Medical College of Virginia Campus, Virginia Commonwealth University, Richmond, VA, USA
e-mail: john.kuemmerle@vcuhealth.org

F. Rieder (ed.), *Fibrostenotic Inflammatory Bowel Disease*,
https://doi.org/10.1007/978-3-319-90578-5_4

4.1 Introduction

In 1942, C.H. Waddington coined the term epigenetics to explain how gene modulation regulates development. Since then research in epigenetics has progressed and such has our understanding of the epigenetic regulation of normal physiology and disease. Disease pathogenesis results from the heritable risk that accrues from alterations in DNA sequence, risk polymorphisms, and from alterations in the epigenome that control gene expression when exposed to environmental change. Epigenetic control of gene expression is exerted through modification of DNA regulatory elements or enhancers that induce transition of condensed heterochromatin, where gene accessibility is limited, to euchromatin, where genes are accessible for transcription regulated by histone modification and DNA methylation status. Gene expression is also controlled by small non-coding RNAs, microRNAs, which post-transcriptionally regulate gene expression. Crohn's disease is a polygenetic disorder with >200 risk loci identified by GWAS. However, understanding the risk of disease development or expression of a specific phenotype of Crohn's disease in a patient is not predicted or understood completely by genetic risk. Study of the epigenetic changes associated with development of intestinal fibrosis in Crohn's disease and fibrosis in other organs, including the lungs, heart, liver, and kidneys reveals patterns common to all. This review will focus on what is known about the mechanisms by which epigenetic risk factors determine the development of intestinal fibrosis in Crohn's disease and compare that to what we know from other fibrotic diseases.

4.2 Genetics

Inflammatory bowel diseases (IBD), Crohn's disease and ulcerative colitis, are polygenic diseases for which >200 risk loci have been identified to date [1, 2]. The most significant genetic associations are with the intracellular bacterial sensor, NOD2, autophagic responses, ATG16L1 and IRGM, and with IL-23R. Taken together this has been interpreted as showing how genetic architecture of Crohn's disease involves both defective innate and adaptive immune responses to intestinal microbiota [1]. To date a deeper analysis of GWAS data has not fully revealed a genomic basis that accounts for individual Crohn's disease phenotypes: inflammatory, fibrostenotic or penetrating [2, 3]. An approach using multi-locus genetic risk scores has improved the genetic risk assessment of IBD but also indicates that in addition to established risk variants other independent variables modulate disease progression [4, 5]. Ethnic variation of associated risk loci does not account for ethnic variations in disease location or behavior or phenotype in Crohn's disease [6, 7]. Purely genetic animal models of Crohn's disease are prone to underestimate the interactions between risk loci, "epistasis" [8]. Epistatic components need to be integrated into large-scale biostatistical models by estimating the contribution of non-genetic factors, termed missing heritability, which can be accounted for by epigenetics [9, 10].

Examination of genetic risk loci by pathway analysis or gene ontogeny identifies groups of polymorphisms likely to play a critical role in pathogenesis of fibrostenosis. TGF-β is a key cytokine that is central to the development of fibrosis. The TGF-β pathway includes identified risk variants in Smad3 and Smad7, variants in the cytokine-activated Jak-Tyk2-STAT3 pathway. Each of these play a role in the regulation of TGF-β expression and function. Polymorphisms in Suppressor of cytokine signaling 3 (SOCS3), the negative regulator of cytokine induced activation of the Jak-Tyk2-STAT3 pathway are also seen in subjects with IBD [11, 12]. Mesenchymal cells: fibroblasts, myofibroblasts and smooth muscle, play a central role in the development of fibrosis as the key cell types are activated and produce TGF-β1 and excess extracellular matrix including collagen and fibronectin [13]. The functional outcomes of mutations in these key GWAS risk loci that mechanistically result in TGF-β1-dependent fibrosis are distinct from the outcomes of mutations leading to initial and sustained inflammation in epithelial and immune cells in the intestine. In the case of TGF-β signaling Smad7 is increased in epithelial and immune cells and inhibits Treg responses. In contrast Smad7 is diminished in affected intestinal subepithelial myofibroblasts and muscle cells allowing sustained TGF-β1 signaling and excess extracellular matrix production, leading to the stricture formation [14–16].

Other loci have been identified that confer risk of fibrostenotic disease that involve other pathways leading to fibrosis in the intestine. The 5T5T polymorphism at the matrix metalloprotein-3 (MMP3) gene increased the risk of developing fibrostenotic complications [17]. The MMPs and tissue metalloproteinases (TIMPs) are key regulators of the balance between extracellular matrix deposition and degradation. Homozygosity for rs1363670 G-allele near IL-12B is an independent risk factor for development of fibrostenosis and for a shorter time to critical stricture formation in the ileum [18]. Other risk alleles have been identified in patients with penetrating disease. It is worth noting that the Montreal classification of Crohn's disease is hierarchical. Patients may express a penetrating phenotype that is the result of underlying fibrostenosis. Thus, studying Montreal Class B2 fibrostenotic Crohn's disease, distinct from B1 inflammatory and B3 penetrating phenotypes is of crucial importance in understanding risk loci and susceptibility of a particular phenotype [19].

4.3 Epigenetics

The identified genetic factors and susceptibility loci account for only 13.6% of disease variability and no more than 25% of the genetic risk in Crohn's disease [1, 7]. Epigenetic processes translate environmental events associated with genetic risk into regulation of chromatin state, shapes the expression of genes, and thereby the activity of specific cell types that participate in disease pathophysiology. Epigenetic mechanisms are emerging as key mediators of the effects of both genetics and the environment on gene expression and disease [20]. Epigenetic modifications represent a fundamental regulatory mechanism that has a profound influence on a

multitude of different phenotypic outcomes in a chromatin-templated environment for both normal and pathological development. In addition to a set of inherited epigenetic marks, there are non-heritable epigenetic marks that are more dynamic and change in response to environmental stimuli [21]. In Crohn's disease interaction of the environment, including the intestinal microbiome and metabolome, with the susceptible patient's genome and immune system, jointly shape the epigenome. These non-genetic effects that alter gene expression and function are implied by the results of multi-locus genetic risk analyses and represent the missing heritability in GWAS [4, 5].

Epigenetics is defined as a "stably inherited phenotype that results from mechanisms other than changes in DNA sequence" [11]. Although initially an individual's epigenome was not thought to be heritable, there is now increasing evidence that epigenetic inheritance can persist for multiple generations [22]. Evidence from a number of lines of investigation demonstrate epigenetic heritability from cell to cell during mitosis, from generation to generation during meiosis, and include true transgenerational inheritance [23], which means transmittance of information from one generation to the next that affects the traits of offsprings without alteration of the sequence of DNA. Such mechanisms have been shown to include incomplete erasure of DNA methylation, parental effects, transmission of distinct RNA types (e.g. mRNA, non-coding RNA, miRNA), and persistence of subsets of histone marks [23]. Epimutations, epigenetic changes that are sustained in the germ line, can be transmitted in a true intergenerational fashion by surviving the developmental reprogramming that erases epigenomic changes present in the parent. This mechanism has been shown to be operative in animal models of liver fibrosis. Remodeling of DNA methylation and histone acetylation in offspring of mice harboring epigenetic changes altering TGF-β1 expression resulting in liver fibrosis is lower in male F_1 and F_2 generations through a process termed suppressive adaptation [24]. Humans with milder non-alcoholic fatty liver disease have hypomethylation of the anti-fibrogenic factor *PPAR-γ* promoter compared to patients with more severe fibrosis lending support to this notion. All these findings suggest transmission of an epigenetic suppressive adaptation that can help offspring better adapt to future hepatic insults that might result in fibrosis. Suppressive adaptation, however, was not seen in the setting of renal fibrosis [24].

Even though all cells within the intestine or an organism share a common genome, gene expression in an individual cell type is regulated by the unique epigenetic events that affect that cell type and may be distinct from neighboring cell types. This can account for the sometimes contradictory epigenetic mechanisms that are identified as regulating gene expression in different cell types such as epithelial, immune and mesenchymal cells. Understanding which mechanisms regulate gene expression in a cell type that are critical to a disease process, e.g. mesenchymal cells and fibrosis, is difficult when based on an epigenetic analysis of DNA obtained from heterogeneous cell populations.

Epigenetic changes that regulate gene expression and function are grouped into four main types: DNA methylation, histone modifications, nucleosome positioning and small or non-coding RNAs. No information on nucleosome positioning as it

relates to fibrosis in Crohn's disease exists to date and therefore will not be discussed further here. The other processes are discussed in greater detail as it relates to the development of fibrosis in general and to what is known about the development of fibrosis in patients with Crohn's disease (Table 4.1).

Table 4.1 Genes that can be regulated by epigenetic mechanisms in the development of intestinal fibrosis

Gene	Expression level in intestine	Epigenetic mechanism			References
		Methylation	Histone modification	miRNA	
Smad3	↑	N/A	HDAC1	miR-21, miR-154, miR-29	[25–28]
Smad7	↓	DNMT1	N/A	miR-21, miR-17-5p	[27, 29]
SOCS3	↓	DNMT1	N/A	miR-19b	[11, 12, 30]
MMP	↑	Promoter	HDAC	miR-17, miR-18a, b & miR-19a, b,	[31, 32]
α-SMA	↑	CpG, DNMT1, DNMT3b	H3K4me1	N/A	[33, 34]
COL	↑	DNMT1, DNMT3b	H4 acetylation, Fli-1 acetylation, H3K4me1	miR-18a, b and miR-19a, b, miR-29	[34–40]
VMP1	↑	N/A	N/A	miR-21	[41]
TGF-β1	↑	Smad7 methylation, Smad4 hypermethylation	H3K4me3↑, H2A. Z↑, ↓H3K9me2 and H3K9me3 on the promoters of ECM genes	miR-21, miR-17 and miR-19a, b	[29, 42]
COX2	↓	Promoter hypermethylation or hypomethylation	↓Histone H3 and H4 acetylation, ↑ in H3K9me3, H3K27me3, and DNA methylation	Reported mostly in cancer research, miR-101, miR-26b, miR-146a, miR-16 and miR-122	[35, 43]
CXCL10	↑	N/A	H3	Unknown	[44]
TIMP1	↑	DNMT1	H3K4me1	miR-17, miR-29, miR-1293	[36]
Spry-1	↓	Promoter hypermethylation	HDAC↑ Spry-1 gene expression	miR-29, miR-21	[37]
PTEN	↓	DNMT1-induced hypermethylation	Its interaction with histone H1 to keep chromatin condensation	miR-21	[42]

(continued)

Table 4.1 (continued)

Gene	Expression level in intestine	Epigenetic mechanism			References
		Methylation	Histone modification	miRNA	
PPAR-α	↓	Promoter hypermethylation	N/A	miR-21, miR-10b, miR-33a	[25, 45, 46]
STAT3	↑	DNMT1	HDACs, SET1, LSD1, EZH2	miR-21, miR-17, miR-29, miR-98	[26, 27, 37, 38, 42, 47]
Thy-1	↑	DNMT1, hypermethylation	H3, HDAC inhibitor	N/A	[43, 44]
IL-27	↓	N/A	N/A	N/A	[48]
NOD2	↓	DNA methylation	H3K4Me2 and H4Ac, H3K27Me3 histone modifications	miR-29, miR-192	[48]
TNF-α	↑	DNA demethylation	Histone acetylation, H3K9 and H3K4 methylation	miR-23a, miR-155, miR-346	[48]
SMT1	↓	DNA methylation	N/A	N/A	[48]
IL-19	↓	N/A	N/A	N/A	[48]

↑ = upregulation, ↓ = downregulation, N/A = study not done

Epigenome-wide association studies (EWAS) have been performed in patients with IBD and provide an analysis of differentially methylated sites or regions in different tissues from the IBD patients within different populations [45]. These findings are difficult to reproduce or understand how they confer risk of disease due to several confounding factors including the selection of different patient populations (adult vs pediatric), the selection of different tissue resources (PBMCs, EBV transformed B cell lines, or colonic biopsies or intestinal mucosal), and selection of different locations (ileum, jejunum, colon, or rectum) [45, 49]. Further EWAS using large populations of IBD patients, who are deeply phenotyped will be needed to integrate what we know from GWAS into a more complete understanding of the pathogenesis of specific IBD phenotypes. More importantly, the combination of advanced, large parallel/next generation sequencing approaches will convert research findings into a translational platform that informs personalized precision medicine.

4.4 Epigenetics and Fibrosis

Fibrosis is characterized by an integrated cascade of cellular and molecular mechanisms that results in excess extracellular matrix production, which is initiated by tissue injury in any organ and lead to destruction of normal tissue structure and

finally organ failure. Although there are a variety of cell types that participate in fibrogenesis, it is the mesenchymal cells that generate the TGF-β-mediated production of collagen-rich scar tissue. Fibrotic disease-related death accounts for ~45% of all deaths in the developed countries. Accumulation of emerging evidence indicates that epigenetic mechanisms play a role in promoting a heritable pro-fibrotic phenotype in mesenchymal cells including fibroblasts, myofibroblasts and muscle cells, which are actively involved in development of fibrosis.

4.5 DNA Methylation

The addition of a methyl group to the 5′ carbon of the cytosine residue by replacement of the hydrogen in position 5 (5MeC) in the context of cytosine-guanine (CpG) dinucleotides that are clustered in CpG islands is the most widely studied epigenetic modification. Sixty to eighty percent of the CpG dinucleotides present in the human genome are methylated [49] and show regional differences in its distribution. About 30,000 CpG islands are present in the human genome, typically extend for 300–3000 base pairs, and are located close to or within 40% of gene promoters. Methylation typically, but not always, represses gene expression by either interfering with the binding of transcription factors to their DNA binding sites or by recruiting methyl-CpG-binding proteins that attract histone and chromatin modifying enzymes. DNA methyltransferases (DNMT)-1 and DNMT-3a and 3b are the primary enzymes responsible for methylation of CpG islands [50]. DNMT-1 is a maintenance methyltransferase whereas DNMT-3a and 3b are de novo methyltransferases. Methylation can be reversed by either active or passive demethylation. The ten-eleven translocation methylcytosine dioxygenase (TET) family of enzymes catalyze active demethylation via oxidation of cytosines forming the 5-hydroxymethyl-2′-deoxycytine (5HMeC) which attracts DNA excision and repair machinery thereby restoring DNA to its demethylated state [51]. This suggests that oxidation is part of a demethylation pathway of DNA. Passive demethylation occurs when maintenance methylation is absent and progressive dilution of 5meC occurs during DNA replication [52].

4.6 DNA Methylation and Fibrosis

DNA methylation status has been examined in a number of disease processes that result in tissue fibrosis including systemic sclerosis, pulmonary and cardiac fibrosis, hepatic fibrosis, and intestinal fibrosis in Crohn's disease [21, 48, 53–58]. Hypermethylation of specific genes as well as global changes in DNA methylation have been identified in these organ systems. Two genomic studies in patients with idiopathic pulmonary fibrosis (IPF) demonstrated extensive DNA methylation changes in the control of IPF gene expression [35, 59, 60]. CpG island methylation changes are present in genes linked to a fibro-proliferative phenotype in IPF and to

myeloproliferative diseases, via miR-17–92 cluster that include increased DNMT-1-mediated feedback affecting DNA methylation and microRNA expression [30, 33]. Notably altered CpG island methylation in the α-smooth muscle actin (α-SMA) promoter is present in pulmonary fibroblasts and myofibroblasts of patients with IPF [34]. A core set of genes known to be related to fibrosis, including several collagens, were differentially methylated in patients with progressive renal fibrosis compared to controls [41]. Recently, in a rat model of hypoxia-induced cardiac fibrosis, global hypermethylation of gene expression was observed along with upregulation of both DNMT-1 and DMNT-3b that resulted in increased collagen and α-SMA [42]. Whether DNA methylation is fundamental to transcriptional repression still remains elusive with some researchers arguing that DNA methylation is a consequence rather than a cause of gene repression [34, 41, 61]. Aberrant DNA methylation is a classic hallmark of cancer and many other diseases, which is composed of loss of DNA methylation and hypermethylation of specific gene promoters. Moreover, whether cyclical demethylation and remethylation processes plays a role in intestinal fibrosis is unknown and awaits clarification with further investigations. Importantly, Watson et al. showed that hypoxia-induced cardiac myofibroblasts could be reversed back to a fibroblast by both silencing of HIF-1α and exposure to 5′-Aza′C, a non-specific DNMT inhibitor [42]. Our recent work has implicated methylation as a regulator of *Smad7* and *Socs3* gene silencing in human myofibroblasts and smooth muscle cells from affected ileum as expression was restored after treatment with the demethylating agent 5′-Aza′C or knockdown of DNMT-1 [27]. These findings indicated the important role of DNA methylation in mesenchymal cell function in the development of intestinal fibrosis in Crohn's disease.

Genome-wide methylation profiling in patients with IBD has identified numerous sites that are differentially methylated between cases and controls [53]. The most highly statistically significant include genes controlling altered immune activation, responses to luminal bacteria and regulation of the Th17 pathway [48]. A significant enrichment in DNA methylation was seen within 50 kb of several Crohn's disease GWAS risk loci including IL-27, IL-19, tumor necrosis factor (TNF), Soluble latent membrane-type 1 (SMT1) and NOD2. In this study by Nimmo and colleagues, methylation status was predictive of disease activity [48]. In pediatric Crohn's disease, Adams et al. provided evidence that 4 of the most differentially methylated regions resided in proximity to the vacuole membrane protein-1 (VMP1) GWAS locus [29]. VMP1 is a putative transmembrane protein that has been reported to be involved in different biological events including autophagy, cell adhesion, and membrane translocation [62]. The microRNA (miR)-21 gene lies within the VMP1 gene. They share a common transcription start site and promoter region but pri-miR-21 possesses its own unique promoter thus VMP-1 and pri-miR-21 can be differentially transcribed. Primary miRNA (pri-miRNA) with about 100 nucleotides are transcribed from miRNA genes in the nucleus by RNA polymerase II and further processed into pre-miRNA by a microprocessor complex. Our own recent work has demonstrated that the increased transcription of pri-miR-21 in muscle cells and myofibroblasts of patients with fibrostenotic Crohn's disease

results in the sustained TGF-β1 signaling that results in excess collagen and extracellular matrix production and fibrosis [62]. This process uniquely characterizes patients with Montreal Class B2 fibrostenotic Crohn's disease as distinct from patients with Montreal Class B1 inflammatory and Montreal Class B3 penetrating Crohn's disease [19, 62, 63]. These various studies have suggested numerous specific gene-specific methylation events occur in different organ systems as well as provided evidence for promising therapeutic targets through the modulation of epigenetic changes (Table 4.1).

4.7 Histone Modifications of DNA and Post-Translational Modifications of Proteins

Histones are also key players in epigenetic regulatory mechanisms. The four core histones, H2a, H2B, H3 and H4 associate as two H2A–H2B dimers and a H3–H4 tetramer and comprise the nucleosome [64]. Adjacent nucleosome octamers are separated by ~50 kb of DNA with the linker histone, H1 interposed between them. Histones are subject to post-translational modifications on their tail regions including phosphorylation, acetylation, methylation, ubiquitination, SUMOlyation, and ADPribosylation. These post-transcriptional modifications contribute to their ability to regulate the transcriptional state of genomic DNA. Generally euchromatin, open or lightly packed chromatin with accessible DNA and actively transcribed genes, and heterochromatin, condensed or tightly packed inaccessible chromatin, are distinguished by different levels of acetylation and/or methylation of specific histone residues and their position along the genome in promoter regions or intron/exon regions [65, 66]. Histone modifying enzymes catalyze the post-translational modification of histones and non-histone proteins. This large group of enzymes include histone acetyltransferases (e.g. p300/CBP) and histone deacetylases (e.g. HDACs), and lysine methyltransferases (e.g. LSD) [31]. Gene transcription regulated by histones is the cumulative influence of multiple histone modifications that result from the activity of histone modifying enzymes. Data from the ENCODE project has identified key histones and their modifications that have become the most highly studied for their ability to control accessibility of chromatin and thereby regulation of gene expression. Unlike DNA methylation, which typically results in transcriptional silencing, histone modifications exert divergent effects depending upon specific conditions and genes, thus adding another layer of complexity to epigenetic regulation of gene expression in fibrosis. While histone acetylation generally plays antifibrotic roles in many fibrotic diseases, histone methylation plays either transcriptional activation or repression depending on the number of methylations (mono, bi, or tri methylations) and the specific residues and their locations (lysine and/or arginine) [31, 32, 66, 67]. The functions of specific histone modifications have been studied in different diseases and are summarized in Table 4.2 [32, 67–79].

Table 4.2 Histone modifications that regulate gene expression with putative functions

Histone mark	Putative functions	Supplemental references
H3K4me1	Associated with active enhancer and other distal elements, but also closed or poised enhancer A marker of primed enhancers and gene expression during embryogenesis It marks regions of DNA methylation loss during normal ageing.	[32, 67, 68]
H3K4me2	Associated with active enhancer, transcription factor binding in genome-wide datasets. Mark of regulatory elements associated with promoters and enhancers.	[69]
H3K4me3	Active enhancer. Mark of regulatory elements primarily associated with promoters/transcription starts. Hall mark of active gene promoters. It promotes rapid gene activation.	[70, 71]
H3K9ac	Mark of regulatory elements with preference for promoters. Associated with gene activation. It can differentiate active enhancers from inactive ones.	[72]
H3K9me1	Preference for the 5′ end of genes, enriched at the transcriptional start site of active genes.	[70]
H3K9me3	Inactive chromatin, repressive mark associated with constitutive heterochromatin and repetitive elements. It binds heterochromatin protein 1 (HP1) which is responsible for transcriptional repression and the actual formation and maintenance of heterochromatin. HP1 also recruits DNA methyltransferase 3b (DNMT3b), demonstrating the interplay between histone methylation and DNA methylation.	[73]
H3K27ac	Mark of active regulatory elements; may distinguish active enhancers and promoters from their inactive counterparts. Like H3K9ac, associated with transcriptional initiation and open chromatin structure.	[74]
H3K27me3	Inactive chromatin. A repressive mark established by polycomb complex activity associated with repressive domains and silent development genes. Critical for the repression of developmental genes. An important mark of the inactive X chromosome (Xi).	[75, 76]
H3K36me3	Elongation mark associated with transcribed portion of genes, with preference for 3′ regions after intron 1. It serves as a mark for HDACs to bind and deacetylate the histones. Involved in defining exons.	[77]
H3K79me2	Transcription-associated mark, with preference for 5′ end of genes. Its methylation is cell cycle dependent.	[78]
H4K20me1	Preference for 5′ end of genes. Associated with transcriptional activation. Also important for cell cycle regulation.	[79]

4.8 Histone Modifications and Fibrosis

The wide variety of reversible histone modifications regulates the structure of chromatin and gene transcription occurring in a context-dependent manner and plays a critical role in determining the gene-protein-phenotype axis. The emerging specific

inhibitors or agonists targeting each individual PTM of histones in biomedical research, typically in the cancer field, shed light on our understanding of how the epigenetic regulation leads to phenotypic changes in vivo due to early intervention of gene transcription activity.

Histone Acetylation

Chromatin state within the nucleus is regulated by the balance of acetylation and deacetylation of histones thereby regulating the access of transcription factors to DNA. Both histone acetylation and deacetylation are linked to the development of fibrosis. It is worth noting that H3 hyperacetylation through decreased expression of histone deacetylase is consistently associated with pulmonary fibrosis [43, 44]. Alteration of HDAC expression in patients with IPF results in TGF-β-induced myofibroblast differentiation, and excess collagen and matrix metalloproteinase-1 production [44]. However, acetylation is variable despite the fact that an increased acetylation of H3 was seen along with decreased acetylation of H3 on lysine K9 and K18 and increased acetylation on lysine K14 and K56 (unpublished data). Acetylation levels of H3 where shown to regulate expression of genes that are key to fibrosis including cyclooxygenase-2, IFN-gamma-inducible protein 10 (CXCL10), and Thy-1 cell surface antigen [36, 80]. In hepatic stellate cells histone acetylation state regulates expression of profibrotic genes including α-SMA, collagen I, tissue inhibitor of metalloproteinases 1 and TGF-β1 via Histone3 lysine4 methyltransferase I [81]. In systemic sclerosis, increased p300 acetyl transferase activity induces acetylation of Fli-1 proto-oncogene thereby relieving the transcriptional repression of collagens IαI and Iα2, the major collagen species in fibrosis [56].

Distinct patterns of histone H3 and H4 acetylation are present in Crohn's disease [82, 83]. Mokry et al. recently provided evidence that many of the GWAS risk loci overlap with DNA regulatory elements in the intestine including Histone H3 lysine 27 (H3K27ac) and p300 which is responsible for H3K27 acetylation and H3K4 monomethylation (H3K4me1) [84]. Sadler et al. have demonstrated that collagen Iα2 expression induced by the cytokines interleukin-1β, TNF-α and TGF-β is regulated by hyperacetylation of histone H4 [85]. We have recently shown a global increase of H4 acetylation on lysine K5, K8, K12, and K16 in myofibroblasts of affected ileum compared to normal ileum in the same patient with stricturing Crohn's disease. In the strictured ileal myofibroblasts an increased expression of both p300 and HDAC1 with the increase in HDAC 1> p300 was noted. This suggests that the balance between HAT and HDAC activities needs to be considered jointly to understand the regulation of histone acetylation in the epigenome. HDAC1 inhibition represents a potential therapeutic approach in IBD patients as seen in cancer treatment. Evidence on the use of HDAC inhibitors with other fibrotic diseases includes lung fibrosis, kidney fibrosis but not yet intestinal fibrosis. The fibrotic signaling pathways common to all fibrotic diseases with characteristic features suggest this may be effective.

Histone Methylation

We have also found a global increase of H3 methylation on lysine K4, K9, K27, K36, and K79 in affected ileum compared to normal ileum in the same patient with

stricturing Crohn's disease (unpublished results). Interestingly, we noticed that histone methylations occur in H4 lysine K20 as mono, bi, tri methylations, H4 arginine 3 methylations on 2a and 2s, and phosphorylation of Serine 1. The underlying mechanism of methylation has not yet been identified but it appears methylation of histone lysines is more discriminative than acetylation of histones (unpublished data). Trimethylation of histone lysine 9 of histone H3 or trimethylation of K27me3 are usually associated with transcriptional silencing.

4.9 MicroRNA

RNA interference of gene expression by microRNA (miR), small ~18–24 nucleotide non-coding single-stranded RNA molecules, is implicated in the epigenetic regulation of fibrosis [45, 86]. In general, miRs post-transcriptionally repress gene expression by targeting mRNA for degradation. miR genes are located throughout the genome. They can be found in introns of coding regions, in introns or exons of non-coding genes or in intergenic regions. In some cases they are transcribed independently from their own specific promoters as is the case with primary microRNA-21 (pri-miR-21) despite its location within the VMP-1 gene [29, 46].

4.10 MicroRNA and Fibrosis

A number of miRs have been identified that have a similar role in the regulation of fibrosis in the lung, liver, heart, kidney or skin in addition to the intestine. While these miRs can have organ and tissue-specific regulation and effects, two are consistently associated with fibrosis and with the expression of TGF-β: miR-21 and miR-29. MiR-21 is pro-fibrotic and is implicated in the transcriptional regulation of Sprouty homolog 1 (Spry-1), phosphatase and tensin homolog (PTEN), peroxisome proliferator-activated receptor-α (PPAR-α), signal transducer and activator of transcription-3 (STAT3) and Smad7 [25, 37, 38, 62, 87]. It is worth noting that miR expression can itself be subject to epigenetic regulation. Transcription of miR-21, for example, is regulated by promoter methylation [38]. During intestinal barrier dysfunction, miR-21 is increased to impair the tight junction integrity and to increase barrier permeability through targeting the Rho GTPase, RhoB [94]. MiR-29a, b, c are anti-fibrotic and are implicated in the silencing of collagen, MMP and Spry1 expression [26, 27, 39, 40, 47, 62, 88–91]. MiR-29 expression is downregulated by the TGF-β-dependent Smad3 transcription factor [88]. The miR17–92 cluster is also an important determinant of fibrosis that is regulated by IL-6 in the fibrotic intestine. Transcribed from this cluster are several miRs that can target key proteins in fibrosis including Collagen IαI (miR-18a, b and miR19a, b), TGF-β (miR-17 and miR-19a, b) and MMPs (miR-17, miR-18a, b and miR-19a, b) [28, 33, 92] (Table 4.3). Unique miRNAs expression profiles in tissue samples and

Table 4.3 Summary of non-coding RNAs associated with intestinal fibrosis in Crohn's disease

microRNA	Human tissue	Expression level	Target	Pathway/ function	Methods	References
miR-21	Ileal CD tissue with different phenotypes	Increased	Smad7, Smad3, RhoB, PDCD4	TGF-β, Th1	qRT-PCR, DNA-ChIP	[37, 38, 93–95]
miR-29/ miR-29b	Mucosa overlying strictures, mucosal fibroblasts, human dendritic cells from CD	Reduced	Collagen I and III, Smad3, IL-12p40, IL-23	TGF-β, EMT	qRT-PCR	[26, 40, 47, 89, 90]
miR-200 family	Intestinal epithelial cells, intestinal biopsies	Decreased	Smad2, E-cadherin, ZEB1	TGF-β, EMT	qRT-PCR	[91]
miR-192	Active UC tissue, colonscopic pinch biopsies from the sigmoid colon	Downregulated	MIP-2α, SIP1, NOD2, Smad3	Cytokine production	qRT-PCR, in situ hybridization	[96–98]
miR-143/145	Intestinal mesenchymal cells	Decreased	IGFBP5, PI3K/Akt	IGF	qRT-PCR	[99]
miR-155	Intestinal myofibroblasts, colonic mucosa pinch biopsy, intestinal epithelial HT29 cells	Increased	FOXO3a, IL-8, IκBα	Th17	qRT-PCR	[100]
miR-19b	Intestinal pinch biopsy, intestinal epithelial cells	Decreased	SOCS3	JAK/STAT	qRT-PCR	[11, 12, 30, 33]
CDKN2B-AS1	Colonic samples, colonocyte cell line	Decreased	TGF-β	TGF-β	qRT-PCR	[101]

peripheral blood were reported between patients with UC and CD to differentiate the diagnosis of IBD [93, 94, 102]. The study of the role of miRNAs in IBD has yielded significant insights with a deeper understanding yet to be gleaned. Even though there is much progression in anti-inflammation treatment of IBD in clinical trials and practice, the frequency of stricture complication post-surgery and after immunotherapy is still high and no cure for targeted fibrosis is currently available [7–10].

4.11 Long Non-Coding RNA and Fibrosis

The understanding of the regulatory role of long non-coding RNA (lncRNA) on gene expression as it relates to fibrosis is emerging. Examination of the transcriptome of lncRNAs in IBD has demonstrated expression profiles that distinguish inflamed and non-inflamed Crohn's disease and ulcerative colitis [103, 104]. The lncRNA CDKN2B-AS1 is associated with both Crohn's disease and ulcerative colitis and is downregulated by TGF-β [101]. From a functional perspective the lncRNA H19 is protective against renal fibrosis whereas Wisp2-super-enhancer associated RNA, Wisper, controls cardiac fibrosis [105, 106].

4.12 Summary

GWAS analysis of Crohn's disease has identified numerous risk loci that account for up to 25% of the genetic risk. Recent investigations of the epigenome indicate differential changes in DNA methylation patterns, histone modifications and differential expression of miRs can further contribute to the "heritable" risk of developing fibrostenotic Crohn's disease. Integration of genetic susceptibility with changes in the epigenome associated with the development of intestinal fibrosis has been demonstrated for several genes key to the development of fibrosis in Crohn's disease including STAT3, Smad3, Smad7 and SOCS3. Preclinical studies from different laboratories have supported the potential of epigenetic therapeutics including DNMTs inhibitors, HDAC inhibitors including butyrate, a natural HDAC inhibitor, and the histone methylation inhibitor EZH2. It is also important to note that the commensal microbiota have a direct impact on the host epigenome but the correlation between the two and Crohn's disease phenotype is as yet unknown.

For progress to be made in Crohn's disease efforts to understand the epigenome and it changes that relates to the identified risk loci and their associated pathways and thus the missing heritability of fibrosis will be needed. This understanding will only improve from our exploration of strictly phenotyped and genotyped patients with fibrosis using well-defined disease-relevant cell populations.

Acknowledgments Supported by DK49691 from NIH: National Institutes for Diabetes, Digestive and Kidney Diseases (JFK).

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

Noam Jacob, Stephan R. Targan, and David Q. Shih

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

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

N. Jacob
F. Widjaja Foundation, Inflammatory Bowel and Immunobiology Research Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA

Vatche and Tamar Manoukian, Division of Digestive Diseases, Geffen School of Medicine, University of California, Los Angeles, CA, USA
e-mail: njacob@mednet.ucla.edu

S. R. Targan · D. Q. Shih (✉)
F. Widjaja Foundation, Inflammatory Bowel and Immunobiology Research Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA
e-mail: Stephan.Targan@cshs.org; david.shih@csmc.edu

F. Rieder (ed.), *Fibrostenotic Inflammatory Bowel Disease*,
https://doi.org/10.1007/978-3-319-90578-5_5

5.1 Introduction

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

5.2 "Inflammatory" Cytokines

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

5.2.1 TNFα

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

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

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

Table 5.1 Cytokine and drug targets in fibrosis

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

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

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

5.2.2 Th1 Cytokines

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

5.2.3 IL-1 Cytokines

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

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

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

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

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

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

5.2.4 Th2 Cytokines

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

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

5.2.5 Th17 Cytokines

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

5.2.6 TL1A

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

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

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

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

5.3 "Regulatory" Cytokines

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

5.3.1 TGFβ

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

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

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

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

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

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

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

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

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

5.3.2 IL-10

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

5.4 Concluding Remarks

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

Acknowledgement This work is supported NIH T32 DK07180-43 (NJ), Specialty Training and Advanced Research (STAR) Program at UCLA (NJ), NIH R01 DK056328-16 (NJ, SRT and DQS), NIH K08 Career Development Award DK093578 (DQS), and the F. Widjaja Foundation Inflammatory Bowel & Immunobiology Research Institute (NJ, SRT and DQS).

Conflict of Interest The authors have declared that no conflict of interest exists.

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Chapter 6
Inflammation-Independent Mechanisms of Intestinal Fibrosis: The Role of the Extracellular Matrix

Debby Laukens

Abstract Current therapies controlling inflammation in patients with Crohn's disease do not modify natural disease progression to stenosis, suggesting that the molecular mechanisms contributing to intestinal fibrosis occur partly independent from inflammation. This may be explained by auto-propagation of fibrosis, accomplished by components of the interstitial, non-cellular environment referred to as the extracellular matrix (ECM). Aside from its function in maintaining tissue integrity, the ECM is a highly dynamic structure that closely communicates with cells, including with those that produce ECM components. Interaction of fibroblasts with the ECM through multi-protein focal adhesions orchestrates a variety of processes including proliferation, migration and activation. In particular, the mechanical properties of the ECM, determined by the degree of 'stiffness' which is typically increased in the stenotic bowel, induces a variety of pro-fibrotic signaling cascades in fibroblasts. Although the mechanical cues translating into the activation of these cells have only begun to be unraveled, mechanotransduction in fibroblasts should be considered as an important inflammation-independent contributor to intestinal fibrosis. In addition, the ECM is a reservoir of growth factors and a source of 'danger signals' that can trigger pro-fibrotic responses in the rigid ECM. This chapter provides an overview of the components of the intestinal ECM, the interaction with fibroblasts, and the inflammation-independent mechanisms contributing to fibrosis including mechanotransduction of fibroblasts and mechanical activation of the ECM. Finally, the potential therapeutic targets in these pathways to tackle fibrogenesis in the intestine are discussed.

Keywords Mechanotransduction · Integrins · Focal adhesions · Extracellular matrix stiffness · Smad signaling and Rho kinases

D. Laukens
Ghent University, Ghent, Belgium
e-mail: debby.laukens@ugent.be

F. Rieder (ed.), *Fibrostenotic Inflammatory Bowel Disease*,
https://doi.org/10.1007/978-3-319-90578-5_6

6.1 The Extracellular Matrix of the Bowel Wall

The extracellular matrix (ECM) found in the intestine refers to the fibrous, hydrated, non-cellular environment that provides structure, compressive and tensile strength and elasticity to the tissue. A compliant ECM in the intestine is particularly important, since the bowel wall is continuously exposed to shear toward the intraluminal chime, stretch and compression forces, and must resist tissue distension in case of inflammation [1]. In addition, the ECM in the intestinal mucosa functions as a scaffold for the epithelial cells, and accommodates the cells residing in the lamina propria. Although it was once thought that the ECM represents a relatively inert mass, increasing evidence supports a very active role of the ECM during various physiological and pathological conditions [2]. It is a highly dynamic structure, both in terms of density and composition, which is crucial to maintain tissue homeostasis. In addition, the ECM actively contributes to the fate of cells; it provides a route for cell migration and controls their polarization, proliferation and differentiation by means of highly regulated cell-ECM interactions. The relevance of well-orchestrated mechanisms of ECM modulation is demonstrated by the large number of pathological conditions associated with genetic defects in ECM components, many of which are embryonic lethal [3, 4]. It is therefore not surprising that the ECM plays a crucial role during recurring cycles of bowel distension, mucosal cell infiltration and wound healing associated with chronic inflammation and disease progression in inflammatory bowel diseases [5].

Two types of ECM can be distinguished in the intestinal mucosa, i.e. the ECM as a dense structure supporting and controlling the epithelial monolayer referred to as the basement membrane, and the interstitial loose connective matrix, providing tissue resistance and incorporating mucosa resident cells. Extracellular matrix constituents of the basement membrane are produced by epithelial cells and stromal fibroblasts [6], whereas those in the mucosa are also produced by resident mesenchymal cells, mainly fibroblasts and myofibroblasts. The submucosa contains loose connective tissue with fibroblasts as the main cell type and is traversed by blood vessels and nerves, in which the fibroblast and smooth muscle cells surrounding the muscularis mucosae are the main producers of ECM.

6.1.1 Major Components of the Mucosal Extracellular Matrix

Water constitutes the majority (up to 90%) of the extracellular space, providing the typical viscosity of the ECM. The water content determines tissue volume and its compressive resistance, whilst creating the space for movement of cells, and the exchange of nutrients and other molecules with the blood supply. The major classes of macromolecules found in the ECM are polysaccharides and proteins, assembled and combined into highly complex structures, mirroring the complexity of functions of the ECM (Table 6.1). Well over 100 core proteins have been found in the colonic

Table 6.1 Major classes of extracellular matrix molecules found in the bowel mucosa

Class	Type	Structure	Examples	Functions
Polysaccharides	GAG (bound to core proteins)		Heparin, heparan sulphate	Sequestering of water
	GAG (not bound to core protein)		Hyaluronic acid	Sequestering of water, matrix integrity and signaling
Proteins	Collagens	Fibril-forming	Collagen I, III, V, XI	Tensile strength
		FACIT	Collagen IX, XII, XIV, XVI	Links fibers to each other and to the ECM
		Sheet-forming	Collagen IV	Scaffold for epithelial monolayer
	Proteoglycans		Decorin, syndecan, versican	Reservoir for growth factors, ECM-cell interaction
	Glycoproteins	Fibril-forming	Laminin, elastin, fibronectin, tenascin, nidogen	ECM assembly, ECM-cell interaction, elastic strength
	ECM-associated proteins		ECM modifying enzymes, growth factors, cytokines, mucus	ECM remodeling, mesenchymal cell activation

GAG glycosaminoglycan, *ECM* extracellular matrix, *FACIT* fibril-associated collagens with interrupted triple helices

ECM, largely categorized as collagens, glycoproteins and proteoglycans. In addition, the matrix contains a wide variety of so-called ECM-associated proteins, since these do not directly contribute to its structural integrity [7].

6.1.1.1 Collagens

Collagens represent the dominant structural units in the ECM and are characterized by the presence of one or more triple helix domains. They are classified as fibrillary (fiber-forming) and non-fibrillary and are designated by Roman numbers. The fiber-forming collagens typically assure the tensile strength of the mucosa. Collagens have a unique protein composition, containing the common motif Gly-Pro-X and Gly-X-Hydroxypro (X designating any amino acid except glycine and proline), which is required to generate the stabilization of the helical structure. Fibrillary collagens self-assemble into triple helices or fibrils, composed of homotrimers or heterotrimers of α chains. More than 40 genes encoding α chains have been identified in the human genome, producing at least 28 different combinations of collagen fibrils [8]. For example, collagen I proteins are encoded by the *COL1A1* and *COL1A2* genes, in which two chains of the *COL1A1* gene product assemble with one chain of the *COL1A2* coding protein to form a fibril.

The generation of collagen fibers occurs through a complex set of pre-and post-translational steps [9]. Following the synthesis of the preprocollagen alpha chains in the rough endoplasmic reticulum and upon transit through the Golgi complex, the signal peptides are lost, generating procollagen alpha chains. These proteins contain a number of additional non-helical N- and C-terminal propeptides that increase the solubility of the protein in the endoplasmic reticulum and will aid in the formation of the helical structure. Upon Golgi transit, numerous modifications will take place, including hydroxylation (e.g. by lysine hydroxylases) and glycosylation. These modifications allow the procollagen alpha chain proteins to twist upon themselves forming the typical triple helical structure. Next, the resulting procollagen fibrils are packed in secretory vesicles that traffic along the microtubules to the membrane. Upon secretion of the procollagens in the extracellular milieu, procollagen proteases will remove the propeptides, reducing their solubility and generating the so-called tropocollagen. Next, multiple tropocollagens will gather and polymerize into collagen fibers under the guidance of oxidation of certain lysine residues by extracellular lysine oxidases, which will eventually place covalent bonds within (intramolecular cross-links) and between the molecules (intermolecular cross-links). This is the critical step that gives the collagen fibers tremendous strength. Finally, the fibers are stabilized via interactions with non-fibrillary collagens also referred to as fibril-associated collagens with interrupted triple helices (FACIT). The higher order configuration of all collagens is a long rod-like structure, that can be identified by the striated effect under the electron microscope, since the assembly of tropocollagens is such that adjacent molecules are displaced approximately 1/4 of their length. Importantly, scar collagen in adults is not so highly organized and only regains a fraction of its initial strength and elasticity.

In the gut, mainly type I, III, IV and V collagen is found, of which type IV collagen is abundantly expressed in the basement membrane, whereas type I collagen is mostly found in the interstitial ECM of the mucosa, and type III in the submucosa [10, 11]. In the fibrotic intestine of patients with Crohn's disease, collagen types I, III and IV are highly increased [10–12].

6.1.1.2 Glycosaminoglycans and Proteoglycans

The most abundant sugars in the ECM are the glycosaminoglycans (GAGs, including heparin and heparan sulphate), which are large unbranched chains of polysaccharides made up of repeating disaccharide units that contain a net negative charge [13]. This negative charge assures that GAGs adopt an extended conformation, and attract divalent ions and water, leading to its high viscosity. Large chains of polysaccharides are usually linked with core proteins to form proteoglycans, and can be found in the ECM or bound to the cell surface. These core proteins covalently link with GAG side chains and are classified based on the core protein, the number of GAGs and its sulphation status. Proteoglycans fill the gaps in between the collagen

structure and, because of their water-retaining properties, they provide hydration to the tissue. Another hallmark of GAGs is their capacity to bind and release growth factors such as fibroblast growth factors, connective tissue growth factor and transforming growth factor β (TGFβ). Decorin, a small leucine-rich proteoglycan closely linked with collagen I fibers, exhibits an extraordinarily scavenging function, binding and inactivating a wide variety of growth factors [14].

Syndecans are a family of four transmembrane proteins that are substituted with covalently attached GAGs on the external surface, which bind ECM [15]. Recent evidence supports a crucial role for syndecans in sensing the mechanical properties of the ECM (see Sect. 6.2.2) [16].

An atypical GAG is hyaluronic acid (also called hyaluronan), built of D-glucuronic acid and N-acetyl-D-glucosamine disaccharide units which does not contain sulphate and is not bound to a protein core. Hyaluronic acid can adopt extremely large molecular weights, ranging from 5 to 10,000 kDa in vivo, and thus represents a major source of tissue hydration. These structures can be degraded by hyaluronidases and oxidative stress, creating disaccharides that act as danger associated molecular patterns (DAMPs) initiating pro-fibrotic functions in mesenchymal cells (see Sect. 6.3.3). Interestingly, hyaluronidases are also produced by bacteria, however their role in tissue hyaluronic acid degradation and intestinal fibrosis remains to be established [17].

6.1.1.3 Glycoproteins

Glycoproteins are a very diverse set of ECM proteins, and harbor a wide variety of functions, ranging from ECM assembly to ECM-cell interaction [18]. Several glycoproteins contain the Arg-Gly-Asp or RGD motif, which is important for attaching cells to the ECM (see Sect. 6.2.1). They can adopt elastic fiber structures and may also bind growth factors that can be released by proteolysis.

Laminins are the most abundant glycoproteins found in the basement membrane. These proteins are secreted and self-assemble into trimers of α, β and γ chains. The laminins found in the basement membrane are attached to the epithelial cells via α6β4 integrin receptors found in the hemidesmosomes and they adopt a distinct expression pattern along the crypt-villus axis. In the villus, the dominant forms are laminin 1 and laminin 5, whereas laminin 2 is primarily found in the crypts. In the small intestine of Crohn's disease patients, this pattern is lost [19].

Fibronectin is the major fibril forming glycoprotein, attaching cells to various ECM components except collagen IV. More than 11 fibronectins have been identified arising from alternative splicing of a single gene transcript. During fibrotic changes, including in Crohn's disease, fibroblasts will produce a different repertoire of fibronectins, such as fibronectin ED-A that drives further fibroblast differentiation [20, 21]. Fibronectins tightly bind to transglutaminase 2, a transamidating enzyme that catalyzes cross-link formation between fibronectin and other types of ECM such as collagen and laminin [22].

6.2 Mechanosensitive Interactions Between the Extracellular Matrix and Fibroblasts

In the absence of inflammatory stimuli, the main driver of fibroblast activation is the stiffness of the ECM (see Sect. 6.3.1). To understand how such auto-propagation of fibrosis may occur, one must be familiar with the molecular interactions taking place between the ECM and effector cells of fibrosis. Fibroblasts closely attach to components of the ECM and live in a mutual relationship, communicating in a bidirectional way. These interactions are central to regulate signaling pathways in response to external signals from the ECM to the fibroblast, and vice versa, to transfer forces generated by migrating or contracting cells to the ECM.

Mechanical sensing by the cell is achieved at the level of multi-protein assemblies at the cell membrane called focal adhesions. The major constituents of these focal adhesions that are most widely studied are the integrins [23].

6.2.1 Integrins

Integrins are noncovalent transmembrane heterodimers, composed of an α and β subunit, both involved in ECM ligand binding [24]. The mammalian genome contains 18 α and 8 β genes, generating at least 24 different types of heterodimers that bind specific ECM ligands. All β integrins, except for β2 and β7, bind to ECM, and the α subunit largely determines the binding preference to either collagens, laminins or RGD-containing ECM components (Fig. 6.1). The intracellular domain of integrins interacts with a variety of proteins, collectively referred to as the focal adhesion complex (Fig. 6.2) [25].

The exact identity of integrins that play a role in mechanosensing and fibroblast activation in the intestine remain to be established. The most important integrins involved in mechanotransduction are the α5β1 and αν class integrins, which both adopt specific and redundant functions. For example, β1 class integrins are involved in the formation of loose and temporary adhesions, whereas the αν class integrins form the large and highly structured focal adhesions, and both are required for full activation in response to matrix rigidity [26]. α5β1 integrins are highly expressed in intestinal fibroblasts [21].

6.2.2 A Role for Syndecans and Transglutaminase 2 in Mechanosensing?

Syndecan-4 is a critical part of the mechanosensory machinery in cardiac fibroblasts and is necessary to from focal adhesions and exert proper wound healing in mice [16, 23]. Syndecans-1, -2 and -3 do not localize to focal adhesions, but evidence

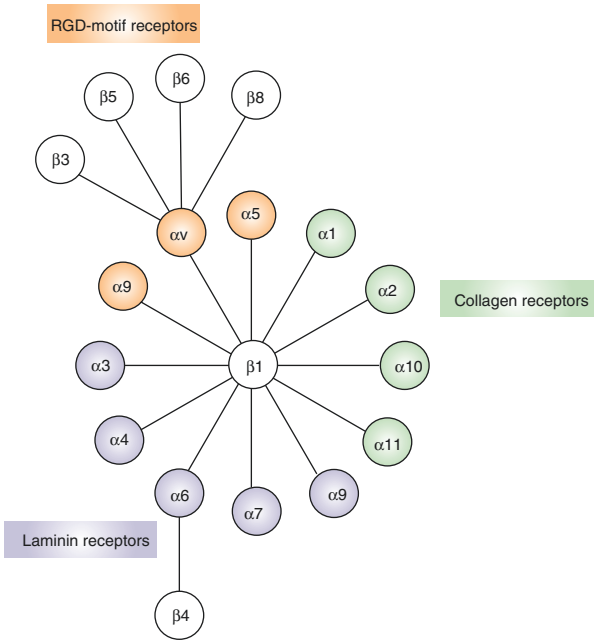

Fig. 6.1 Integrin receptors binding to the extracellular matrix. Adapted from Hynes [24]

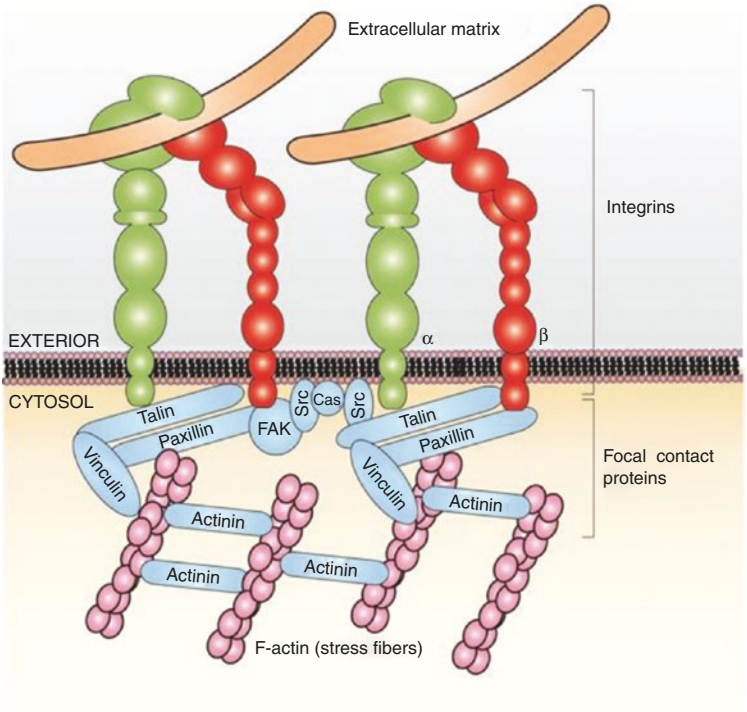

Fig. 6.2 Schematic structure of a focal adhesion. *FAK* focal adhesion kinase, *Cas* Crk-associated substrate family of scaffold proteins, *Src* Src kinase family

suggests that at least syndecan-1 is involved in mechanosensing in cardiac fibroblasts, and was also found to be increased in the stenotic bowel of patients with Crohn's disease [27]. An important role for syndecan-1 in ECM remodeling is demonstrated by impaired intestinal wound healing in mice deficient for syndecan-1, and its decreased expression in patients with ulcerative colitis [28].

Aside from its function in cross-linking fibronectin proteins and thereby solidifying the ECM, transglutaminase 2 is also found within the cell, and acts as a β1 and β3 integrin binding co-receptor for fibronectin, accumulating mainly in focal adhesions [29]. However, whether this enzyme plays a specific role in mechanosensing remains to be established.

6.2.3 Focal Adhesions: The Sites of Mechanotransduction

Focal adhesions are dynamic force-responsive protein complexes made up of concentrated transmembrane receptors, mainly integrins (Fig. 6.2). F-actin stress filaments in the cytosol of the cell are attached to the β subunit of the integrins through a number of adaptor proteins, including vinculin, talin and paxilin. As such, a direct link is created between the ECM and the termini of the F-actin cytoskeleton. Upon mechanical stimulation of the focal adhesion site, adaptor proteins will mediate the recruitment of a variety of downstream signaling molecules (see Sect. 6.3.1.2).

In resting conditions, fibroblasts do not contain stress fibers, and form only loose contacts with the ECM through nascent integrin adhesions, in which integrin binding to ECM is typically weak and short-lived, enabling cells to make and break the interactions [30]. Upon tissue injury however, fibroblasts rapidly transform into an activated cell type, secreting mainly fibronectin and ED-A fibronectin, and start migrating over the injured tissue in an attempt to restore tissue integrity [31]. Migration of the fibroblast itself induces traction forces on the ECM, aligning the fibers along its path. At this point, the fibroblast will form stress fibers, but it will not necessarily acquire the typical molecular features of what is usually considered as a differentiated myofibroblast. In general, a myofibroblast is considered differentiated by its neo-expression of α smooth muscle actin (αSMA) and the formation of F-actin/myosin-containing stress fibers, which renders the cytoskeleton more contractile. Therefore, the initial myofibroblasts exhibiting stress fibers without induced αSMA and myosin expression are sometimes referred to as proto-myofibroblasts [32]. In general, the early events in wound closure are linked with the formation of proto-myofibroblasts, acting on medium stiff substrates and maintaining migration capacities. In contrast, differentiated myofibroblasts are found in late contracting wounds, where they are generated by a combination of mechanical stimulation via the focal adhesions and the presence of TGFβ. For as yet unknown reasons, the ECM deposited during wound healing is never as compliant as it was before, and the contracting wound ECM is less organized and more rigid [30]. The subsequent resistance in the matrix in turn leads to further reinforcement of the ECM-

myofibroblast interactions, by increasing the number of focal adhesions, and increasing the differentiation of the proto-myofibroblast [33].

6.3 Mechanical Activation of Myofibroblasts by the Stiffness of the Extracellular Matrix

For a long time, tissue stiffness has been regarded as a consequence of fibrosis, however since a number of years, it is believed to be an active contributor to fibrosis. The existence of signals in the ECM that actively participate in stimulating further ECM synthesis, even in the absence of inflammation, was elegantly demonstrated by the fact that fibrotic ECM isolated from patients with idiopathic pulmonary fibrosis can induce fibroblast differentiation in vitro [34]. In addition, although transient elastography (Fibroscan) and invasive fibrosis assessment in the liver correlate linearly, increased stiffness of the matrix has been shown to precede fibrosis in the liver in rats [35].

At least two mechanisms may contribute to sustaining intestinal fibrosis even when mucosal healing is accomplished, which both convey to increased matrix stiffness. Firstly, increased stiffness of the regenerated ECM will further drive differentiation of fibroblasts, resulting from the translation of mechanical forces of the ECM to biochemical activity within the cell. Secondly, the ECM is a vigilant reservoir for growth factors and DAMPs, that can be released upon mechanical stretching of the non-elastic ECM. Whether ECM stiffness also impairs growth factor binding and neutralization by peptidoglycans and glycoproteins is unknown.

6.3.1 Extracellular Matrix Stiffness

Stiffness of the ECM is measured as a force, describing the Young's elastic modulus, i.e. the ratio of the amount of stress exerted to bring about a strain (deformation over the initial length), and is thus measured in Pascal (Pa = Newton/m^2) (Fig. 6.3). Stiffness of the matrix is determined by the abundance of fibrillary collagens, and the degree of ECM cross-linking, which is mediated primarily by the activity of transglutaminases, lysine hydroxylases and lysine oxidases [36]. In addition, the concentration of proteoglycans and hyaluronic acid regulates matrix hydration and thereby increases resistance to compression.

Tissue fibrosis is often linked with increased matrix stiffness, an increased abundance of ECM fibrils, and increased ECM cross-linking activity, often induced by TGFβ [37–39]. The Young's modulus of the healthy bowel is 1.9 kPa, whereas Crohn's strictures reach 16.7 kPa [12]. Lysine oxidase enzyme expression is increased in rat colitis, and correlates with enhanced matrix stiffness [39–42]. It must be noted however, that stiffness of a tissue is not only caused by ECM stiffening but also by inflammatory oedema [42].

Fig. 6.3 The Young's elastic modulus. *ECM* extracellular matrix

6.3.1.1 Modeling Extracellular Matrix Stiffness

Most data generated to demonstrate the relationship between ECM stiffness and cell fate were determined in vitro, whereas in vivo data are mostly circumstantial. Although these in vitro conditions are not perfect, they have provided some important basic insights in cell response approximations in vivo. Standard in vitro culturing surfaces have a stiffness in the GPa range, and therefore one can assume that fibroblasts grown on untreated cell culture surfaces represent proto-myofibroblasts. To maintain a dormant cell type and create the ability to evoke mechanical stresses in vitro, culture dishes are typically coated with various types of materials.

Gels that mimic the higher order fibrous material such as collagen type I, Matrigel, and fibronectin have been used frequently. These proteins self-assemble, and the level of cross-linking of for example collagen matrices can be modified by increasing the protein concentration or including glutaraldehyde or other chemicals [43]. Photochemically-induced fibrin crosslinking also yields limited stiffness ranges [44]. Of note, most of the cross-linking strategies act by targeting primary amines and carboxylates of the matrix, which also contain adhesion receptors for cells. Of particular interest for culturing intestinal fibroblasts, decellularized small intestinal submucosal biomaterial is available [45].

Synthetic hydrogels have been developed (e.g. polyacrylamide hydrogels and polyethylene glycol), in which the mechanics can be tuned and tethered with ECM ligands and growth factors. Such matrices have been used to demonstrate the process of durotaxis, the migration of cells toward a gradient of stiffness [46], and mechanical responses of fibroblasts [47]. As for non-synthetic materials, polyethylene glycol hydrogels are also cytocompatible, and can therefore be used for growing cells in a three dimensional matrix.

An important limitation is that some substrates are not biodegradable by for example ECM degrading proteases produced by the cell. In addition, it is very difficult to univocally uncouple matrix stiffness from ligand density, surface chemistry or porosity of the matrix [48].

6.3.1.2 Extracellular Matrix Stiffness and Mechanotransduction

Although the exact identity of the proteins that actually sense mechanical forces is unknown [49], the downstream signaling events induced by substrate stiffness can affect the cells attached in different ways; it determines cell growth, resistance to apoptosis, migration and differentiation [34, 50] (Fig. 6.4).

Mechanotransduction induced by ECM stiffness occurs because the cytoskeleton is a dynamic network in which tension (so-called pre-stress) is built and adapted continuously trying to create a balance between the cytoskeletal stiffness

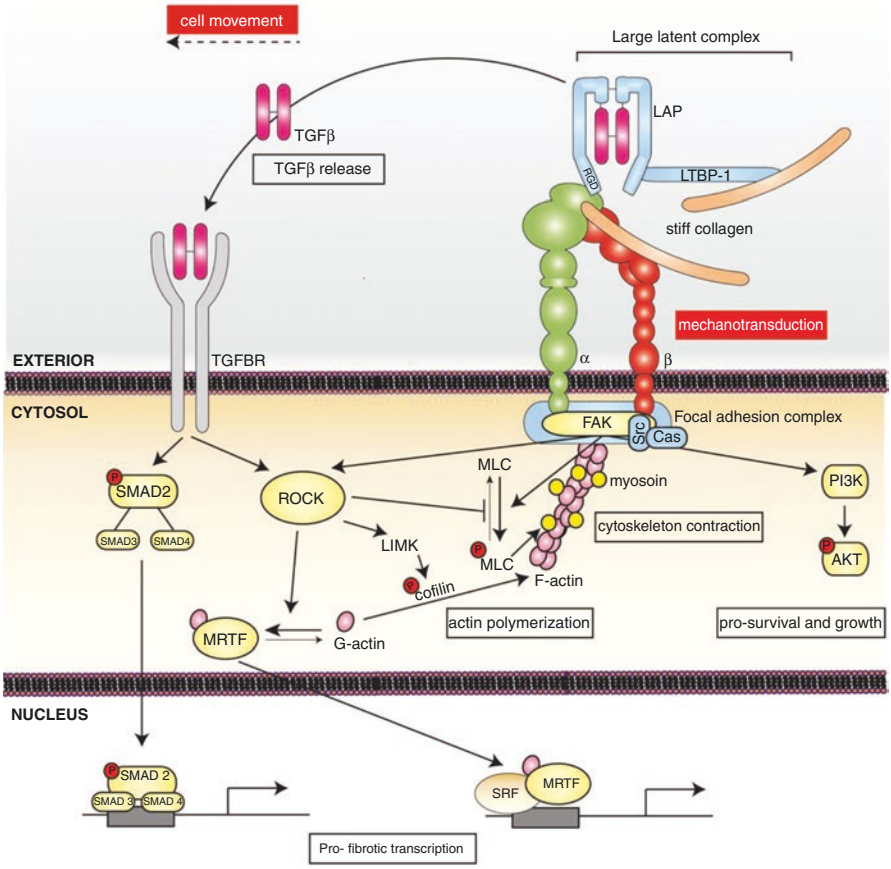

Fig. 6.4 Mechanotransduction and TGFβ release in response to matrix stiffness. *TGFβ* transforming growth factor beta, *TGFBR* TGFβ receptor, *LAP* latency-associated peptide, *LTBP-1* latent TGFβ binding protein 1, *FAK* focal adhesion kinase, *Cas* Crk-associated substrate family of scaffold proteins, *Src* Src kinase family, *ROCK* Rho kinase, *LIMK* LIM domain kinase, *MLC* myosin light chain, PI3K phosphatidylinositol 3-kinase, *AKT* AKT serine/threonine kinase 1, *SRF* serum response factor, *MRTF* myocardin-related transcription factor, *SRF* serum response factor, *SMAD* SMAD family member

and ECM stiffness [51]. Cells adhered to the ECM pull on the substrate via their cytoskeleton, and respond to any resistance through the cytoskeleton by increasing the number and size of focal adhesions, as well as the cytoskeletal pre-stress developed in the cell itself. This will lead to an increase in the number of F-actin/myosin stress fibers and downstream pro-fibrotic signaling, including the deposition of ECM, and the release of active TGFβ from the ECM by traction (see Sect. 6.3.2). This process thus creates a positive feedback loop, which is probably central for continued and sustained function of myofibroblasts [32]: ECM tension increases the production of TGFβ and ECM components, activation of αSMA expression and enhancement of pre-stress. It must be noted that mechanical stress alone is not sufficient to induce myofibroblast differentiation, since active TGFβ is required [33, 52]. Fascinatingly, studies of wound healing during development have shown that scar tissue does not develop in foetal wounds. One of the reasons could be the relative low expression of TGFβ in the foetus compared to adults [53]. Thus, autocrine production of TGFβ by myofibroblasts is of great importance for preserving the fibrogenic activity once the inflammatory stimulus has decreased [54].

On the biochemical level, the increase in the number of F-actin/myosin stress fibers and downstream pro-fibrotic signaling is translated from the ECM to the cell by the activation of myosin light chain (MLC) and cofilin phosphorylation. Two kinase systems that regulate these phosphorylation activities are controlled by focal adhesion kinase (FAK): the calcium-dependent MLC kinase (MLCK) and the Rho/Rho kinase (ROCK) system. ROCK is activated by the small Rho/GTP, which leads to the phosphorylation of LIM kinases (LIMK) and the subsequent inactivation of cofilin, an actin depolymerizing factor. In addition, ROCK increases the phosphorylation of MLC by inactivating MLC phosphatase. The net result of MLCK and ROCK activation is an enhanced F-actin fiber formation, and contraction of this network by myosin.

Increased polymerization of F-actin from the globular (G) actin monomers will mediate the major pro-fibrotic events upon FAK activation. These G-actin monomers bind and thereby sequester the myocardin-related transcription factors (MRTF) A and B to the cytosol. Once liberated from G-actin, MRTF will translocate to the nucleus, act as a cofactor for the serum response factor (SRF) and transactivate pro-fibrogenic gene expression, including *ACTA1* (coding for αSMA). In addition, FAK will determine fibroblast viability via the induction of the phosphatidylinositol 3-kinase/Akt survival signaling pathway [55]. Together with the anti-apoptotic activities of TGFβ that is released, myofibroblasts will proceed to thrive in the stiffened ECM [56].

Colonic fibroblasts grown on a matrix that has similar stiffness found in stenotic tissue leads to the development of a pro-fibrogenic phenotype characterized by increased proliferation, increased MLCK and ROCK activity, αSMA expression. Interestingly, mechanotransduction in intestinal fibroblasts also lead to the repression of interleukin 1 β, a potent pro-inflammatory cytokine [12, 57].

6.3.2 Mechanical Control of TGFβ Bioavailability

Transforming growth factor β1 is perhaps the most recognized and potent factor driving fibroblast-to-myofibroblast transition, inducing pro-fibrotic signaling via the canonical Smad2/3 pathway, and via the non-canonical pathway resulting in the activation of Rho-MRTFA signaling and F-actin/myosin contraction (Fig. 6.4).

Transforming growth factor β (TGFβ) is secreted in the extracellular environment in a biologically inactive (latent) form, forming a complex (the large latent complex) with TGFβ latency-associated propeptide (LAP) and latent TGFβ binding protein 1 (LTBP-1) [58]. Latency-associated propeptide contains an RGD-motif that binds integrins, mostly αv integrin [59]. Thus, the ECM-integrin complex provides a reservoir for TGFβ ready to be exposed by stretching the ECM [60, 61]. This can occur for example when a fibroblast is moving, or by its contraction when balancing pre-stress levels. However, liberation of TGFβ does not take place in a compliant, mechanically-resistant ECM. In case of compliant ECM, myofibroblast contraction will result in dragging the latent complex along with the cell. In contrast, when bound to stiff ECM, the integrin-mediated force can trigger a conformational change in the LAP, releasing TGFβ into the environment. The consequent binding of active TGFβ to the transmembrane TGFβ receptors type I and II leads to the assembly of a receptor complex inducing serine/threonine kinase activity. This leads to the activation of the transcription factors Smad, forming a complex with co-smads, that translocate to the nucleus and transactivate a number of pro-fibrotic genes including *ACTA1*, collagens and more than 60 other genes [62].

Also in the absence of myofibroblasts, stretch to the ECM will result in the liberation of TGFβ. This was illustrated in tendon, which is continuously exposed to variable mechanical tension [63]. The mechanical threshold for the initiation of this process is tissue dependent, and may thus be induced whenever the physiological stiffness is exceeded.

Finally, TGFβ is sequestered and bound to several proteoglycans such as decorin, thrombospondin and fibronectin. This either results in its neutralization or activation, and therefore the tissue concentration of these scavenging proteins will also determine the bioavailability of TGFβ [64].

6.3.3 Release of Danger Associated Molecular Patterns from the Extracellular Matrix

Mechanical tissue damage in the absence of inflammation, or an impaired restored ECM may contain DAMPs that originate from the ECM, which subsequently activates toll-like receptors (TLRs) on the cell surface of a variety of innate immune cells, including fibroblasts [65]. Toll-like receptors are central innate receptors, and

downstream signaling involves the activation of the transcription factor nuclear factor kappa-light-chain-enhancer of activated B cells triggering a pro-inflammatory response.

For example, in its physiological, unfragmented form, hyaluronic acid is biologically inert. Following modulation by hyaluronidases during tissue injury, the emerging small molecular weight fragments stimulate TLR2 and TLR4 [66]. Fibroblasts from patients with inflammatory bowel disease express a hyaluronic acid degrading enzyme that induces pro-fibrotic signaling [67], and the deposition of hyaluronic acid is an early event in mouse models of colitis [68]. Likewise, alternative forms of fibronectin can be generated by alternative splicing or by mechanical stretch, which may serve as ligands for TLR4 stimulating myofibroblast differentiation and increasing ECM stiffness [34, 69].

6.4 Extracellular Matrix Interactions: Potential Therapeutic Targets for Intestinal Fibrosis

For as yet unknown reasons, the standard therapies efficiently controlling inflammation in patients with Crohn's disease do not alter the natural progression of the disease toward stenosis [70, 71]. Supporting the hypothesis that intestinal fibrosis becomes auto-propagative, it was shown that repressing inflammation in an infection-induced model of enteritis did not prevent the progression to fibrosis [72]. Such interpretation greatly influences the way we will adapt therapeutic management of the disease, and supports the use of add-on therapy to inhibit the progression of fibrosis [57].

Myofibroblasts are the major contributors to intestinal fibrosis, mediating tissue repair by producing ECM components, and contracting the ECM to facilitate wound closure. Interestingly, both processes in these cells are highly prone to mechanical stimulation, and therefore the pathways involved in mechanotransduction open opportunities for therapeutic intervention (Table 6.2).

Injecting synthetic biomaterials or decellularized ECM scaffolds in wounded tissues may show potential to reduce ECM stiffness and liberate the auto-propagative function of myofibroblasts. This strategy has been evaluated in porcine models of myocardial infarction and were shown to improve cardiac function and stimulate regeneration of the tissue [73–76]. Inhibiting the cross-linking enzymatic activity also seems an interesting strategy to decrease ECM stiffness. Inhibiting lysine oxidases using the antibody simtuzumab, or chemical inhibition of transglutaminases showed promising results in animal models of fibrosis [77, 78].

Considering to simply diminish the amount of ECM that was built up in the stenotic intestine, protease-based degradation of ECM may represent an interesting strategy to reduce the stiffness of the ECM. Most compelling evidence of reducing fibrosis using this strategy comes from the injection of collagenase in Dupuytren's contractures [79]. In addition, increasing the cellular re-uptake of collagen by endocytosis-mediated degradation of ECM may yield interesting therapeutic strategies [80].

Table 6.2 Potential therapeutic targets based on interfering with mechanotransduction of myofibroblasts

Process interference	Target	Mechanism	Tools	Reference
Matrix stiffness	ECM	Providing compliance to the stenotic ECM	Synthetic hydrogels or porcine ECM	Seif-Naraghi et al. [73], Wassenaar et al. [75], Yoshizumi et al. [76]
	ECM	Degradation of ECM	Collagenase	Holzer and Holzer [79]
Cross-linking of ECM	LOX	Inhibiting ECM cross-linking	Simtuzumab	Barry-Hamilton et al. [77]
	TG2	Inhibiting ECM cross-linking	NTU283, NTU281	Johnson et al. [78]
Growth factor inhibition	ECM	Antagonizing growth factors in ECM	Recombinant decorin	Hill et al. [81]
Mechanotransduction	αv integrin	Inhibiting TGFβ release and αv integrins in focal adhesions	CWHM 12, [Cilengitide]	Henderson et al. [59]
	α4	Inhibiting α4 integrin in focal adhesions	Natalizumab	Targan et al. [86]
	RhoA/ MRTF	Inhibiting MRTF signaling downstream of FAK activity	CCG-100602, CCG-203971	Johnson et al. [87]
	ROCK	Inhibiting ROCK downstream of FAK activity	AMA0825	Holvoet et al. [57]
	FAK	Inhibiting focal adhesion signaling	PF-562271	Fan et al. [88]
Survival of myofibroblasts	FAK	Stimulating myofibroblast apoptosis	–	Xia et al. [55]

TGFβ transforming growth factor beta, *LOX* lysine oxidase, *TG2* transglutaminase 2, *ROCK* Rho kinase, *FAK* focal adhesion kinase.

Targeting the bioavailability of TGFβ in stenotic tissue may hold a strong scientific rationale, since it plays a central role in preserving a differentiated myofibroblast phenotype [54]. Strategies to increase growth factor affinity to the ECM, for example by delivering recombinant decorin molecules, may reduce the bioavailability of a variety of pro-fibrogenic mediators [81]. However, it needs to be considered that TGFβ is a major immunoregulator, and any interference in the activity of TGFβ may hold potential risks in patients with chronic inflammatory disorders, illustrated by the observation that TGFβ1-deficient mice develop severe multi-organ inflammation [82]. In fact, inducing TGFβ signaling by inhibition of the negative TGFβ-regulator Smad7 holds major potential to dampen inflammation in Crohn's disease [83].

Of particular interest is the inhibition of mechanotransduction of myofibroblasts in the stenotic bowel. Blocking αv integrin using the small molecule CWHM 12 has proven efficiency in attenuating liver and lung fibrosis [59]. Likewise, αvβ5 and αvβ3 on myofibroblasts could be targeted using Cilengitide, an small molecule integrin inhibitor that was developed to reduce angiogenesis, however its use in two models of liver fibrosis actually worsened the fibrogenic response [84]. The humanized αvβ6 integrin-blocking antibody STX-100, which inhibits the activation of latent TGFβ via epithelial cells, is currently being examined in a phase II trial in patients with idiopathic pulmonary fibrosis [85]. Interestingly, an antibody directed against α4 integrin (natalizumab) has been developed to induce disease remission in patients with Crohn's disease [86]. Although the primary rationale is to inhibit the intestinal homing of T cells, it may show potential to inhibit focal adhesion activation in myofibroblasts in patients who develop stenoses.

More downstream in the mechanotransduction process, inhibition of the RhoA/MRTF pathway using CCG-100602 and CCG-203971 was successful in reducing experimental intestinal fibrosis and downregulating matrix stiffness [87]. In line with these results, we have successfully used a small molecule inhibitor of ROCK, showing also therapeutic potential in murine models of intestinal fibrosis [57].

Inhibition of FAK induces apoptosis of myofibroblasts in vitro [55], and some evidence points to an anti-fibrotic effect of pharmacological inhibition of FAK in a model of post-myocardial infarction [88].

Taken together, an emerging number of studies support the notion that intestinal fibrosis is able to propagate in the absence of inflammation. Although this process is not fully understood, stiffness of the ECM is probably a prime contributor to autopropagation of intestinal fibrosis, and therapeutically targeting the matrix stiffness, or inhibiting downstream integrin- or TGFβ-signaling events represent interesting strategies that warrant further investigation. Together, this may raise some optimism as to inhibit ongoing fibrosis accumulating in patients with IBD.

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Chapter 7
Fat and Fibrosis

Ren Mao and J. Calvin Coffey

Abstract Wrapping of the mesenteric fat (creeping fat) represents a characteristic feature of Crohn's disease (CD). As a powerful producer of fatty acids, cytokines and adipokines, creeping fat plays an important role in regulation of immunity and inflammation. Increasing evidence points towards a link between creeping fat and intestinal inflammation in CD. Early data from macroscopic findings showed a significant relationship between creeping fat and connective tissue changes including fibrosis and muscular hypertrophy. Emerging mechanistic data indicate a link between creeping fat and intestinal fibrosis in CD. Data on fibrosis in other organs could provide clues to address the mechanistic role of distinct components of creeping fat in the pathogenesis of intestinal fibrosis. Future studies will provide essential new information and could lead to novel therapeutic agents for the prevention or treatment of IBD-associated fibrosis.

Keywords Creeping fat · Crohn's disease · Stricture · Fibrosis · Adipose tissue

R. Mao (✉)
Department of Gastroenterology and Hepatology, The First Affiliated Hospital of Sun Yat-sen University, Guangzhou, Guangdong, China

Department of Gastroenterology, Hepatology and Nutrition, Digestive Diseases and Surgery Institute, The Cleveland Clinic Foundation, Cleveland, OH, USA

Department of Pathobiology, Lerner Research Institute, The Cleveland Clinic Foundation, Cleveland, OH, USA

J. Calvin Coffey
Department of Surgery, Graduate Entry Medical School, University Hospital Limerick, 4i Centre, University of Limerick, Limerick, Ireland
e-mail: Calvin.Coffey@ul.ie

F. Rieder (ed.), *Fibrostenotic Inflammatory Bowel Disease*,
https://doi.org/10.1007/978-3-319-90578-5_7

7.1 Introduction

Crohn's disease (CD) and mucosal ulcerative colitis (MUC) are conventionally described as mucosal inflammatory conditions [1]. In general, in IBD, surgery is reserved for failure of medical therapy to control symptoms, or the development of complications [1]. The rate of surgery could thus be an excellent reflection of the success or failure of non-surgical treatment. Since its original description in 1932, the medical and surgical management of CD have changed considerably, but overall resection rates remain largely unchanged [2–6]. Eighty percent of patients with ileocolic CD will require resection on at least one occasion. Of these, at least 20% will require repeat resection, usually within the first 5 years of the index resection [1, 3, 5]. In contrast, surgery (i.e. total colectomy) rates have decreased for mucosal ulcerative colitis indicating that current pharmaco-therapeutic treatments are more successful (at least in MUC) in the control of symptoms and the prevention of complications [6–9]. The failure of pharmaco-therapeutic treatment regimens to reduce resection rates in CD suggests we need to refresh our approach to investigating this disease [1, 3].

Unlike in CD, mucosal ulcerative colitis appears to commence at the mucosal level and progresses to involve deeper intestinal levels [6–8]. It appears that adipose-related events (i.e. within the mesentery and submucosa) are relatively less important at an etiological and pathobiological level. Notwithstanding that, data are emerging demonstrating a relationship between cellular and molecular events within mesenteric lymph nodes, and inflammation in the adjacent intestinal mucosa [10–13]. It appears that mesenteric based events are also important in mucosal ulcerative colitis, albeit from an indirect and immunological perspective [10–13].

Recent clarification of mesenteric anatomy in general has highlighted a relationship that has long been suspected, but little understood, between the mesentery and intestine in CD [1, 11, 14, 15]. The intestinal submucosa is a further (and often overlooked) site of exposure to adipose activities. It is in this context investigatory focus in the mesentery and submucosa has increased in CD, but less so in MUC [1, 9, 11, 14–20].

The following chapter aims to explore the relationship between adipose events and fibrosis in IBD. Given the importance of acute and chronic inflammation in leading to fibrosis, the chapter will explore the relation between adipose and inflammatory events, and how these contribute to intestinal fibrosis. The chapter commences with an overview of the role of the mesentery in Crohn's disease in particular. Cellular and molecular mechanisms by which fat-based activities may contribute to fibrosis are then discussed. The chapter finishes on a number of proposals for future directions.

7.2 Creeping Fat/Mesenteric Fat in IBD

Increasing evidence points to a link between alterations in mesenteric fat, and intestinal fibrosis in Crohn's disease [1, 19–21]. Before delving into this, a short review of the anatomical relationship between the mesentery and intestine is important.

During embryological development intestinal endoderm develops within mesoderm at the intestinal margin of the mesentery (Fig. 7.1) [22]. Whilst epithelial layers are derived from the endodermal germ layer, the remaining mesenchymal layers of the intestine derive from mesenteric mesenchyme. This histological relationship is retained into adulthood when mesenteric and intestinal connective tissue are continuous at the region where mesentery intersects the intestine [10, 23].

The important anatomical relationship between the mesentery and intestine was recently updated. As the mesenteric organ is continuous, so too is the zone of intersection between it and the intestine (the intestinal hilum) [10, 15, 23]. This is the zone across which all signals from the intestine must pass to reach the body, and vice versa. Continuity between the small intestinal mesentery and right mesocolon mean a large mesenteric tissue mass occurs at the ileocaecal region [14, 24, 25]. The mesentery houses lymphatic channels and nodes, which are distributed in tandem with the arterial supply to the intestine, and are numerous at the ileocaecal region [24]. Adipose tissue accumulates first around vessels, leading to the development of vascular pedicles [14, 26]. This combination of mesenteric properties (lymphatic and adipose) correlate to a large degree with the macroscopic distribution of Crohn's disease in general (i.e. ileocaecal CD is commonest subtype followed by segmental Crohn's colitis) [1].

Creeping fat is pathognomonic of Crohn's disease [1, 4]. It is an extension of mesenteric fat, that begins at the intestinal hilum and progressively extends over the intestinal surface. It appears to be more prominent in the small bowel compared with the colon, in patients with Crohn's disease. This may relate to the fact that appendices epiploicae (fat globules) normally accumulate on the colonic surface to begin with.

One hypothesis is that creeping fat exerts a break-like effect on inflammation in CD [27]. If that were the case, then mucosal and mural mesenchymal disease manifestations would be expected to decrease, wherever mesenteric disease increased. The reverse is the case and mesenteric, mucosal and mural disease manifestations all increase in tandem in Crohn's disease. This and other observations have meant the concept of protecting against inflammation, or exerting a break-like effect on inflammation, has been largely superseded by the hypothesis that the mesentery exerts a net pathobiological effect in Crohn's disease [1, 19]. Examination of resection specimens demonstrates a mesenteric transition zone where creeping fat commences, and gradually worsens (Fig. 7.2). Examination of the opened specimen indicates corresponding mucosal and mural transition zones. Coupling of mucosal, mural and mesenteric disease is further reflected in the fact that mucosal ulceration almost always commence at the mesenteric (and not the anti-mesenteric) axis of the intestinal circumference (called axial polarity). This is also the zone where the mesentery intersects the intestinal circumference.

Further studies support the concept that mesenteric fat exerts a net pathobiological role in CD. Li et al. demonstrated that increases in mesenteric fat are independently predictive of recurrence requiring surgery (i.e. surgical recurrence) [17, 28]. We recently showed that fat wrapping greater than 50% of the intestinal surface, independently predicted surgical recurrence in CD patients of different phenotypes (manuscript in press).

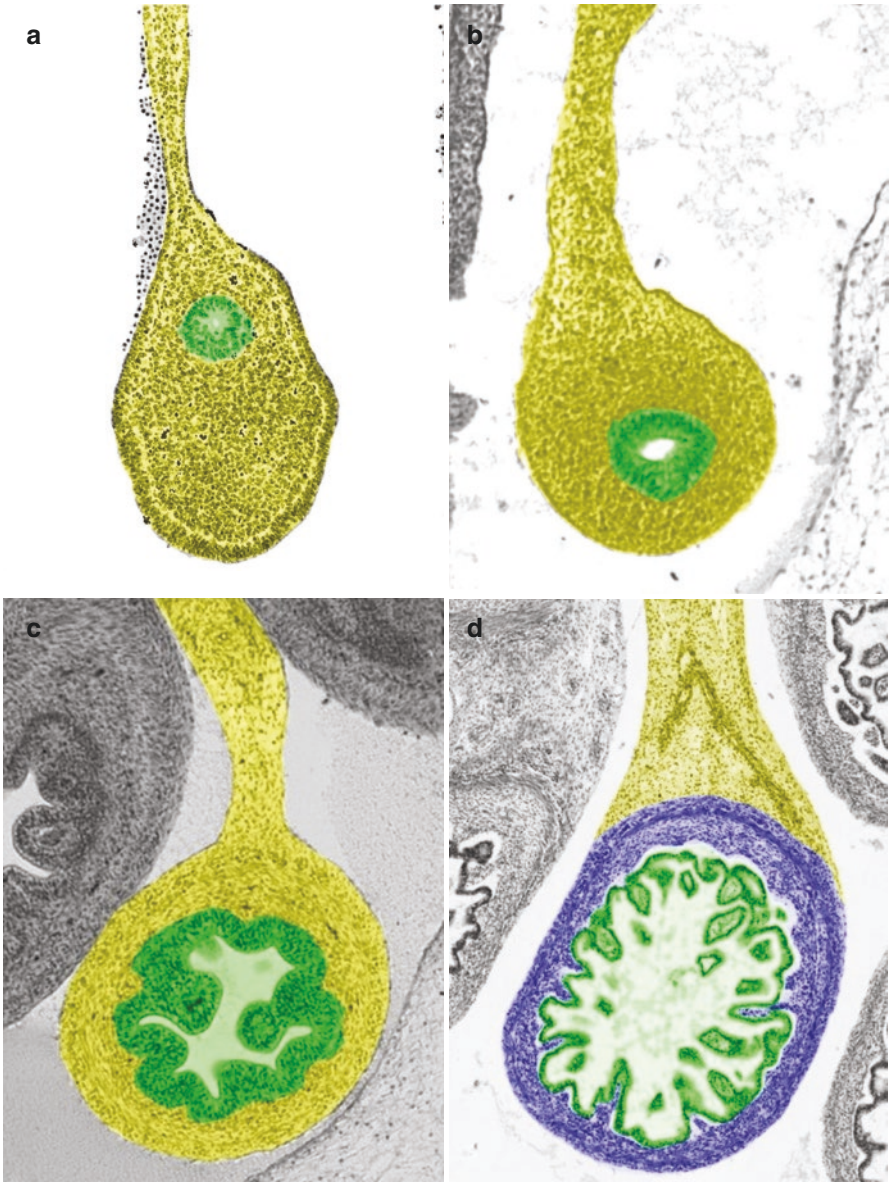

Fig. 7.1 Relationship between mesenteric mesoderm and endoderm at (**a**) 33 days (**b**) 44 days (**c**) 59 days (**d**) 84 days of embryonic development. Colour code, yellow represents mesenteric mesoderm, green represents endoderm and blue represents mesodermal component of intestinal wall. Courtesy of Dr. Kevin Byrnes, images taken from Carnegie Collection, Washington & Maastricht University (http://virtualhumanembryo.lsuhsc.edu)

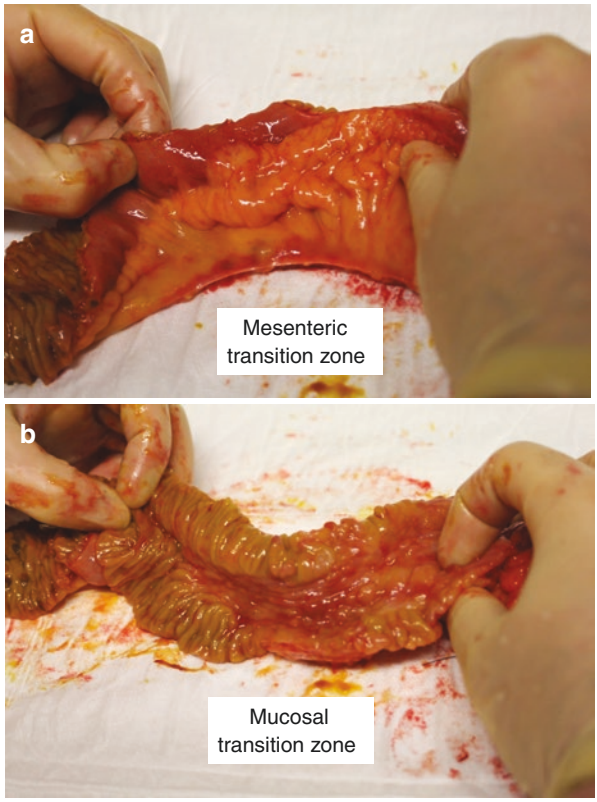

Fig. 7.2 The (**a**) mesenteric and (**b**) mucosal transition zones. (**a**) at the mesenteric transition zone mesentery changes gradually from non-diseased to severely diseased with advancement over the intestinal surface (creeping fat), thickening of the mesentery and loss of the normal angulation at the intestinal. (**b**) After opening the same specimen, the mucosa changes in tandem from non-diseased to severely diseased

The above macroscopic correlations (i.e. that mucosal, mural and mesenteric disease manifestations increase in tandem) are underpinned by overlapping intestinal and mesenteric histopathological abnormalities in Crohn's disease. Firstly, a histopathological overlap occurs between the mesentery and submucosa in CD (Fig. 7.3). Both intestinal submucosa and nearby mesentery are hallmarked by profound mesenchymal abnormalities including adipocyte hyperplasia and collagen deposition. This points to an overlap in cellular and molecular events that could in turn explain the characteristic transmural appearance of CD [1]. According to this hypothetical model submucosal inflammatory changes progress inwards towards the mucosa, and outwards to the intestinal serosal surface. Mesenteric inflammatory changes progress inwards from the serosal surface. When both inflammatory fronts meet, inflammation is transmural.

If the mesentery is of pathobiological relevance in CD then its resection should lead to improved outcomes for patients undergoing resectional surgery. The surgical convention is to divide the mesentery flush with the intestine, and thus retain the mesentery, during surgery from CD. We and others have data demonstrating that if the mesentery is included as part of a surgical resection then endoscopic and surgical recurrence rates appear to be reduced (manuscript in press, and Li Yi, personal

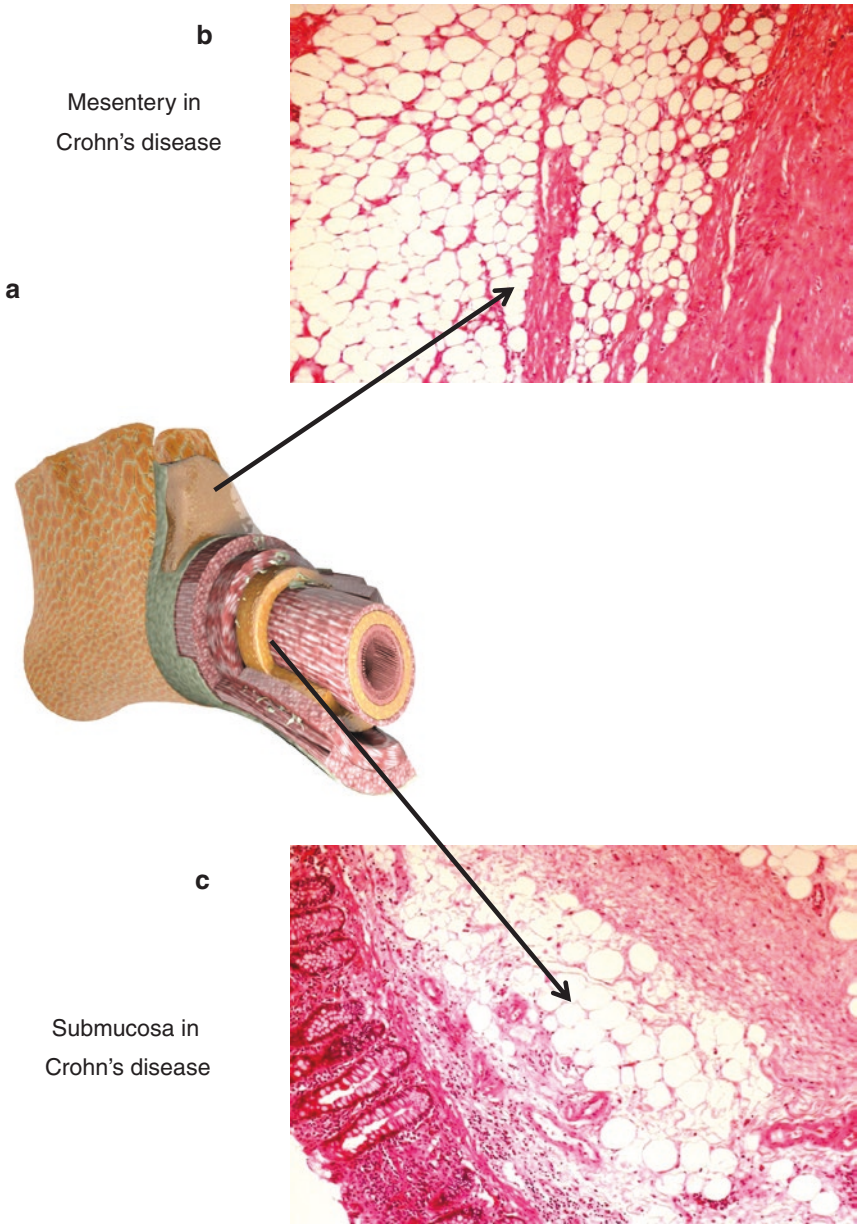

Fig. 7.3 (**a**) Image from 3D digital model demonstrating relationship between mesentery and contiguous intestine. (**b**) Photomicrograph (stained with haematoxyin and eosin) showing adipocyte hyperplasia and connective tissue thickening in the mesentery in Crohn's disease. (**c**) Photomicrograph (stained with haematoxyin and eosin) showing adipocyte hyperplasia and connective tissue thickening in the submucoa in Crohn's disease

communication). These preliminary findings support the development of a multicentre randomized international trial in CD, comparing outcomes between groups that undergo conventional resection versus one where the mesentery is included [17].

The mesentery is not usually inflamed in true mucosal UC, where disease is mostly mucosal. This is not to say that the mesentery does not have a pathobiological role in this condition. Increasing evidence points to key immunological activities within mesenteric lymph nodes and increasingly data is emerging pointing to a relationship between immunological events in mesenteric nodes (cellular and molecular events), and mucosal events in nearby contiguous intestinal mucosa. Notwithstanding this, the mesentery in uncomplicated MUC (when a perforation has not occurred) appears normal at macroscopic and histological levels. This helps in its differentiation from CD.

As relatively little is known regarding the mesentery in CD, there is a paucity of data on mesenteric cellular events. It is known that mesenteric adipocytes produce substantial amounts of TNF-α and CRP [21, 27]. Takahashi et al. recently showed that interactions between intestinal epithelia and adipocytes can induce inflammatory-type responses in an autonomous fashion, i.e. they do not require immunologic inputs to do so [29]. It is suggested that fibrocytes may also play a role. These have the capacity to differentiate into either adipocyte or fibroblast [30]. Until recently the origin of cells responsible for mesenchymal changes found in CD was unknown. Emerging data suggest that fibrocytes exploit the mesenteric platform to gain access to the intestinal surface [11, 30]. At the serosal side of the intestinal wall they use the connective tissue continuity between mesentery and intestine to access deeper intestinal layers. Fibrocyte tracking along intestine-mesenteric mesenchyme could explain many aspects of Crohn's disease (including transmural and trans-mesenteric disease manifestations) [1, 11].

7.3 Creeping Fat and Strictures

Although emerging data support a role for creeping fat in the pathogenesis of intestinal inflammation, there are very limited data linking creeping fat to intestinal fibrosis. Early data from macroscopic findings indicates that the presence of creeping fat is associated with muscularis propria hyperplasia and clinically apparent stricturing disease [31]. In a study investigating 20 patients undergoing ileal resection for CD and 20 normal controls, serosal fat wrapping was present in all cases. The extent of fat wrapping correlated significantly with the degree of chronic inflammation [31]. In a consecutive and unselected group of 27 intestinal resections performed on 25 patients with Crohn's disease confirmed by histology, fat-wrapping was identified in 12 of 16 ileal resections and in seven of 11 large bowel resections. It correlated with transmural inflammation, and there was a significant relationship between fat-wrapping and other connective tissue changes, including fibrosis,

muscular hypertrophy and stricture formation [32]. These findings suggest that serosal connective tissue changes including fat wrapping in CD are related to local effects of underlying chronic inflammatory infiltrates including fibrosis.

7.4 Potential Mechanisms of Fat Influencing Fibrosis/ Smooth Muscle Cell Hyperplasia in Organs other than the Gut

Although data from macroscopic findings showed the presence of creeping fat is associated with muscularis propria hyperplasia and clinically apparent stricturing disease [31], there is essentially no data explaining this phenomenon. However, data from fibrosis in other organs could provide clues to address the fundamental knowledge gap about the mechanistic role of distinct components of creeping fat in intestinal fibrosis.

7.4.1 Cardiac Fat and Fibrosis

The adipocyte-derived hormone leptin may induce cardiac fibrosis by promoting endothelial dysfunction, m-TOR pathway activation and oxidative stress [33, 34]. Recently, data are accumulating pointing to an association between pericardial fat and occurrence of atrial fibrillation (AF). In atrial fibrillation atrial myocardial fibrosis appears to be prominent and may lead to functional abnormalities [35]. In the Framingham Heart cohort study, pericardial fat volume was an independent predictor of AF development even after adjusting other AF risk factors [36].

As occurs in the case of creeping fat in Crohn's disease, epicardial fat (EAT) occurs between visceral pericardium and epicardium in direct contact with adjacent myocardium. As a result of direct contact, there is no anatomical or histological barrier that may prevent crosstalk between epicardial fat and adjacent muscle [37]. The secretome of human EAT obtained from patients undergoing coronary bypass surgery, but not from subcutaneous fat (SAT), rapidly leads to increased fibrosis in atrial organo-culture [37]. Among the adipo-fibrokines secreted by EAT, activin A (a member of the TGF-β superfamily) may be an important mediator of this pro-fibrotic effect. Indeed, supplementation of culture media with recombinant human activin A enhanced fibrotic events whilst anti-activin A antibody neutralized these. Histological analyses of human myocardium has identified fibrosis at the interface between adipose and myocardial tissues. Interstitial fibrosis of the neighboring myocardium was often marked in these studies [37].

Pangenomic transcriptomic studies identify a specific transcriptomic signature in human EAT compared with SAT. EAT includes peri-atrial, peri-ventricular and pericoronary fat. Over 400 genes are commonly expressed across these fat depots.

Amongst these are genes involved in extracellular matrix remodeling (associated with collagen IV, VI, thrombospondin 3, laminin alpha 2, fibronectin 1 genes), inflammation and thrombosis [38].

7.4.2 Infrapatellar Fat Pad and Osteoarthritis (OA)

The effects of local adipose tissue on nearby joints has also come under the spotlight in the recent past. Eymard et al. [39] showed that intra-articular adipose tissues (IAATs) have a distinct histological phenotype compared with subcutaneous adipose tissue. Fibrosis and vascularity are increased in all IAAs compared with SAT. IAATs adipocytes are smaller in size compared with subcutaneous counterparts. Differential expression of genes involved in developmental signalling, lipid handling and general metabolism is apparent when IAAT and SAT are compared. IAATs secret more IL-6, IL-8 and prostaglandin E2 compared with SAT.

The infrapatellar fat pad (IFP), located in the knee joint, is an intra-capsular and extrasynovial structure. Barboza et al. [40] noted prominent fibrosis in this fat depot. Mice fed a high-fat diet for 20 weeks develop osteophytes and early structural changes in cartilage. Obesity-associated changes in IFP tissue are associated with increased expression of genes involved in fibrosis and extracelluar matrix production.

7.4.3 Perivascular Fat and Atherosclerosis

Perivascular fat (PVAT) is directly contiguous with vascular adventitia. It has been suggested PVAT may exert a pathobiological effect in atherosclerosis development and that this is facilitated by contiguity between PVAT and blood vessel [41]. PVAT plays an important role in local vascular homeostasis. Factors released from PVAT may contribute to inflammation, smooth muscle proliferation, and promote atherosclerotic or neointimal lesion growth [42]. Findings in rodent models show that aging and diet-induced obesity enhance the ability of PVAT to stimulate proliferation of human smooth muscle cells [43].

7.4.4 Creeping Fat and Intestinal Fibrosis in CD

Within creeping fat, activated adipose tissue lies in close proximity to both adipose derived stromal tissue and immune effector cells. This histological relationship (in combination with the fact that activated adipose tissue secretes a spectrum of fatty acids, adipokines and cytokines) plays a role in shaping local immune responses in nearby intestinal mucosa. The adipocyte-rich microenvironment within the

creeping fat directs the local macrophage compartment to the M2 subtype. These cells in turn have an important pro-fibrotic role mediated in part through their production of pro-fibrotic factors such as TGF-β [44, 45]. Rieder et al. [46] recently reported that creeping fat derived mediators such as free fatty acids (FFA), but not adipokines, induce a differential and selective fibrogenic response by human intestinal fibroblasts (HIF) and human intestinal muscle cells (HIMC) as demonstrated by increases in matrix secretion, IL-6 production, α-SMA expression and cell migration. Interestingly, bFGF and FFA synergistically increased HIF and HIMC proliferation in a p38MAPK-dependent but NFkB- and MyD88-independent manner. Moreover, results show that ECM released by activated HIMC selectively and specifically promotes migration of adipocytes (Ad) and pre-adipocytes (Pre-Ad). This could provide a molecular basis for the advancement of mesenteric fat along the surface of the intestine (i.e. creeping fat). Adipocytes and their precursors expressed integrins α1, 2, 4 and αV as well as integrins β1 and αVβ5. Blockade of integrin β1inhibited the increased adipocyte and pre-adipocyte migration induced by ECM. Combined, these findings point to a feedback loop between mesenteric fat and intestinal muscle that could explain the association between creeping fat and stricture formation in CD. Challenges in this field include the the lack of high fidelity animal models.

7.5 Future Outlook

Fat to mesenchymal interactions appear to be a critical driver of tissue remodeling in multiple organs, including the intestine. Future studies should focus on specific features of CD that are strongly associated with changes in the mesentery, i.e. creeping fat, axial polarity, transmural inflammation and mesenteric shortening [30]. Special emphasis should be put on the zone of intersection between the mesentery and the intestine. Although this region is a least two meters in length, it is remarkably understudied in general [47, 48]. Additionally, the area of transition between creeping fat and the serosal surface not affected by creeping fat should be examined as it may provide direct clues about the effect of fat on intestinal smooth muscle. Novel animal models are necessary to elucidate mechanisms of creeping fat formation or fat to mesenchymal interactions in vivo. This in combination with human ex vivo culture systems could yield novel medical therapies in this field.

Future diagnostic studies could exploit the relationship between the mesentery and intestine by determining the appearance of the mesentery on cross sectional imaging [48]. As mesenteric abnormalities are largely pathognomonic of Crohn's disease, then it is likely that imaging approaches evaluating the mesentery will provide important diagnostic information. Endoluminal approaches (combining radiological and endoscopic technologies) are likely to generate the highest yields [47, 48].

At present, surgery continues to be reserved for patients in whom medical treatment fails to control symptoms, or who develop complications of IBD. The promising results obtained in studies in which the mesentery was also included during resection prompt the question as to whether surgery (and mesenteric resection) should be introduced earlier in the management of IBD. This was not the case in the past as complication rates following surgery were too high. However, recent clarification of mesenteric anatomy has enabled a comprehensive standardization of colorectal surgery, which in turn could facilitate the earlier introduction of mesenteric-based resectional surgery [26, 49, 50]. Mesenteric resection should also be further evaluated through randomized, double blinded and multi-institutional studies.

In tandem with the above studies, investigation should be led into developing new pharmacotherapeutic (i.e. non-operative) approaches to IBD, using fat targeted therapy. These would be greatly facilitated by the development of animal models that mimic the relationship between the mesentery and intestine in IBD, with a high level of translatability. Any such model should reproduce the connective tissue continuity that occurs between the intestine and mesentery and the cellular (i.e. fibrocyte-based) and molecular events that lead to mesenchymal abnormalities.

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Chapter 8
Environmental Factors and Their Influence on Intestinal Fibrosis

Claudio Bernardazzi, Fernando Castro, and Heitor S. de Souza

Abstract Multiple endogenous and exogenous factors have been implicated in the development of inflammatory bowel disease (IBD), comprising Crohn's disease and ulcerative colitis; these factors may initiate and maintain the chronic inflammatory process, potentially resulting in intestinal fibrosis. Several distinct mechanisms are involved in the tissue response leading to excessive extracellular matrix deposition in IBD. This process involves the complex and dynamic interactions of a network of several genes and molecules, forming a microenvironment that favors the development of fibrosis. In addition to inflammation, alternate contributors have been implicated in intestinal fibrogenesis, including microbiota and the action of microbe-associated molecular patterns and other pattern recognition receptors, as well as damage-associated molecular patterns (DAMPs), dietary factors, and natural and synthetic compounds. These elements have been shown to act directly or through epigenetic changes, usually interfering with the immune response and mechanisms of tissue repair, which may ultimately cause fibrosis. Further investigation of specific environmental triggers and the epigenetic molecular network underlying the pathogenesis of IBD may help in the prevention of and in the development of a more effective treatment for intestinal fibrosis.

Keywords Inflammatory bowel disease · Environmental factors · Intestinal fibrosis · DAMPs · Epigenetics · Intestinal microbiota

C. Bernardazzi · F. Castro
Serviço de Gastroenterologia e Laboratório Multidisciplinar de Pesquisa, Departamento de Clínica Médica, Hospital Universitário Clementino Fraga Filho, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brazil

H. S. de Souza (✉)
Serviço de Gastroenterologia e Laboratório Multidisciplinar de Pesquisa, Departamento de Clínica Médica, Hospital Universitário Clementino Fraga Filho, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brazil

D'Or Institute for Research and Education, Rio de Janeiro, RJ, Brazil

F. Rieder (ed.), *Fibrostenotic Inflammatory Bowel Disease*,
https://doi.org/10.1007/978-3-319-90578-5_8

8.1 Introduction

Inflammatory bowel disease (IBD), comprising Crohn's disease (CD) and ulcerative colitis (UC), represents a group of chronic and potentially progressive and destructive diseases primarily affecting the gastrointestinal tract. Currently, these complex diseases are believed to originate from an inappropriate response of the mucosal immune system to the commensal intestinal microbiota in a genetically susceptible individual [1]. Notwithstanding positive developments in modern therapy for IBD, there is still an unmet need for better medical treatments, particularly regarding chronic complications such as fibrosis.

Several factors have been implicated in the development of IBD, such as genetic background, smoking, indiscriminate use of medications, dietary habits, and bacterial infection; these factors may trigger and sustain chronic inflammation and ultimately result in the development of intestinal fibrosis [2]. Considered as a consequence of the excessive production of extracellular matrix (ECM) by activated mesenchymal cells, intestinal fibrosis often results in intestinal obstruction in the context of IBD, particularly among patients with CD [3].

8.2 Environmental Factors Contributing to the Development of Intestinal Fibrosis

Intestinal damage and repair involve multiple mechanisms that converge in the recruitment of mesenchymal cells and the generation of activated myofibroblasts, resulting in excessive ECM deposition. Excessive ECM deposition is caused by an imbalance between MMPs and tissue inhibitors of metalloproteinase, resulting in fibrosis [4]. These abnormalities are, in great part, thought to result from the action of several mediators that control ECM deposition [5]. Although the inflammatory process is a major activator of mesenchymal cells, these cells can also be activated by other pathways, such as microbe-associated molecular patterns and other pattern recognition receptors, autocrine and paracrine signals, and damage-associated molecular patterns (DAMPs) [6, 7]. Despite the relative improvement in the control of intestinal inflammation achieved in recent years, especially after the advent of biologic therapy, fibrosis continues to represent an important medical problem, frequently requiring surgery.

8.2.1 Smoking

Although the exact mechanism by which tobacco is involved in intestinal inflammation is not clear, smoking is a well-known influential factor in IBD, usually worsening CD, but offering relative protection from UC [8]. Cigarettes are

composed of several chemical elements, some of which have been shown to affect different targets by acting on DNA through epigenetic influences in the intestinal cells and even by modulating the microbiota in the intestinal lumen [9, 10]. From a clinical perspective, a recent study addressing the impact of smoking in IBD identified an increased risk of surgery among current smokers with CD, while former smokers with UC have an increased risk of colectomy [11]. On the other hand, another recent study involving the Sydney IBD Cohort database confirmed the detrimental effects of smoking in CD but failed to demonstrate substantial benefits from smoking in UC [12]. Interestingly, in a recent study, investigators analyzed the association between cigarette smoking and pancreatic fibrosis and concluded that aryl hydrocarbon receptor (AhR) ligands found in cigarette smoke increase the severity of pancreatic fibrosis in an experimental model of chronic pancreatitis in mice through the up-regulation of IL-22. In addition, in humans, higher levels of IL-22 have been identified in serum samples from smokers compared to nonsmokers [13]. AhR activation has been shown to deliver anti-inflammatory signals dependent on the induction of regulatory T cells [14] and to promote IL-22 production by Th17, Th22 and gamma-delta T cells [15]. In the context of CD, the low expression of AhR found in lamina propria mononuclear cells [16] has recently been associated with elevated levels of Smad7, an inhibitor of transforming growth factor beta 1 (TGF-beta 1) activity, which in turn regulates AhR expression [17]. Although the exact mechanism by which smoking affects mucosal immunity is yet to be determined, abnormal signaling mediated by AhR may represent a potential link between the environment and the consequences of inflammation, including fibrogenesis.

8.2.2 The Influence of Diet

In recent decades, the way food is prepared and offered has progressively changed, and the consumption of processed foods, high in fat and sugar and with large amounts of salt, has increased. In addition to the increased risk of cardiovascular diseases, diabetes and obesity, the widespread consumption of Western-like diets deficient in fiber and micronutrients but frequently containing artificial additives has been shown to affect the intestinal microbiota [18] and has been associated with the development of intestinal diseases, including IBD and colorectal cancer [19–21].

A variety of luminal elements, such as dietary components, interact with epithelial cells to cause pro- or anti-inflammatory responses, which are usually mediated by cell surface receptors. The interplay between cigarette smoking and immune cells, previously discussed in this chapter, has been attributed to abnormal AhR activation, for example. After the discovery that several natural and synthetic ligands can activate AhR, including environmental, dietary, and endogenous aromatic compounds [22], this transcription factor has become a target of major interest in research because it constitutes a potential connection between the environment and

immune-mediated diseases [23]. In fact, AhR plays an important role in the differentiation, activation, and proliferation of many immune and non-immune cells and is known to convey protective signals in the intestine; moreover, it has low expression levels in lamina propria mononuclear cells in CD [16].

Vitamin D has been demonstrated to induce tolerogenic dendritic cells and has been regarded as an important regulator of mucosal immunity [24]. Exposure to sunlight with natural ultraviolet (UV) radiation is essential for the availability and functionality of this vitamin. In IBD patients, it has been proposed that low sunlight exposure constitutes a risk factor, particularly for CD [25, 26]. This observation is in agreement with the notion that the incidence of IBD is higher in the northern hemisphere, where UV exposure is significantly lower [27]. Although sunlight may also result in high temperatures, which are shown to be associated with an increased risk of intestinal obstruction in cystic fibrosis [28], neither ambient temperature nor humidity has been associated with the development of IBD. On the other hand, other environmental changes such as ambient air pollution, a common product of industrialization, have been epidemiologically associated with the emergence of IBD. For example, exposure to nitrogen dioxide and sulfur dioxide has been suggested to increase the risk of early-onset IBD [29]. Furthermore, in an ecological study, total air emissions of criteria pollutants have been associated with increased rates of hospitalizations for IBD in adults [30].

Increased intestinal iron uptake, which occurs in hereditary hemochromatosis, leads to iron deposition in the liver, heart, and pancreas, resulting in tissue injury and, ultimately, fibrosis. In this context, iron is thought to cause tissue damage via the generation of free radicals that contribute to the peroxidation of lipid membranes, organelle fragility and cellular toxicity, which determine cell death and the activation of stellate cells [31]. During iron overload or in chronic inflammatory diseases, circulating ferritin levels become elevated, and these levels may reflect either the body's iron stores or the body's inflammatory status [32]. Some data, however, suggest that ferritin may have functions other than simply storing iron or passively indicating inflammation. In fact, ferritin has been shown to be able to regulate the expression of alpha-smooth muscle actin, a key indicator of the myofibroblastic phenotype transformation of stellate cells [33]. Another study on hepatic stellate cells has shown that ferritin may activate an iron-independent signaling cascade, resulting in nuclear factor kappa B (NF-kappa B) activation and increased expression of the hepatic proinflammatory mediators IL-1 beta, iNOS, RANTES, I kappa B-alpha and ICAM1 [34]. Regarding the gut, it is unclear to date whether iron overload and radicals do play a role in intestinal fibrosis.

8.2.3 Participation of the Gut Microbiota

The intestine is colonized by trillions of microorganisms that interact symbiotically with the host to participate in metabolic functions, energy production and nutrition [35]. This interaction is also capable of modulating the local and systemic immune response in both physiological and pathological conditions, thus playing a pivotal role in maintaining homeostatic equilibrium [36]. This process is known to rely on the recognition of microbial-associated molecular patterns (MAMPs) by pattern recognition receptors (PRRs), including Toll-like receptors (TLRs) and Nod-like receptors (NLRs), found in immune and non-immune cells of the intestine [37]. The activation of these PRRs leads to the induction of innate and adaptive immune responses through the activation of intracellular signaling pathways, including NF-kappa-B [38]. Among the non-immune cells of the intestine, studies have shown that fibroblasts express TLRs and NLRs, and once activated, they may contribute to intestinal fibrosis [39, 40]. In another investigation using human fibroblasts, the enhancement of collagen production was observed in *E. coli*- but not *S. aureus*-stimulated cells. Such results support the notion that *E. coli* can directly interact with TLR4 expressed on the surface of fibroblasts to induce collagen production in those cells. Moreover, these data may represent an important piece of evidence connecting infection by gram-negative bacteria to fibrogenesis [41].

In the healthy gut, the microbiota is modulated by different mechanisms involving epithelial and immune cell molecules, while the immune response is mutually regulated by the microbiota, with specific microorganisms shaping the growth of distinct T cell subsets [37]. For instance, *Bacteroides fragilis* is capable of inducing type 1 T helper (Th1) cells, whereas Clostridia induces Treg cells [37], and segmented filamentous bacteria induce Th17 cells [42]. Although the mechanism by which the intestinal microbiota induces Tregs remains relatively obscure, a role for microbial metabolites has been proposed [43]. Among these metabolites, short-chain fatty acids (SCFAs), the product of the microbial fermentation of dietary fibers, not only represent an important energy source for epithelial cells but also possess anti-inflammatory properties [44] that may be mediated by Treg differentiation [45]. The relevance of luminal nutrients for homeostatic balance has been reinforced by evidence showing, for example, the beneficial effect of butyrate, a SCFA, in experimental colitis [46]. Furthermore, in regard to intestinal fibrosis, the administration of butyrate through enemas was also shown to be effective in a diversion colitis model by preventing inflammation and collagen deposition [47]. Therefore, the intestinal microbiota, which is constantly modulated by dietary factors, is directly implicated in the regulation of intestinal immunity, thus actively affecting homeostatic control [48].

8.2.4 Role of Damage-Associated Molecular Patterns

In addition to the well-established role of MAMPs, such as flagellin, lipopolysaccharide (LPS), peptidoglycans and zymosan, which drive the inflammatory response of the host's mucosal immune system, "sterile" components also contribute to the inflammatory process. These components consist of damage-associated molecular patterns (DAMPs), which are released into the extracellular environment; DAMPs originate from dying or stressed cells and comprise non-pathogen-derived molecules such as ATP, interleukins, chemokines, growth factors, and extracellular matrix-derived proteins. Recently, the potential contributions of immunogenic endogenous DAMPs have been investigated as distinct stimuli that appear to maintain and amplify mucosal inflammation in IBD [49].

Analysis of the differential expression of inflammatory and fibrogenic genes and their regulation in experimental colitis has shown that although an effective anti-inflammatory therapy reduces the inflammatory factors, the levels of fibrotic mediators likely remain increased afterward [50]. This example coincides with a common and concerning clinical outcome for patients with CD and has been proposed as a mechanism by which fibrosis may be stimulated even in the absence of inflammation. For example, ECM fragments resulting from tissue damage, including hyaluronan, have been regarded as DAMPS and as key elements driving fibrosis through inducing proinflammatory chemokine and cytokine production by inflammatory monocytes and macrophages [51]. In intestinal fibroblasts from patients with CD, high-molecular-weight hyaluronan that has been depolymerized to low-molecular-weight fragments has been implicated in the maintenance of inflammation and fibrosis in a cyclic and amplifying fashion [52].

Adenosine triphosphate (ATP) is a well-known energy storage molecule produced in cellular respiration that supplies the metabolic needs of cells. Primarily identified and studied in matters of metabolism, ATP has also been regarded as a signaling molecule, particularly when released in the extracellular space. Different immune and non-immune cells can express a membrane receptor capable of binding the ATP molecule as a ligand. This family of receptors is known as purinergic receptors, of which P2X7 is the most studied. In the gut, P2X7 is present in epithelial and lamina propria cells and is up-regulated by an inflammatory microenvironment, especially during the Th-1 type of immune response [53]. In addition, to induce apoptosis and autophagy in human epithelial cells [54], the ATP-P2X7 pathway is implicated in the production of proinflammatory cytokines [55], and because it associates with pannexin-1, P2X7 has also been proposed to be involved in NLRP3 inflammasome activation [56]. Overexpression of P2X7 has been demonstrated in the intestinal mucosa of human IBD, particularly in CD [57], and in experimental colitis, where prophylactic blockade of P2X7 prevented inflammation and resulted in remarkably less collagen deposition within the colonic wall [58]. Whether ATP-P2X7 signaling is directly involved in intestinal fibrogenesis is currently unknown, but this pathway has been shown to participate in mechanisms of amplification and perpetuation of the inflammatory process of IBD.

8.2.5 *Inflammatory Mediators and the Potential Link to Environmental Factors*

When patients with CD present with excessive deposition of ECM in affected regions of the gut, they develop the predominantly fibrotic, stricturing disease phenotype. This outcome is invariably preceded by a long-term process, which provides a proinflammatory milieu consisting mostly of cytokines secreted by Th1 and Th17 cells [50].

TGF-beta, one of the components of the inflammatory process in IBD, plays a special role due to its pleiotropic actions, which include a profibrogenic effect, and because of its potential links with environmental factors via AhR. Mucosal myofibroblasts from strictured CD intestinal segments present with elevated TGF-beta transcripts and increased collagen production but reduced migration ability [59]. As previously discussed, despite the high concentrations in intestinal mucosa in CD, TGF-beta cannot effectively dampen inflammation, likely due to the inhibitory effects of high levels of Smad7 on TGF-beta signaling in lamina propria mononuclear cells [60]. Interestingly, an inverse correlation between Smad7 and AhR levels has been demonstrated in CD lamina propria mononuclear cells [16]. Furthermore, TGF-beta 1 has been shown to induce AhR in normal intestinal LPMCs, while treatment with Smad7 antisense oligonucleotide can restore TGF-beta 1 signaling, enhancing AhR expression in intestinal LPMCs in CD [17].

As a transcription factor, AhR has been shown to play an important role in the immune response, shifting the balance between Treg and Th17 cells [61]. The ability of AhR to bind several different ligands, ranging from environmental contaminants, such as polycyclic aromatic hydrocarbons, to natural phyto-flavonoids or synthesized chemicals, with different affinities and functional outcomes represents an important example of the link through which environmental stimuli may modulate the immune response [62]. In this regard, it is interesting to note that the gut is constantly exposed to food and dietary products that may contain phytochemicals including polyphenolic and flavonoid compounds, tryptophan and its derivatives, quercetin, curcumin, and resveratrol, which have been shown to elicit AhR signaling pathway responses [63]. These data suggest that exogenous stimuli may interfere with the immune system response, possibly affecting inflammation and fibrogenesis, at least indirectly.

IL-1 alpha, a member of the IL-1 family, has been implicated in the pathogenesis of acute and chronic inflammatory disorders [64]. Of note, IL-1 alpha is expressed in intestinal epithelial cells and has also been recognized as a DAMP, capable of triggering sterile inflammation [65, 66], and is thus potentially involved in intestinal inflammation [67]. Recently, IL-1 alpha has been shown to induce proinflammatory cytokine responses from human intestinal fibroblasts and to act as an early mediator and re-activator of intestinal injury in experimental colitis. Hence, investigators concluded that IL-1 alpha constitutes a mediator of epithelial-fibroblast interactions, playing an important role in the pathogenesis of IBD [68].

8.2.6 Epigenetic Modifications

Recent information has supported the hypothesis that there are epigenetic interactions between host DNA and environmental triggers that determine the phenotypical expression of complex diseases, including IBD. Distinct epigenetic mechanisms, in addition to DNA methylation, have been associated with the development of IBD, including histone changes [69] and the differential expression of microRNAs (miRNAs) [70]. Nevertheless, DNA methylation studies have provided the most common epigenetic modifications correlated to the pathogenesis of IBD [71].

Recent epigenetic profiling has revealed the DNA methylome signatures of colon tissue from patients with IBD [72, 73]. In a study analyzing epigenetic changes in fibrogenesis, chromatin modifications were associated with type I collagen gene activation in the endothelial to mesenchymal transition, typical in intestinal fibrosis [74]. In another study, using a genome-wide approach to define the DNA methylome and the transcriptome of intestinal fibroblasts, investigators identified differentially methylated regions underlying molecular interactions that appear to lead to fibrostenotic CD [75].

Although specific patterns of miRNA have been identified in mucosa and serum in CD, the mechanism by which they interact with target genes remains relatively obscure [76]. Nevertheless, a recent study has reported an inverse correlation between miR-124 and AhR protein levels in CD-induced inflamed intestine. These results appear to indicate that miR-124 induces intestinal inflammation in CD by interfering with AhR modulation of proinflammatory cytokines [77].

8.2.7 Accidental or Iatrogenic Exposure to Drugs, Derivatives, and Radiation

Olmesartan medoxomil, an angiotensin II receptor blocker used for the management of arterial hypertension, has been associated with a sprue-like enteropathy since 2012 [78]. Ever since, several additional cases of olmesartan-associated enteropathy have been reported, but the underlying pathogenic mechanism remains unknown. As olmesartan has a high affinity for the AT1 receptor, it has been hypothesized that AT1 receptor saturation by olmesartan could drive circulating angiotensin II to bind only the AT2 receptor. This, in turn, would initiate a pro-apoptotic effect in enterocytes, leading to villous atrophy, in addition to increased intraepithelial lymphocytes and a thickened subepithelial collagen layer in the

duodenum of patients. The fibrogenic component of this enteropathy has been proposed to result from the action of elements of the renin-angiotensin system, which can stimulate the conversion of fibroblasts into myofibroblasts, resulting in increased collagen fiber deposition in the tissue [79].

Nephrogenic systemic fibrosis (NSF), an acquired disorder occurring almost exclusively in patients with renal dysfunction [80], is characterized mostly by progressive skin fibrosis but is also capable of affecting internal organs [81, 82]. In addition to renal failure, NSF was recently associated with gadolinium-based contrast (GBC) exposure, which includes agents typically used in magnetic resonance imaging studies [83, 84]. The clinical presentation of NSF consists of an acute phase immediately after exposure to GBC and a chronic phase characterized by progressive fibrosis. In the first phase, GBC is thought to trigger a systemic inflammatory response involving iron mobilization, followed by a progressive, chronic phase in which fibrosis develops [85]. Although the mechanism by which GBC causes NSF is unclear, it is interesting to note the increased tissue infiltration of ferroportin-expressing fibrocytic cells and the tissue accumulation of iron observed in NSF [86, 87]. In particular, at the cellular level, the disruption of iron metabolism by GBC agents may be capable of causing tissue injury by triggering oxidative stress [88]. This information may be relevant to IBD, not only due to the routine use of magnetic resonance enterography for follow-up in patients with fibrostenotic Crohn's disease but also because of the potential implications of the mechanisms underlying chronic intestinal inflammation and fibrosis.

Ionizing radiation from medical and environmental sources results in various biological effects and represents an important reason for health-related concerns. Radiation-induced toxicity has been associated with long-term disability because of several potential complications, including fibrosis, related to cancer treatment. Although radiation fibrosis frequently refers to the development of a neuro-muscular disorder [89], other organs may also be affected. For example, radiation pneumonitis and fibrosis have been associated with the involvement of the lungs [90]. Radiation enteritis represents another important complication of ionizing radiation, usually occurring as a consequence of radiotherapy for pelvic cancer treatment. Of note, the chronic form of enteritis may emerge up to several years after exposure. From a histopathological perspective, radiation enteritis has been characterized by progressive obliterative endarteritis with significant submucosal fibrogenesis [91]. Recently, delayed effects of ionizing radiation, including fibrosis, have been associated with the production of reactive oxygen and nitrogen species, as well as changes in redox signaling [92]. Although none of the abovementioned accidental or iatrogenic exposures have been directly associated with IBD, their involvement with fibrogenic responses may shed light on new mechanisms that also possibly cause intestinal fibrosis (Fig. 8.1).

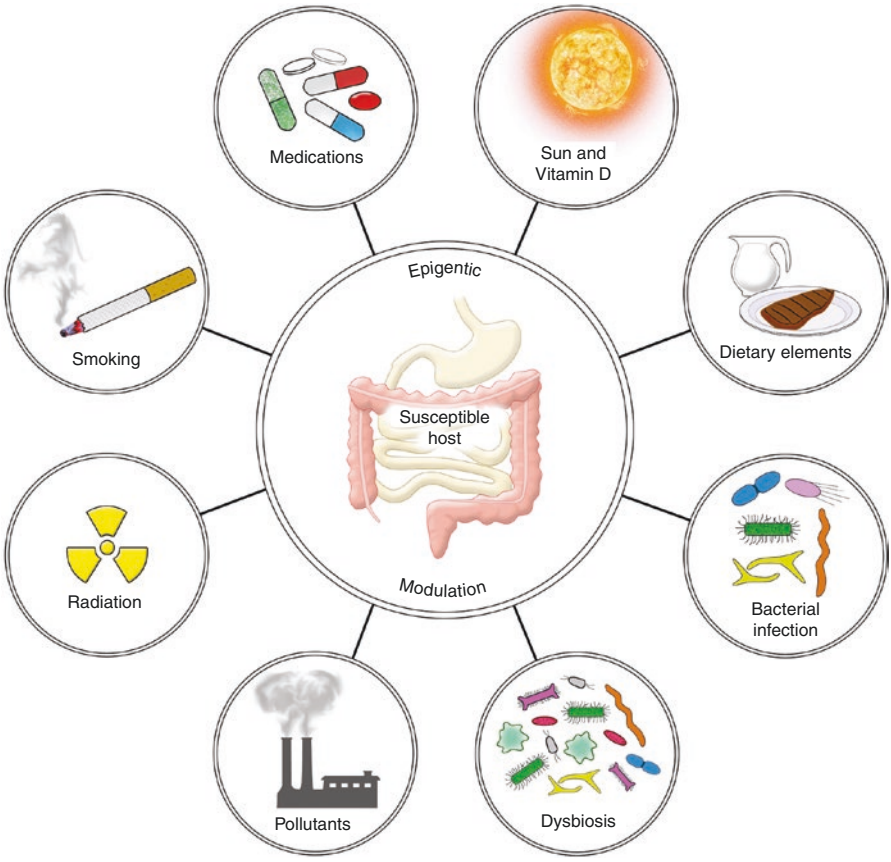

Fig. 8.1 Schematic illustration showing the influence of environmental factors on the susceptible host, potentially leading to the development of chronic intestinal inflammation and, ultimately, to intestinal fibrosis

8.3 Conclusion

Multiple mechanisms are involved in the tissue response that culminates in excessive ECM deposition in IBD. This complex process encompasses the dynamic interactions of several genes and molecules, creating a milieu in which fibrosis may ensue, even in the absence of active inflammation. Although the inflammatory process per se constitutes a major activator of fibrogenic mechanisms, other important pathways have been identified in the gut that include the action of microbe-associated molecular patterns and other pattern recognition receptors, as well as DAMPs. The vast surface and functions of the gut provide a special environment for the participation of luminal contents in immune homeostasis, including the microbiota and its associated products and daily ingested food and its components. In addition, environmental pollutants, dietary habits, food additives, and natural and synthetic compounds have also been shown to modulate epigenetic mechanisms, which are likely to play a critical role in the development of fibrosis (Fig. 8.2). It is likely that the

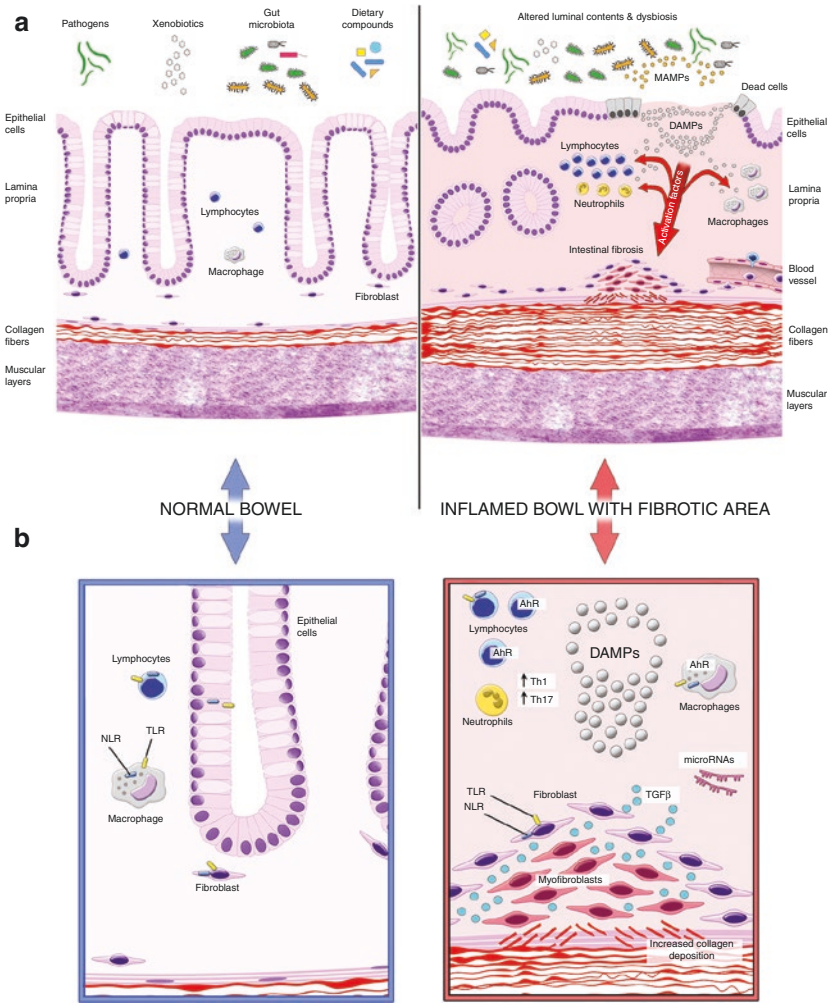

Fig. 8.2 The role of environmental factors on mechanisms of fibrogenesis. The loss of epithelial barrier integrity favors the mucosal exposure to increased microbial-associated molecular patterns (MAMPs) of potential pathogens and a dysbiotic microbiota, in addition to xenobiotics and dietary components, inducing the release of pro-inflammatory mediators, with leukocyte recruitment, and tissue damage. Additional inflammatory response occurs due to the release of damage-associated molecular patterns (DAMPs) which, in conjunction with pro-inflammatory mediators, activate pro-fibrotic signals, stimulating epithelial-mesenchymal and endothelial-mesenchymal transition, driving the differentiation of cell precursors into myofibroblasts, resulting in the overproduction of extracellular matrix (**a**). Activation of toll-like receptors (TLR) and NOD-like receptors (NLR) in immune and non-immune cells can elicit Th1 and Th17 responses, which modulate pro-fibrotic stimuli through the release of several mediators, including TGF-beta. The aryl-hidrocarbon receptor (AhR) is an important immune regulator, shifting the balance between Treg and Th17 cells. AhR is functionally associated with the TGF-beta pathway, and has the ability to bind to several different ligands present within the gut lumen, thus representing a link between environmental signals and the immune and fibrogenic response. Environmental triggers can also affect the host DNA determining the phenotypical expression of IBD, through epigenetic mechanisms including DNA methylation and the differential expression of microRNAs (**b**)

discovery of specific environmental factors and a better understanding of how they interact with the epigenetic molecular network may help in the prevention of IBD and hopefully in the reverse of early stages of intestinal fibrosis in IBD.

Author Contributions All authors provided approval for this chapter; Claudio Bernardazzi and Fernando Castro contributed equally to this work.

Conflict of Interest Statement The authors declare that there is no conflict of interest regarding the publication of this chapter.

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Chapter 9
Animal Models and Sources of Mesenchymal Cells in Intestinal Fibrosis

Dominik Bettenworth

Abstract Intestinal fibrosis is a common complication in patients with Crohn's disease (CD) that often results in an impaired quality of life of affected patients. In the absence of specific anti-fibrotic medical therapy, patients with stricturing CD often have to undergo invasive endoscopic treatment approaches or surgical intervention. Beside the lack of medical treatment options for stricturing CD, the diagnostic work-up is hampered by the limited accuracy of methods for detection, characterization and grading of intestinal fibrosis. Therefore, functional studies as well as studies evaluating novel diagnostic and therapeutic approaches for stricturing CD are urgently needed and require appropriate animal models as a prerequisite. Over recent years, several animal models for intestinal fibrosis have been established that allow for evaluation of experimental fibrosis at different stages and in the context of different pro-fibrotic triggers. In the following chapter, a variety of animals models will be presented, specific advantages and disadvantages will be emphasized and the overall relevance and applicability of these models to study human fibrostenotic IBD will be discussed.

In the second part of the chapter, different sources of mesenchymal cells, one of the key executor of intestinal fibrogenesis, will be discussed. In addition to well known mechanism such as proliferation and migration of fibroblasts, novel aspects such as cellular transdifferentiation including epithelial- and endothelial to mesenchymal transition will be described. Finally, novel techniques to traffic cellular fate will be displayed.

Keywords Animal model · Intestinal inflammation · Trinitrobenzene sulfonic acid · Dextran sodium sulfate · T cell transfer · Senescence accelerated mice P1/Yit mouse · *Salmonella typhimurium* · Radiation · Postoperative fibrosis · Heterotopic intestinal transplant model · Mesenchymal cells · Fibroblast · Myofibroblasts · Epithelial to mesenchymal transition · Endothelial to mesenchymal transition · Bone-marrow stem cell · Stellate cells · Pericyte · Fibrocyte

D. Bettenworth
Department of Medicine B, Gastroenterology and Hepatology,
University of Muenster, Muenster, Germany
e-mail: dominik.bettenworth@ukmuenster.de

F. Rieder (ed.), *Fibrostenotic Inflammatory Bowel Disease*,
https://doi.org/10.1007/978-3-319-90578-5_9

9.1 Animal Models of Intestinal Fibrosis

Animal models may never completely display the complex pathophysiology of human disease and are inevitably associated with methodological limitations. However, they represent one of the best available approaches to emerge the pathophysiological understanding as well as to test novel diagnostic and therapeutic approaches. Furthermore, preclinical data gained from animal models can serve as a valuable and cost-saving rationale for subsequent studies in human patients. Animal models for colitis have been introduced and studied for several decades, however, these models have been predominantly applied to evaluate the involvement of the immune system, the microbiota and inflammatory alterations in the context of inflammatory bowel disease (IBD) [1–3]. While not every colitis model is per se appropriate to equally study intestinal fibrosis, there is a growing body of evidence demonstrating that several of the available colitis models qualify as models of intestinal fibrosis as well [4, 5]. In the following charpter, available fibrosis models will be categorized according to the induction of fibrosis.

Spontaneous models of intestinal fibrosis are particularly promising since they do not depend on exogenous stimulation. The senescence accelerated mice (SAM) P1/Yit mouse starts to develop a spontaneous enteric inflammation in the ileum within 10 weeks after birth and reveals a 100% penetrance of fibrosis by 30 weeks after birth [6]. Beside the ileal disease location, which is the most frequent location in human CD patients, SAMP1/Yit mice depict further CD-like alterations such as a transmural and segmental injury accompanied by perianal lesions including fistula development as well as granulomas in the mucosa and submucosa [6, 7]. Microbial factors are not crucial for this model as SAMP1/Yit mice do not develop inflammation under pathogen-free conditions. Functional studies have revealed that the inflammation in the early phase of this model appears to be mediated by $CD4^+$ cells leading to a Th-1-like cytokine profile [8] while the chronic phase is characterized by Th2 responses [9]. A major drawback of this model is the low breeding rate and the limited commercial availability.

Targeted manipulation of genes is a widely used approach to study the impact of inflammatory pathways related to IBD. With regard to intestinal fibrosis, several *genetically induced models* are available. The targeted disruption of the IL-10 gene in mice leads to a spontaneous chronic enterocolitis [10, 11]. While a rather mild colonic inflammation is observed in IL-10-deficient mice under pathogen-free conditions, animals under conventional housing conditions show mucosal inflammation in the upper and lower intestinal tract as well as systemic signs of inflammation such as anemia [10]. Colitis manifestation is dependent on $CD4^+$ T cells and mediated by IFN-γ since anti-INF-γ antibody administration was elegantly shown to ameliorate the disease course in IL-10-deficient colitic mice [12]. Colitis induction in this model can be accelerated and aggravated by oral administration of cyclooxygenase isoform-selective inhibitors such as piroxicam which act through a blockade of endogenous prostaglandin production [11]. Importantly, the molecular disease pattern of this model changes over time from a Th1-driven phenotype in early stages

towards a predominant Th2-driven phenotype with increased IL-4 and IL-13 synthesis in later disease stages [13]. Of note, recent work has pointed out the relevance of IL-13 in the pathogenesis of intestinal fibrosis [14, 15]. This fact together with the observation that increased ECM levels were found in the intestine of IL-10-deficient mice, emphasize the applicability of this model to study intestinal fibrogenesis [16].

TGF-β1 is a core mediator for initiation and perpetuation of fibrogenesis in general and has also been identified as a key driver for intestinal fibrogenesis in IBD patients [17, 18]. Accordingly, genetically-engineered mice owing a TGF-β overexpression represent a promising research tool to study fibrogenesis. Vallance et al. demonstrated that mice treated by TGF-β1 gene transfer via rectal enema delivery will first develop inflammatory alterations of the intestine within 2 weeks [19]. Subsequently, these animals present with marked fibrotic alterations including massive collagen deposition, myofibroblast infiltration and colonic wall thickening and obstruction in up to 50% of treated mice. Fibrotic alterations in this model appear focally along the colon and mortality rates of this model are below 10% [19].

The cytokine monocyte chemoattractant protein 1 (MCP-1) is well known to play a crucial role in mediating fibrotic alterations in different organs such as the lung, the kidney and the liver [20–22]. MCP-1 is capable to attract different cell types including monocytes, T and NK cells. Furthermore, MCP-1 expressionw as found to be enhanced within the submucosa and muscularis propria of CD patients as compared to healthy controls [23]. Intramural gene transfer via an adenovector endoding for murine MCP-1 resulted in transmural inflammation and induction of fibrosis reflected by an increased collagen accumulation from day 3–21 post gene transfer [24]. Furthermore, profibrotic markers such as TGF-β1 and tissue inhibitor of matrix metalloproteinase 1 (TIMP-1) were found to be increased at day 7 post vector transfer. In contrast, T and B cell-deficient RAG2$^{-/-}$ mice did not respond with collagen deposition upon gene transfer pointing at a crucial role for lymphocytes in this model [24].

Chemically-induced models of fibrosis depend on the external administration of variable agents that induce an inflammatory response of the intestine through intestinal epithelial injury or direct activation of immune cells. As a consequence of a epithelial barrier defect, microbiota can penetrate the colonic wall, interact with local immune cells of the lamina propria and thus initiate or maintain inflammation which in turn may futher fuel fibrogenesis.

The trinitrobenzene sulfonic (TNBS) acid-induced intestinal fibrosis model is one of the most commonly applied chemically-induced models [25]. Rectally administered TNBS (usually diluted in ethanol to induce epithelial damage) acts as a hapten causing a T cell dependent transmural inflammation [26, 27]. Repetitive rectal TNBS application of increasing doses results in a chronic colitis accompanied by intestinal fibrosis that may become evident by luminal stenosis and bowel dilatation [28]. In this chronic stage, the disease pattern is characterized by elevated levels of Th2 cytokines and TGF-β1. More specifically, TGF-β1 expression was shown to be at least partially dependent on IL-13 [4]. In addition, application of an antisense oligonucleotide directed against the pro-inflammatory transcription factor NF-κB

was shown to ameliorate TNBS-induced fibrogenesis indicating that this modell is NF-κB dependent [29]. However, this effect may also be mediated by the anti-inflammatory action of a therapeutic NF-κB blockade. Finally, mast cells and neuropeptides such as substance P are linked to fibrogenesis in the TNBS-induced colitis model since a mast cell blockade and neuropeptide antagonism were shown to result in a reducted fibrotic alterations of TNBS-challenged mice [30, 31]. A major limitation of the TNBS model is that fibrotic alterations are restricted to areas of TNBS installation and depend on the hapten dosing. While low doses may be insufficient to induce an appropriate degree of inflammation, higher doses may cause high mortality rates. In addition, appropriately susceptible mouse strains are required to successful apply the TNBS-induced model. Regrettably, the commonly used C57Bl/6 WT mice does not appear to be particular susceptible to TNBS-induced fibrosis.

Oral administration of dextran sodium sulfate (DSS), a complex polymer of glucose, in the drinking water of mice results in a experimental colitis, mediated by toxic DSS effects leading to impaired proliferation of epithelial cells, break down of the epithelial barrier and recruitment of macrophages [25]. In addition, chronic DSS administration in some mouse strains induces marked fibrotic alterations with increased TGF-β and MMP-2/-9 expression [32, 33]. One major advantage of this chemically-induced fibrosis model is the easy route of DSS-application as compared to TNBS for example. On the other hand, the degree of intestinal fibrosis is modest and fibrosis will not be found in the ileum. The penetrance rate of fibrosis may be increased by extending or repetition of DSS administration [5], however, due to its toxic nature, the overall transferability of this model to human IBD may be limited.

Several years ago, it was reported that peroxynitrite, an oxidant and nitrating agent, rectally administered into the colons of rats lead to narrowing of the colonic lumen and signs of stenosis at day 21 [34]. Furthermore, histopathological analyses showed transmural colitis and thickening of the muscularis mucosae and muscularis propria reflecting manifest fibrosis.

The vast majority of animal models for IBD were intended to study the role of the intestinal immune responses during inflammation. Later on, some of these models such as the DSS-induced colitis model were extended to assess the chronic phase of intestinal inflammation. Additionally, this extension allows for investigation of intestinal fibrosis. The T cell transfer-induced colitis represents the most commonly used *immune-mediated model for intestinal fibrosis*. In this model, the intravenous injection of naïve $CD45RB^{high}$ $CD4^{+}$ T cells into immunodeficient SCID mice results in a strong transmural colitis [35, 36]. In addition to an inflammatory cell infiltrate containing neutrophils, lymphocytes and macrophages, a narrowing of the intestinal lumen can be observed due to the accumulation of stromal cells reflecting fibrotic alterations of the T cell transfer model [37]. This observation has been confirmed by histopathological studies, however, the overall fibrosis development in this model appears to be rather less frequent.

There is a growing body of evidence, that microbioata is a key driver of intestinal fibrogenesis in human IBD patients as well as in experimental models of fibrosis [38].

Accordingly, several experimental approaches have been used to establish *bacteria-induced models of fibrosis*.

Peptidoglycan-polysaccaride (PG-PS), a bacterial cell wall polymer induces a transmural colitis when injected into the colonic wall of rats [39]. After a chronic inflammatory phase of 3 weeks, a fibrotic thickening of the intestinal wall with associated adhesions can be observed. Furthermore, increased tissue levels of TGF-β1 and IGF-I were found [40]. While this model allows reproducible investigation of different stages of fibrosis, it is technically challenging and is only partially transferred to mice by now [41].

Similarly to the latter model, a fecal suspension of various aerobic (*Lactobacillus ssp., Enterobacter aerogenes, Klebsiella pneumoniae and Streptococcus viridans*) and anerobic bacterial strains (*Clostridium ramnosum, Bacteroides fragilis and Bacteroides uniformis*) was found to induce fibrosis when injected into the colonic wall of rats. Treated animals showed signs of chronic inflammation and fibrosis with stricture development. Additionally, TGF-β1 and collagen synthesis were significantly enhanced [42]. Subsequent work demonstrated an increased production of TGF-β1 stimulated Smad2/3 phosphorylation and enhanced ALK5, TIMP-1, and α2 type 1 collagen gene expression [43]. Slight modifications of this models indicated that inoculation with single anaerobic strains such as *Clostridium ramosum or Bacteroides fragilis* (but not with aerobic) strains was able to induce collagen deposition. These observations emphasize the impact of commensal gut microbiota on TGF-β1 and collagen production and the impact on intestinal fibrogenesis in general.

In addition, oral administration of live bacteria such as *Salmonella enterica* serovar Typhimurium can be used to establish intestinal fibrosis in mice. In this model, 24 hours after antibiotic pre-treatment with streptomycin, the ingestion of *Salmonella typhimurium* results in colitis development, which can be accompanied by marked fibrotic alterations of the cecum and colon that further aggravated over the following 3 weeks [44]. Functionally, it was shown that the *Salmonella* virulence factors *Salmonella* pathogenicity islands (SPI)-1 and SPI-2 are essentiell for fibrosis induction in this model. Furthermore, enhanced production of TGF-β1, connective tissue growth factor (CTGF) and IGF-I was documented accompanied by increased fibroblast accumulation [44]. Targeted elimination of the inflammatory stimulus by antibiotic treatment may be used to assess the specific impact of inflammation on fibrogenesis at different time points. This model is easy to perform and shows good reproducibility in various mouse strains as well as in genetically-modified mice. However, the relevance to human fibrostenotic IBD may be limited by the fact that *Salmonella* infection does not contribute to stricture development in human CD patients.

A specific subset of *E. coli* with acquired virulence factors was identified being capable to adhere and invade the intestinal epithelium which in turn leads to an inflammatory response in human CD patients. 21 days after oral gavage of so-called adherent invasive *E.coli* (AIEC) to CD1 mice pretreated with streptomycin, a transmural inflammation of the caecum with edema and crypt hyperplasia was observed. Similarly treated C57Bl/6 mice additionally developed submucosal ulcerations as

well as thickened mucosa indicative of crypt hyperplasia. Macroscopic assessment of ileal specimen from both strains revealed epithelial destruction and crypt ulcerations. Colonic and ileal inflammation in CD1 and C57Bl/6 mice was characterized by a significant increase in TNF-α, INF-γ and IL-17. Furthermore, immunohistological analyses using Masson's trichrome and picrosirius staining visualized progressive ECM deposition being more pronounced in caecal than in colonic samples from AIEC-treated mice. In accordance with these histomorphological changes, increased expression levels of TGF-β, CTGT and IGF1 were detected [45].

Based on the phenomenon that colonic exposure to therapeutic radiation can result in relevant intestinal fibrosis, *radiation-induced models of intestinal fibrosis* were invented. Radiation-induced thickening of the bowel wall is characterized by an enlarged submucosa, increased proliferation rates of fibroblasts and smooth muscle cells as well as enhanced accumulation of collagen and other ECM elements [46]. Core features of radiation-induced intestinal fibrosis are vascular sclerosis with endothelial dysfunction and chronic ulcers. Of note, the degree of fibrosis can increase up to 26 weeks following radiation and is mainly influenced by the applied radiation dose, fraction size as well as the time between several radiation procedures [47, 48]. Two different experimental settings for this approach have been proposed. In the first model, a small bowel segment is resected, exernally irradiated and the re-implanted into the animal [49, 50]. In an alternative approach, a 4-cm segment of the distal ileum is transferred into the scrotum after a orchiectomy has been performed; since this location is easy accessable for radiation [51]. The radiation results in an acute inflammatory response with colitis und ulcer development and induction of fibrotic alterations in the stromal compartment. This multicellular-mediated response is partially dependent on inflammation with microvascular insults being an early stimulus that is accompanied by subsequent hypoxia [52]. Subsequently, common fibrotic alterations including profibrotic cytokine production such as TGF-β1 and CTGF, fibroblast proliferation and increased collagen production are observed [53]. In addition, activation of mast cells, Rho-associated kinase (ROCK) signaling pathways, endothelial dysfunction and activation of capsaicin-sensitive nerves represent additional, more specific response of the irradiated bowel [54].

It is a well-known clinical observation that up to 40% of CD patients being treated by intestinal resection will suffer from a symptomatic recurrence within the first 3 years after surgery, that may further culminate in postoperative fibrosis development and anastomotic stenosis [55]. Recently, an animal *model for postoperative fibrosis* has been described. Rigby et al. performed a ileocecal resection in IL-10-deficient mice and WT mice. In contrast to the WT control group, IL-10-deficient mice developed inflammation-driven fibrosis at the proximal site of the anastomosis [56]. Importantly, germ-free housed IL-10-deficient mice did not develop fibrosis after ileocecal resection indicating that the innate immunity is part of this model [57]. Furthermore, in a model of colonic resection, an enhanced myofibroblast growth and differentiation at the anastomosis was observed [58]. In addition to re-stenosis of the anastomosis, occurrence of postsurgical adhesions reflect another

aspect of postoperative fibrosis. Serosal abrasion of the murine cecum can be easily performed and adhesions could be verified within 6 days [59]. Interestingly, executors of the adaptive immune system such as $CD4^+$ Th1 are involved in adhesion development by regulating chemokine production and leukocyte trafficking. In addition, a protective role of microbial polysaccharides and IL-10 regarding adhesion formation was observed [60, 61].

Recently, an experimental model of tracheal transplantation to study bronchiolitis obliterans was transferred into a *heterotopic intestinal transplant model* to elucidate intestinal fibrosis. To this aim, 3 cm small bowel resections of rats were transplanted into the neck of recipient rats [62]. The intestinal transplants were shown to be viable for 3 weeks. Beginning from day 2 after transplantation, loss of crypt architecture and lymphocyte infiltration was observed and resulted in fibrotic narrowing of the intestinal lumen by day 21. Additionally, collagen expression, TGF-β synthesis and IL-13 production were significantly increased over time [62]. While this model is considered to be rather artificial, it was shown to be appropriate to evaluate anti-fibrotic potential drug candidates [63, 64].

9.2 Relevance and Applicability of Experimental Models of Fibrosis to Study Human Fibrostenotic Inflammatory Bowel Disease

Clinical trials in fibrostenotic IBD patients are challenging due to several reasons. By now, no ideal target for intestinal fibrosis has been identified and the optimal timing of a anti-fibrotic treatment is unknown. In addition, appropriate biomarkers to facilitate an early risk stratification are not available and the ideal route of administration for anti-fibrotic drug candidates is unknown [65]. Therefore, the use of animal models is inevitable to further elucidate intestinal fibrogenesis and to evaluate novel diagsnotic and therapeutic approaches in order to improve quality of life of IBD patients with fibrostenotic complications.

Depending on the specific study hypothesis, one has to decide which model to apply. Of note, most of the above mentioned models have been established to study different aspects of the immune systems in the context of experimental colitis. Giving the experimental set-up, it is obvious that these models do not imply all pathogenic components of human patients suffering from intestinal fibrosis. For example, as chemically-induced models of fibrosis largely depend on an impaired epithelial barrier function upon administration of toxic substances, these models may not reflect the physiological insult that leads to intestinal fibrosis but may be particulary suitable to study the role of the innate immune system during fibrosis development. Furthermore, with regard to the commonly used TNBS model, variability of successful colitis induction depending on the optimal amount of TNBS may be challenging. Genetically-induced models of fibrosis usually feature single gene modification that do not reflect the underlying polygenetic alterations in IBD

patients. Additionally, from the practical point of view, several genetically-altered mice are commonly available on a C57/BL6 background representing a mouse strain that is generally less susceptible to fibrogenesis.

For investigation of the role of T cells in the context of IBD related fibrogenesis, the T cell transfer model appears to be promising. With regard to infection-induced models of fibrosis one should consider the difficulty to distinguish between the impact of the applied pathogens as a pro-fibrotic driver and the commensal microbiota of the animals. Moreover, *Salmonella typhi*, the commonly used bacterial species to induce experimental fibrosis has not been shown to be associated with the pathogenesis of human IBD.

With regard to the heterotopic intestinal transplant model, the rather artificial approach may be a significant limitation of this model and the immune response towards intestinal resections may be slightly different depending on the individual mouse strain used. Furthermore, the speed of fibrosis development as well as the absence of vasculature indicate significant differences compared to the pathophysiology of fibrosis in human IBD patients.

The SAMP1/Yit mouse model possesses particularly high relevance to human fibrostenotic IBD given the ileal disease localization and appearance of stricture development as well as the absence of an external stimulus. However, the hampered availability of these animals limits a broader use of this promising model.

The gut microbiota-induced model bears the advantage of direct dependence on bacteria-derived components and may therefore be particularly appropriate to study the impact of microbiota on fibrosis development. Finally, postoperative models using anastomotic fibrosis imply a high relevance to human fibrostenotic CD since the same surgical approach is used as in human patients and may therefore allow to study the outcome of routine bowel resection.

In summary, available murine models of intestinal fibrosis are valuable tools to gather relevant information for human IBD patients with stricturing disease. Independent on the model used, as fibrosis is primarly a consequence of chronic inflammation, differentiation of anti-inflammatory from anti-fibrotic treatment effects should be considered.

9.3 Sources of Mesenchymal Cells in Intestinal Fibrosis

Mesenchymal cells are key effector cells in fibrogenesis and can present as smooth muscle cells, fibroblast and myofibroblasts [66]. The latter, myofibroblasts, respresent an activated or differentiated form of fibroblasts [67]. While fibroblasts are resident in the interstitium of all human tissues and organs, a persistent stimulus such as injury or inflammation results in fibroblast activation (also referred to as disease-activated myofibroblasts) [68]. In addition, activation can be facilitated by various autocrine and paracrine signals as well as by microbiota- or damage-associated molecular patterns and ligation of other pattern recognition receptors [38, 69]. As a

consequence, fibroblasts become susceptible to inflammatory stiumulation due to an increased expression of receptors for pro-inflammatory cytokines including TNF-α [70]. Upon inflammation-mediated activation, myofibroblasts proliferate and produce various molecules that contribute to both, the perpetuation of local inflammation as well as to ECM protein deposition [32]. Over recent years, different sources of myofibroblasts have been identified that will be described in the following.

One key aspect of intestinal fibrogenesis is the *proliferation of fibroblasts*. Interestingly, it was demonstrated that fibroblast proliferation rates per se are increased in human CD and UC patients as compared to fibroblasts from healthy control patients [71]. Furthermore, is was shown, that various growth factors (known to be increased in the inflamed gut) including insulin-like growth factor I (IGF-I), basic fibroblast growth factor (bFGF), epithelial growth factor (EGF), connective tissue growth factor (CTGF), platelet-derived growth factor (PDGF) as well as pro-inflammatory cytokines such as IL-1β, IL-6 and TNF-α result in enhanced fibroblast proliferation in vitro [71–73]. Transforming growth factor (TGF)-β1 can further fuel fibroblast proliferation, however, not by direct stimulation of proliferation rates but by enhancing PDGF receptor, CTGF and IGF-1 synthesis [40]. Finally, direct interaction between fibroblasts and different cell types including mast cells, eosinophils or T cells can further foster fibroblast proliferation [74].

Within the inflamed gut, a chemotactic gradient triggers the active influx of various cells including fibroblasts that can most likely migrate from all layers of the intestinal wall as long as the gradient persists. The extent of *fibroblast migration* largely dependents on the duration and severity of the underlying inflammation. Among various soluble factors that stimulate fibroblast migration, fibronectins appears to be the most potent one [75, 76]. In addition to fibronectin, other paracrine and endocrine pathways to stimulate intestinal fibroblast migration have been identified [77]. Finally, in vitro experiments with (human CD) fibroblasts indicate an reduced migration of these cells after pro-inflammatory stimulation with TNF-α and IFN-γ; an effect that may help to maintain these fibroblasts in situ during fibrosis development [77].

Mesencyhmal *stellate cells* are characterized by a low mitogenic activity and their involvement in the retinoic acid metabolism. These cells are known to be crucially involved in liver fibrosis [78] and differentiate into fibroblasts and mediate ECM accumulation [79]. Interestingly, cells with retinoid-rich lipid droplets and a stellate-like morphology have also been identified within the submucosal layer [80]. Stellate cells isolated from human IBD patients differentiate into fibroblasts much faster (reflected by an accelerated α-SMA acquisition), proliferate faster and secrete enhanced amounts of ECM components than cells from healthy control patients [81].

It is well-known that adult bone marrow *stem cells* can differentiate into various adult lineages including fibroblasts [82]. Indeed, the ability of bone-marrow-derived cells to engraft in damaged tissue is increased and these cells were found to transdifferentiale into intestinal pericryptal fibroblasts in humans and animals [83]. Notably,

a substantial amount of myofibroblasts may be derived from the bone marrow as elegantly shown in IL-10-deficient mice [84].

Furthemore, in the experimental TNBS-induced model of colitis, transplanted bone marrow cells were found to increase with disease severity and contributed to recovery from inflammation through differentiation into activated fibroblasts [85]. Similarly, tissue repair following previous DSS challenge can be accelerated by intravenous administration of immortalized stem cells and does not require prior ablation of the immune system [86].

There is compelling evidence that in addition to the above-mentioned classical sources of fibroblasts, more recently identified mechanisms are also crucially involved in intestinal fibrogenesis. For example, *epithelial-to-mesenchymal transition (EMT)* results in a significant alteration of the epithelial cell phenotype and function towards that of mesenchymal cells [87]. While TGF-β1 represents the strongest inducer of EMT, IGF-1 and -2, EGF, FGF-2 as well as TNF-α, fibronectins, fibrin and reactive oxygen species can further influence this process [87, 88]. As a consequence, affected cells do not longer express epithelial markers such as E-cadherin, catenins and cytokeratins but constitute a spindle shape morphology and synthesize characteristic fibroblast proteins such as fibroblast-specific protein (FSP)-1, α-SMA, vimentin and secrete collagens and fibronectin [87, 89]. Furthermore, these cells display an altered capacity of migration as well as infiltration and show an increased resistence to apoptosis while the mitosis rate is reduced [87]. EMT has been detected in renal, pulmonary and liver fibrosis and the overall impact of EMT on fibrogenesis may be significant since up to 30% of fibroblast in renal fibrosis are assumed to be EMT derived [90]. By now, there is no direct proof for EMT in human fibrostenotic IBD patients, however, EMT was demonstrated in experimental intestinal inflammation with a change of epithelial cell function towards the production of collagen [91]. Furthermore, given the increasing evidence of EMT in fibrotic organs other than the gut, it is highly suggestive that EMT represents a general feature of organ fibrosis also being present in intestinal fibrosis [92].

Similarly to epithelial cells, endothelial cells are able to transform into mesenchymal cells (also referred to as *endothelial-to-mesenchymal-transition (EndoMT)*). For example, it was reported that embroyogenic stem cells that differentiate into endothelial cells may change their phenotype towards a mesenchymal differentiation such as smooth muscle cells (SMC) [93]. Additionally, adult endothelial cells of the bovine aortic or pulmonary artery origin were shown to maintain their ability to transdifferentiate into SMCs [94]. In the context of wound healing experiments, microvascular endothelial cells were found to transdifferentiate into spindle-shaped mesenchymal cells under persistent inflammatory conditions. For experimental cardiac fibrosis, it was estimated that endothelial cells represent one-third of the total pool of tissue-infiltrating fibroblasts [95]. Several similarities between EMT and EndoMT have been reported: TGF-β1 is known to be a potent driver of EndoMT as well, while Insulin-like-growth factor-II and pro-inflammatory cytokines such as

IL-1β and TNF-α are able to foster EndoMT transdifferentiation [96]. EndoMT has recently been demonstrated in colonic fibrosis of TNBS-treated mice and in microvessels of the IBD mucosa [97]. Therefore, in line with the evidence of several key inducers of EndoMT within the gut, it is most likely that EndoMT is involved in intestinal fibrogenesis as well [97].

Vascular smooth muscle cells (vSMC) encompasses arteries and veins while capillaries are surrounded by single cells called *pericytes* [98]. Both cell types comprise similar cytoskeletal components such as α-SMA and desmin and are located between the endothelium and the interstitium. Pericytes regulate various functions during inflammation and also mediate ECM degradation [99]. It has been demonstated that pericytes can be considered as an additional source for fibroblasts during tissue repair. In addition, pericytes were reported be involed in inflammation-associated tissue fibrosis as these cells can detach from vessels and differentiate into a collagen type-I-producing fibroblast-like cell [100]. This observation may explain why increased levels of ECM deposition during the initial phase of fibrogenesis accumulate around blood vessels.

By now, there are only a very few reports on the involvement of pericytes in intestinal fibrogenesis, which may be at least partially explained by the lack of appropriate in vitro models. In the TNBS-induced colitis model, it was shown that vSMCs as well as pericytes can be successfully collected from the bone marrow of mice [85]. Hower the exact role of these cells during intestinal fibrosis is still unknown.

Fibrocytes represent bone marrow-derived circulating mesenchymal cells expressing hematopoietic and mesenchymal markers such as CD34 (stem cell marker), CD45 (leukocyte antigen) and CD14 (monocytic marker) [101]. In addition, fibrocytes secrete typical fibroblast components such as collagens and α-SMA as well as ECM-modifying enzymes. Fibrocytes were furthermore shown to be able to differentiate into fibroblasts in vitro and in vivo [102].

Under inflammatory conditions, fibrocytes leave the bone marrow and move directly towards the site of inflammation in a CCR2-dependent pathway. Subsequently, fibroblasts can transdifferentiate into several cell types including epithelial, endothelial, neuronal cells and mesenchymal cells [103]. Fibrocytes are characterized by the expression of CD90 and the absence of CD34 and CD45 expression as well as monocyte markers. In contrast, cells expressing CD34 and CD45 or myeloid antigens (e.g. CD11b and CD13), and owing the ability to synthesize collagen are defined as fibrocytes and differ from resident leukocytes, dendritic cells, endothelial cells and tissue resident fibroblasts [101].

Fibrocytes have been identified as an important driver of tissue fibrosis in experimental models of pulmonary, cardiac, renal and vascular fibrosis [104–106]. Uniformely, fibrocyte inhibition resulted in a decreased amount of myofibroblasts and collagen synthesis. In addition to experimental data, fibrocytes have been identified in several human diseases including scars and keloids, asthma, nephrogenic fibrosis, systemic sclerosis, atherosclerosis, chronic pancreatitis, chronic cystitis,

and tumor-associated stromal reaction [107–109]. Given the fact that all these disorders are associated with persistent inflammatory infiltrates, it is most likely that fibrocytes are also involved in intestinal fibrosis as well.

In face of the various above-mentioned sources of mesenchymal cells and their dynamic role in intestinal fibrogenesis, it is crucial to track the cellular fate in experimental settings to eludicate the cellular origin and function in vivo. Traditional histological evaluation of the intestine usually requires the death of the animal and therefore does not allow a longitudinal evaluation. Genetically-engineered mice represent a promising tool to study mesenchymal cell fate. For example, α-SMA-GFP mice were used to visualize α-SMA$^+$ cells in a recently established α-SMA promotor driven cre-loxP mediated expression of yellow fluorescent protein mice (aSMACre;YFP$^{f/f}$ mice) [110]. In the context of renal fibrosis, the specific function of proliferating myofibroblasts was nicely studied by of the use of mice in which the viral thymidine kinase was expressed under the control of the α-SMA promotor [110]. In addition, the impact of pericytes was successfully studied by using mice in which yellow and red fluorescent protein, respectively, were expressed under the control of Cspg4 and Pdgfrb genes—both being fairly specific pericyte markers [110, 111]. Finally, the role of EMT was addressed by using transgenic reporter mice using γGT-Cre [110].

In addition to genetic modifications, it was shown recently that non-invasive MRI may be helpful to assess the fate of mesenchymal stem cells [112, 113]. For example, Kraitchman and collegues demonstrated that the role of magnetically-labelled mesenchymal stem cells (MCSs) in cardiac fibrosis can be evaluated using an 1.5 T MRI scanner [113]. However, recent work by Chen et al. indicated that assessment of iron-oxide labeled MCSs can be confounded by iron-oxide particles that were ingested by macrophages [114]. With regards to the intestine, recently a novel approach to evaluate the fate of implanted mesenchymal cells was introduced employing cell sheets. Here, magnetically and fluorescently labeled bone-marrow derived mesenchymal cells were applied to a cell sheet and administered to a murine fistula model. Subsequently, cellular fate was successfully monitored in vivo by a 4.7 T MRI and confocal laser endomicroscopy [115].

9.4 Conclusions

The pathophysiological understanding of intestinal fibrogenesis has significanyly increased over recent years. Multiple animal models are available to test novel anti-fibrotic compounds. Beside classical sources such as fibroblast proliferation and migration, several other sources of mesenchymal cells have been identified including stem cells, EMT, EndoMT and pericytes. Further studies evaluating the fate and specific contributions of these cell types to fibrotic alterations are warranted and may pave the way to a targeted therapeutic manipulation to stop or ameliorate intestinal fibrosis (Fig. 9.1).

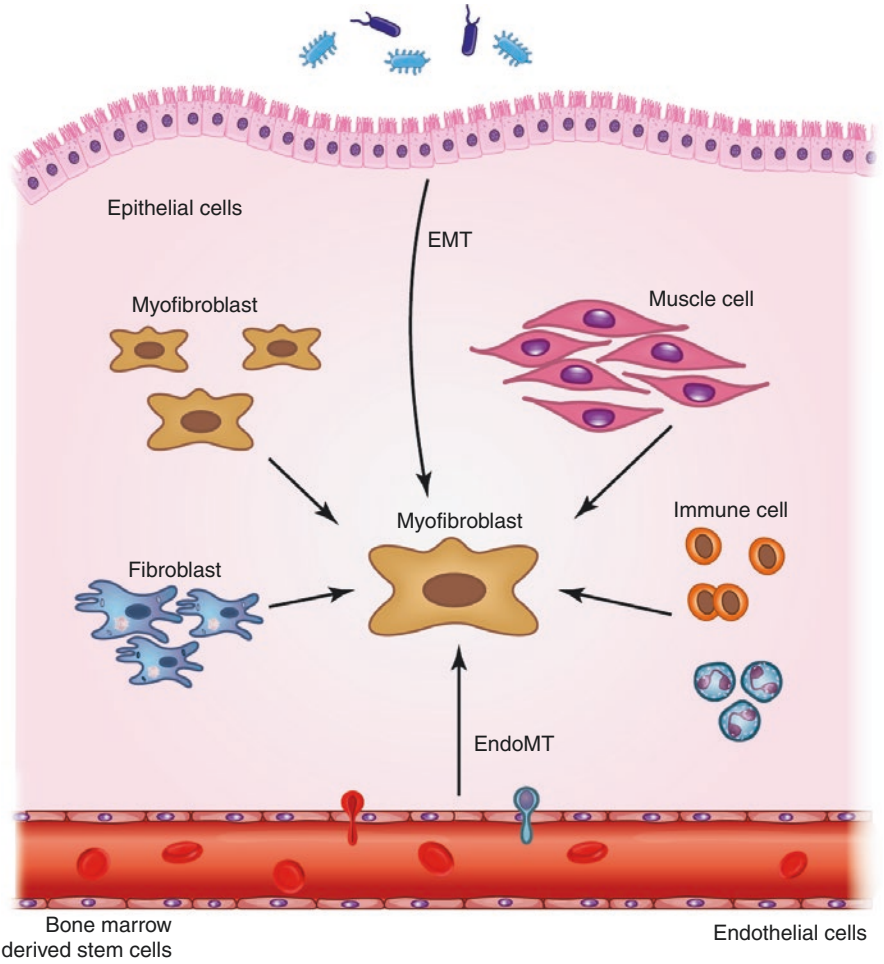

Fig. 9.1 Sources of fibroblasts in intestinal fibrogenesis. In addition, to proliferation and migration, fibroblasts can derive from precursors such as intestinal stellate cells and fibrocytes or via epithelial- or endothelial-to-mesenchymal transition (EMT, EndoMT). Additionally, fibroblasts can generate form circulating fibrocytes or from bone marrow stem cells. By now, the role of pericytes in intestinal stricture formation is unclear

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Chapter 10
Fibrosis in Ulcerative Colitis

Fernando Magro and Tatiana António

Abstract Intestinal fibrosis is a classic complication in Inflammatory Bowel Diseases where chronic inflammation and abnormal tissue repair together lead to a compromised bowel function. Although fibrosis and stricture formation are acknowledged features of Crohn's disease courses, these complications remain poorly studied in ulcerative colitis (UC). The relevance of this topic has long been ignored, despite the well-known prevalence of stenosis in UC, its clinical impact in motility and the importance of assessing stricture malignancy.

Fibrosis in UC is now perceived as a dynamic and reversible process. However, still no proper antifibrotic therapy exist, mainly due to the very limited pathophysiological insights.

This chapter aims to review the current knowledge about fibrosis development in UC, outlining disease basic concepts, epidemiology, histopathologic features and clinical consequences.

Keywords Ulcerative colitis · Inflammatory bowel diseases · Fibrosis · Stenosis · Myofibroblasts · Extracellular matrix

10.1 Ulcerative Colitis

Ulcerative colitis (UC) is, along with Crohn's disease (CD), a chronic inflammatory bowel disease where the intestinal permeability is disturbed by an inappropriate immune response. Both diseases share many epidemiological and clinical features. Nevertheless, UC distinguishes itself by being less prone to complications and by its gastrointestinal distribution, which is continuous and begins in the rectum, spreading proximally, but not reaching the ileum. Therefore, inflammation is worse in the distal colon [1, 2].

Overview of fibrosis in Ulcerative colitis

F. Magro (✉) · T. António
Faculty of Medicine, University of Porto, Porto, Portugal
e-mail: fm@med.up.pt; taa@med.up.pt

F. Rieder (ed.), *Fibrostenotic Inflammatory Bowel Disease*,
https://doi.org/10.1007/978-3-319-90578-5_10

The extent of the colonic mucosal involvement can either be limited to the rectum (proctitis), which accounts for about one third of all patients [3, 4], or progress distally, called left-sided colitis where the inflammatory process reaches the splenic flexure. When disease activity goes beyond that location and is present throughout the colon, UC is classified as extensive colitis, the most common presentation at onset in children [5]. In general, the illness' natural course is mild, marked by periods of flares and spontaneous remission of variable duration and the prognosis is usually good for the first 10 years after diagnosis [6–8].

The colonoscopy of UC reveals a diffuse, uniform inflammation with loss of the visible vascular pattern and haustral folds, as well as a granular, erythematous appearance of the mucosa. Friability is also noticeable, since the mucosa bleeds either when touched or spontaneously. Pseudopolyps may be present in long-standing UC [2, 5], but fissures, granulomas and transmural lymphoid aggregates are absent from UC patient's colon [1]. Multiple endoscopic biopsies allow the understanding of disease distribution. Findings of discontinuous mucosal impairment with sparing areas and ileal involvement favor CD diagnosis, as is a cobblestone mucosal pattern and longitudinal, irregular ulcers [1, 2]. Histologically, UC usually appears to be confined to the most superficial layers of the colon. Microscopic evidences include crypt architectural distortion, atrophy and abscesses, along with infiltration of plasma cells (plasmacytosis), lymphocytes and granulocytes [1, 5, 6].

Furthermore, some clinical manifestations are the hallmark of UC: bloody diarrhea with or without mucous secretion, fecal urgency and tenesmus [2, 7, 9]. On the other hand, CD is more likely to present with frequent abdominal pain and perianal lesions [9]. The natural course of UC can also be accompanied by frequent evacuations of blood or mucus, variable abdominal pain, overall malaise, fatigue and less commonly fever and weight loss, depending on the extent and severity of the disease [2, 6, 9]. Local complications may encompass strictures, abscesses, fistulas and cancer. Additionally, colonic dilatation (toxic megacolon) and massive bleeding may occur in the most acute fulminant form of UC [5, 10].

Besides, 10–30% of patients with UC will experience extraintestinal manifestations of the disease, which comprise musculoskeletal problems such as arthritis and osteoporosis, eye pathologies, primary sclerosing cholangitis, skin conditions like erythema nodosum, pyoderma gangrenosum and aphtous stomatitis, as well as anemia and coagulation abnormalities [2, 6, 11, 12].

Ultimately, the disease progresses towards a fibrotic pathway and, consequently, colonic failure, which appears to be a self-sustaining process that can endure even in the absence of inflammation [13].

10.1.1 Epidemiology

Overall, Inflammatory Bowel Diseases (IBD) are associated with an industrialized and westernized lifestyle, being more common in Europe and North America. Their prevalence and incidence are increasing over time and geographically, becoming a global disease [14]. The fact that IBD incidence is now much higher in prior low

incidence countries such as Asia, the finding that the disease is more typical in urban areas versus rural regions and the observation that migrating from a lower prevalence area of IBD to a higher prevalence area increases a person's disease risk all suggest that environment and lifestyle have an important role in the etiology of IBD [3, 14, 15]. UC is the most prevalent form of inflammatory bowel disease and it seems to affect both men and women in an equal manner [3, 6, 14]. The highest annual incidence of UC is found in Europe, reaching 24.3 cases per 100,000 persons [14]. This idiopathic illness is mainly diagnosed in adults between 20 and 40 years of age, although it can have its onset at any age [14–16].

Chronic inflammation in these patients eventually brings about fibrosis. This process is most of the times clinically silent and only becomes symptomatic in about 5% of the individuals with UC [17]. Benign strictures, a complication for which fibrosis is believed to contribute, occur in less than 5% of UC cases [18–20].

10.1.2 Etiology

Despite the fact that UC pathophysiology is still not fully understood, today it is widely accepted that UC does not result from a single cause, but instead it is an outcome of a multifactorial mechanism involving the immune system, environmental factors, gut commensal microbiota and genetic susceptibility [5]. These factors promote an inappropriate immune response that is accompanied by adverse clinical outcomes.

Family history is a fairly important risk factor for UC development, especially with affected first-degree relatives [21]. Genome-wide association studies (GWAS) have found 163 risk loci for IBD, 23 of those being specific for UC. These polymorphisms may disturb innate and adaptive immunity and other mechanisms that assure intestinal homeostasis [22]. However, the fact that concordance rate for UC among monozygotic twins is only about 16% denotes the great impact of non-genetic aspects in disease risk [23]. This puts a spotlight on environmental factors, particularly the ones affecting bacterial colonization of the intestine. Evidence that germ-free animals do not develop UC highlights the relevance of commensal enteric microorganisms in disease pathogenesis [24, 25]. In fact, microbiota seem to play a major role in both disease onset and severity, as well as in determining disease phenotype as UC or CD [21].

Thus, the exposure of genetically susceptible individuals to antigens of the commensal microbiota leads to a persistent immuno-mediated intestinal disorder. Nonetheless, it remains undetermined what exactly triggers chronicity in UC [10].

10.2 Fibrosis in Ulcerative Colitis

Intestinal fibrosis is frequently associated with chronic intestinal inflammation in many enteropathies and it's often observed in both main forms of IBD. It is seen as a process of long-lasting illness, where persistent tissue damage and healing result in barrier dysfunction followed by scar tissue formation [19, 26].

Fibrosis is characterized by an imbalance favoring deposition of collagen-rich extracellular matrix (ECM) over breakdown of ECM components. In UC, it is usually marked by a local increase in the mesenchymal cell pool together with thickening of the muscularis mucosa [27, 28].

Since inflammation is only rarely detected in the muscularis propria and because strictures are much less frequent in UC (1–11.2%) when compared to their fairly high incidence in CD, fibrosis was thought to be limited to the mucosa and submucosa in UC subjects [18, 20, 27, 29]. These fibrotic changes have been ignored over the years, despite their clinical relevance in leading to a stiffened colon unable to perform peristalsis or resorb fluids [30, 31]. Moreover, CD is well-known for being a transmural disease where strictures may originate from the muscular layers, whereas UC was formerly believed to be confined to the inner layers [19]. This made stricture formation in UC much harder to explain and hence mechanisms other than excessive deposition of ECM have been proposed in the formation of benign strictures in UC, namely the hypertrophy and contraction of the muscularis mucosae, which was found to narrow the lumen of the large bowel [20, 32].

It was only then suggested that disease activity might not be strictly confined to the mucosa as previously thought, but instead can affect the entire thickness of the bowel wall. This is in line with the findings of an enhanced collagen deposition throughout all the layers of the colonic wall [27, 29, 30].

10.2.1 Pathogenesis

It is widely acknowledged that fibrosis is an outcome of inflammatory damage to the tissue followed by healing impairment [33]. It has been stated that inflammation is required for the initiation of fibrosis [34]. This is supported by evidence that fibrosis follows inflammation distribution and it's never found in segments apart from the ones affected by inflammation [35]. On the other hand, inflammation seem to play only a minor role in fibrosis progression [10]. In fact, there are intestinal diseases, like celiac disease, where chronic inflammation is present but fibrosis and strictures do not occur. Furthermore, anti-inflammatory therapies have failed to prevent or reverse intestinal fibrosis. These observations highlight the existence of an independent mechanism underlying fibrosis other than inflammation [36, 37] Not only it is not yet clear what drives chronicity in UC, but also it is still undefined what prompts strictures formation. Since not all individuals develop intestinal fibrosis and the ones who do, display a variable extent of it, we can postulate that there is a genetic factor that determines susceptibility [10, 38].

It is still unclear if UC and CD share the same fibrogenic pathways [28]. Yet, in both situations, an inflammatory environment seems to be a prerequisite for the intestinal fibrotic process to begin. Inflammation leads to injury of the epithelium with ECM disassembly and release of chemokines and other cytokines, which in turn determines the recruitment and activation of immune and non-immune cell

types. Immune cells migrating to the injured site include both macrophages and neutrophils as part of the immediate innate response, and T lymphocytes as part of the adaptive immune response [34, 36, 39].

Damage additionally extends to the lamina propria by local activation of ECM-degrading enzymes, like elastases and matrix metalloproteinases (MMPs) that allow further infiltration of immune cells. Moreover, collagenases reinforce destruction of the ECM by fibronectin and collagen degradation. Eventually, this continuous cycle of epithelial damage, repair, angiogenesis and lymphangiogenesis is responsible for the loss of epithelial cells and mucosal ulceration (Fig. 10.1) [19, 29, 34].

In this manner, damaged mucosa and submucosa becomes exposed to a profibrotic milieu of soluble mediators and enzymes. In UC these include proinflammatory cytokines such as IL-13 [40], IL-17 [41] and IL-33 [42] and several growth factors among which are the insulin-like growth factor-1 (IGF-1) [43–45], the transforming growth factor-β (TGF-β) [31, 46], the platelet-derived growth factor (PDGF) [47], the basic fibroblast growth factor (b-FGF) [20, 43] and the tumor necrosis factor-α (TNF-α) [31, 45].

This microenvironment encourages local mesenchymal cells to actively differentiate and dedifferentiate between three acknowledged phenotypes: fibroblasts

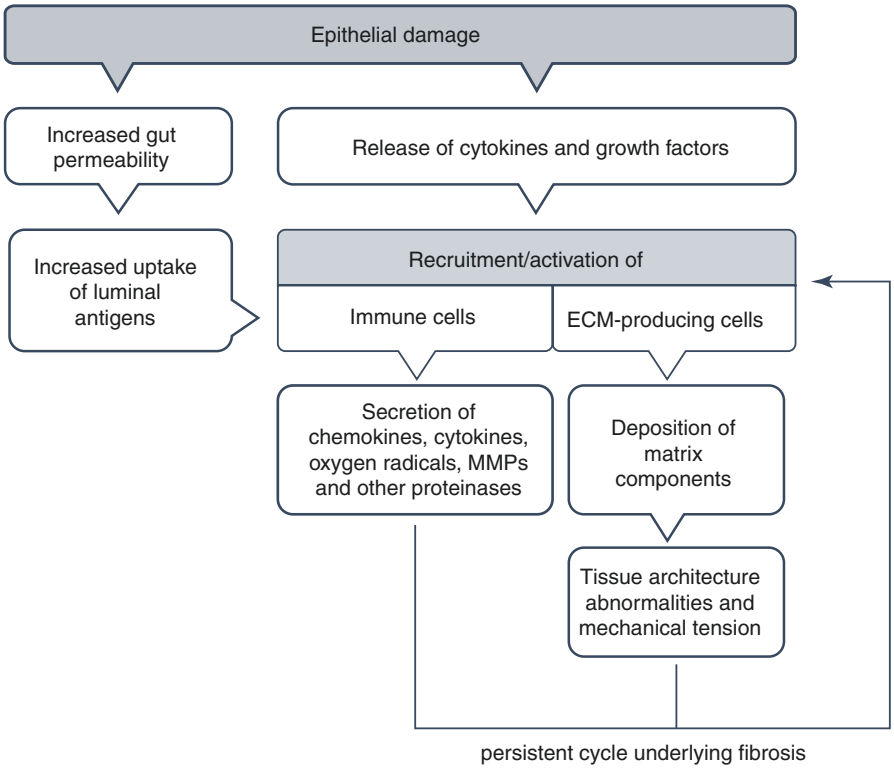

Fig. 10.1 General representation of downstream events that underscore fibrosis in UC

(α-smooth muscle actin (α-SMA) negative, vimentin positive, desmin negative), myofibroblasts (α-SMA positive, vimentin positive, desmin negative) and smooth muscle cells (α-SMA positive, vimentin positive, desmin positive) [48]. The great contractile ability of activated myofibroblasts, their capacity to migrate and secrete ECM components as well as growth factors, make them the main player in tissue remodeling and fibrosis. In the active UC mucosa, the number of α-SMA positive cells is increased, especially at the margins of deep ulcers [34, 49]. Myofibroblasts can become activated through different pathways, by means of paracrine signals from immune and nonimmune cells, as the ones mentioned above, by autocrine signaling, by pathogen-associated molecular patterns that come from the interaction of microorganisms with Toll-like and NOD-like receptors, and also by products from cell injury named damage-associated molecular patterns [17, 50]. Myofibroblast activation represents an acquisition of a pro-repair and pro-fibrogenic cell phenotype, inciting their proliferation (expansion in number) and dramatically increasing their secretion of numerous molecules, including mediators that further sustain local inflammation and ECM components deposition [43, 51].

Among these components, collagen comprises the major scar protein, with a predominance of subtypes I and III. Collagen type I provides tensile strength and mechanical stability to the tissue, whereas collagen type III is known for its elastic and flexile properties. In UC, both these subtypes of collagen together with fibronectin have been found in increased levels transmurally, i.e., not only in the mucosa and submucosa, but also in the muscularis externa [27, 29].

Normal wound healing begins with deposition of new matrix components predominantly collagen type III, which in time is replaced by collagen type I [52, 53]. Some studies also found this increased collagen type III:I ratio to exist in inflamed colonic specimens from UC patients, which relates to the initial state of fibrosis development. Moreover, the areas where this increase occurs overlap with regions of an inflammatory cell infiltrate rich in TGF-β1 and IGF-1. This ratio later changes in favor of collagen type I as the fibrotic process matures [27, 54].

Besides local proliferation, myofibroblasts may arise from a wide variety of sources: they can either migrate from neighboring tissue, be recruited from circulating precursors like fibrocytes or bone marrow stem-cells, differentiate from intestinal stellate cells and pericytes, or derive from epithelial or endothelial-to-mesenchymal transition [26, 48, 51, 55].

Under physiologic conditions, tissue repair is a self-limited controlled process. Eventually the epithelial barrier becomes fully restored, MMPs break down the fibrotic matrix and myofibroblasts become inactivated or undergo apoptosis, but still very little is known about the signals that control this process [53, 56]. In UC, however, repair mechanisms are disturbed. Instead, persistent deposition and cross-linking of matrix components modify the ECM leading to its stiffening. Since immune and nonimmune cells can sense the surrounding matrix via integrin mediated mechanisms, mechanical tension by itself can drive cells to a proliferative and activated phenotype, leading to a vicious cycle of profibrotic events (Fig. 10.1) [33, 57].

Although strictures are much less frequent in UC than in CD and often associated with longer disease duration, they can still have a significant impact on the disease course and lead to serious clinical complications. Above all, a better understanding

of stricture pathogenesis in UC is crucial when considering the risk of cancer, even though only a minority of strictures in UC are indeed malignant. It is difficult to perceive whether colonic cancer emerges from a pre-existing benign stricture or if it is in fact a malignant growth from the beginning. Benign strictures are frequently asymptomatic and associated with long-lasting disease. The time elapsed from UC diagnosis to stenosis detection is usually about 15 years [18, 20, 58].

The mechanisms for the development of benign strictures in UC remain poorly understood. Some authors believe that hyperthrophy and contraction of the muscularis mucosae is the most likely phenomenon to be responsible for the colonic narrowing related to strictures. However, the ability of the reported lesions to revert spontaneously and the inclusion of muscular hyperthrophy cases in the study suggests that these may not be in fact true strictures [18, 59]. Indeed, several inflammatory mediators are known for promoting growth and function alterations of smooth muscle cells [17]. Nevertheless, muscular thickening of the muscular layer does not seem to be the major culprit of stenosis in UC as not all stenotic specimens display a thickened muscularis propria [20]. Additionally, a more rigorous approach found stenotic cases to be linked to greater ulcer scars when comparing to nonstenotic specimens, even at segments of the colon without stenosis, denoting that stenotic subjects are more prone to fibrosis development [20]. Furthermore, b-FGF, an important proliferation factor for mesenchymal cells, was found to be highly expressed at stenotic sites, along with myeloperoxidase, in cases where neutrophils appear to be the dominant inflammatory cell type. This further supports the hypothesis that colonic stenosis in long-lasting UC is owed to fibrosis, probably by b-FGF-positive neutrophils, inducing proliferation of myofibroblasts [20]. The reason why stenosis and strictures are common complications of CD but are rather rare in UC is still uncertain. UC confinement to the most superficial layers of the colon was initially pointed out as one possible explanation, but several studies have later reported ECM deposition in all layers of the colonic wall [29, 30]. Further studies are needed to explain this observation.

10.2.2 Clinical Consequences of Fibrosis

The abnormal tissue architecture that arises from fibrosis may disturb the normal function of the epithelium and eventually trigger the development of symptoms [26]. Peristalsis and fluid reabsorption are compromised, and may lead to the abdominal pain and diarrhea often experienced by patients with UC. Fibrosis in UC has been a quite overlooked topic and therefore its serious clinical implications have been far underestimated. This is surprising as the importance of the ECM deposition in the disease course, its role in stricture formation, the ensuing obstruction and motility problems that may result and the crucial need of distinguish between benign and malignant strictures are well-known phenomena [30, 31, 33]. Clinical complications are likely related to the accumulation of scar tissue in the intestinal wall of UC subjects and therefore the smaller diameter of the colon. This comes with an increase in wall stiffness and patients with long-standing illness normally present with a

narrowed colon with diffuse loss of haustration, acquiring a “lead pipe” appearance. Clinical findings suggest that these structural alterations entail loss of colonic elastic properties and decrease of contractility and compliance. Moreover, a reduced tone of the descending colon is found in these patients after meals. Consequently, looser faeces or even diarrhea will ensue. A particularly severe form of dysmotility in UC is anorectal dysfunction, expressed by fecal urgency, incontinence and tenesmus [28, 32, 33, 60]. Generally, the clinical picture correlates with the disease extent and distal involvement, as colorectal lesions usually display more and earlier symptoms [10].

It is true that these symptoms can be more severe during the active phases of UC and indeed it is well established that inflammation itself is able to affect motor and perceptive functions of the colon [61–63]. However, even when inflammation subsides and disease is quiescent, patients’ symptoms still persist and because of that one can hypothesize that fibrosis of the large bowel wall could contribute to all of the above clinical presentations [32, 64, 65].

10.3 Conclusion and Future Questions to Be Addressed

The concept of fibrosis is now one of a dynamic and reversible process. In order to provide reliable therapeutic options to manage fibrosis in UC, some matters are yet to be addressed, as pathophysiological insights are still very limited. First, there is a need to determine the key effectors in myofibroblast activation and the markers that identify the activated form of the mesenchymal cell. Second, it would be crucial to determine which factors rule the transition from an inflammatory to a fibrostenotic phenotype, which could potentially also be used as a specific target for antifibrotic therapy.

Limitations to the study of fibrosis and stricture formation include the lack of good animal models that truly represent the chronic, polygenetic nature of the disease and the need of reliable biomarkers that would make monitoring fibrosis in clinical trials possible [22, 66].

Several compounds have been proposed as potential antifibrotic drugs [13, 67], but no specific therapy is yet available. The fibrotic mechanisms are highly complex and multifactorial and a multi-target approach would likely be the best strategy. The self-perpetuating nature in UC highlights the urge for drugs that would allow the prevention and reversal of intestinal fibrosis. As our knowledge on fibrostenotic mechanisms progresses, this target will come within reach.

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Chapter 11
Histopathology of Intestinal Fibrosis

Ilyssa O. Gordon

Abstract Fibrostenotic inflammatory bowel disease classically refers to Crohn's disease, where stricture and submucosal fibrosis are frequent and defining histopathologic features. Descriptions of histologic fibrosis in ulcerative colitis have existed for over half a century, but are not currently part of the clinical diagnostic framework. Histologic scoring systems for fibrosis in inflammatory bowel disease are varied and highlight the need for improved histopathologic correlation, given recent advances in our understanding of the pathophysiology of intestinal fibrosis.

Keywords Fibrosis · Histopathology · Muscularis mucosae · Submucosa · Stricture Inflammatory bowel disease · Histologic fibrosis score

11.1 Histopathology of Fibrosis in Crohn's Disease

Submucosal fibrosis is a pathologic hallmark of Crohn's disease. Normal submucosal collagen and adipose tissue are replaced by fibrous tissue which contracts the submucosal area (Fig. 11.1). Strictures are areas of marked submucosal fibrosis, along with hyperplasia of the muscularis mucosae, which can become so thick as to obliterate the submucosa [1] (Fig. 11.2). Expansion of the muscularis mucosae, including hyperplasia, architectural disarray, and collagen deposition, accounts for about half of the increased wall thickness of an ileal stricture in Crohn's disease [2]. Strictures also often contain hypertrophic nerves (Fig. 11.3). Submucosal arteries and veins often have fibromuscular hyperplasia in strictured areas [2] (Fig. 11.4). Deeper in the bowel wall, muscularis propria hypertrophy can be seen, and along with disorganization and fibrosis, leads to an overall thickened muscularis propria, although this feature is not a diagnostic hallmark [3]. On gross examination, creeping fat or fat wrapping, is a common finding and a major pathologic feature, characterized by fat extending along the antimesenteric border.

I. O. Gordon
Cleveland Clinic, Cleveland, USA
e-mail: gordoni@ccf.org

F. Rieder (ed.), *Fibrostenotic Inflammatory Bowel Disease*,
https://doi.org/10.1007/978-3-319-90578-5_11

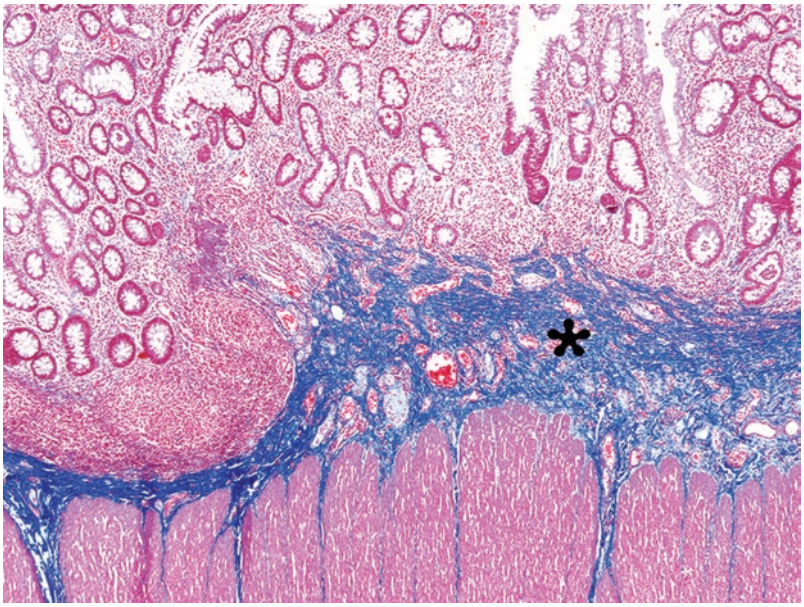

Fig. 11.1 Submucosal fibrosis in Crohn's disease. Fibrous tissue is present within the submucosal area (black star) (Masson Trichrome, original magnification 4×)

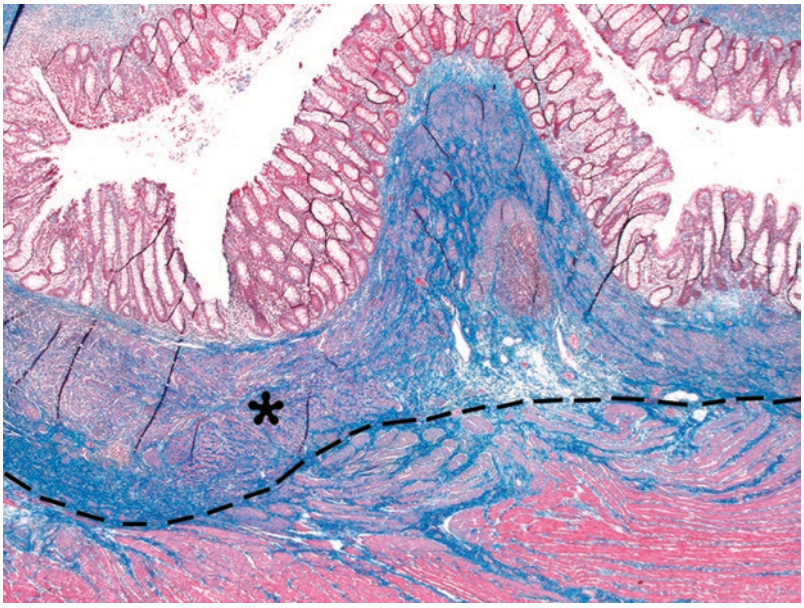

Fig. 11.2 Stricture in Crohn's disease. In addition to submucosal fibrosis, marked hyperplasia of the muscularis mucosae (black star) can obliterate the submucosal area. The dotted line demarcates the submucosa above from the muscularis propria below. (Masson Trichrome, original magnification 4×)

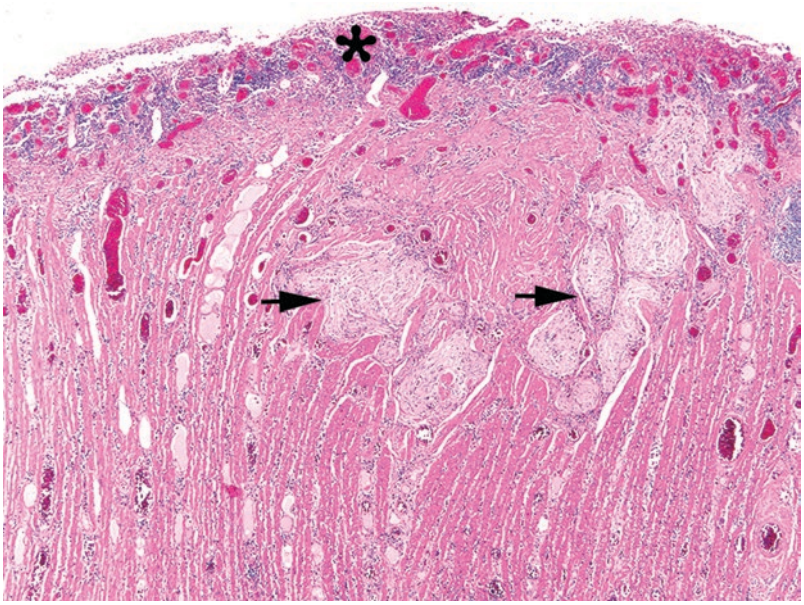

Fig. 11.3 Stricture in Crohn's disease. Hypertrophic nerves (black arrows) are present in the submucosa of this ulcerated stricture (black star indicates ulcerated luminal surface) (Hematoxylin & Eosin, original magnification 4×)

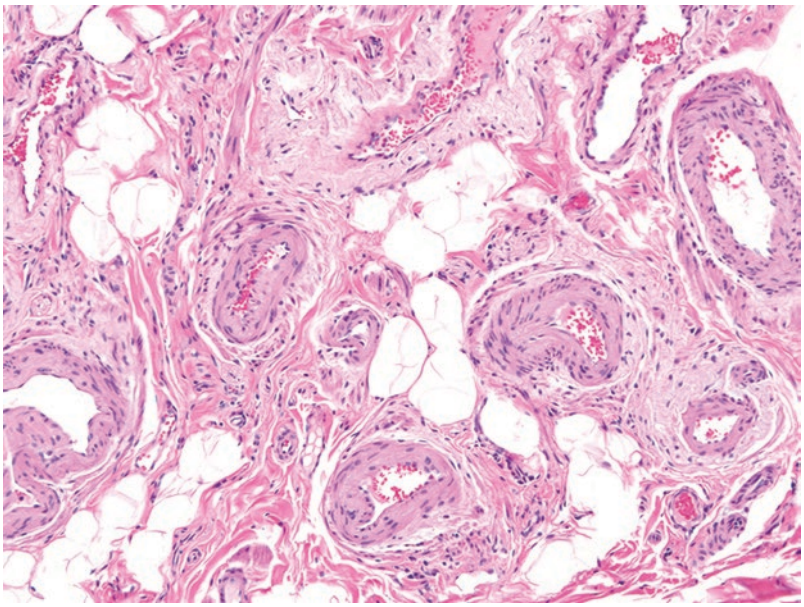

Fig. 11.4 Stricture in Crohn's disease. Fibromuscular hyperplasia of submucosal arteries and veins (Hematoxylin & Eosin, original magnification 10×)

It is not known whether hyperplasia of the muscularis mucosae and nerves occur as a result of a prolific response following ulcerating injury (i.e. aberrant wound healing [4]) or if they develop in response to other factors, such as mesenchymal growth factors, related to inflammation or gut bacteria; although a combination of these factors is most likely involved [5–7]. For example, intestinal fibroblasts express Toll-like receptor-4, which is acted upon by bacterial lipopolysaccharide (LPS) to activate a nuclear factor kappa B pathway, resulting in collagen contraction [7]. LPS also enhances connective tissue growth factor expression by decreasing expression of the transforming growth factor-beta inhibitor, smad-7 [7]. Other TLRs, including TLR-9, as well as chemokines, such as CXCL8, and cytokines also play a role in mesenchymal-bacterial interactions at the molecular level [6].

11.2 Histopathology of Fibrosis in Ulcerative Colitis

Standard descriptions used in diagnostic pathology do not include fibrosis as a histologic feature of ulcerative colitis. Furthermore, as ulcerative colitis is defined as having pathologic features restricted to the mucosa and superficial submucosa, the presence of a stricture in UC is immediately concerning for an infiltrative carcinoma, rather than a benign process. Looking to the published literature, however, 71% to 100% of all clinically detected strictures in UC patients were benign [8–10]. The risk of a stricture being due to malignancy is associated with longer duration of disease and location of the stricture, with rectal strictures being more common (68%), and therefore more often benign (90%), and strictures of the right colon being malignant 87–100% of the time [8, 11].

Histologic studies of benign strictures in UC have of necessity been performed on resection specimens. Goulston et al. compared the thickness of the muscularis mucosae and inner layer of muscularis propria in benign strictured and non-strictured UC areas, and found 40-fold and 20-fold thickening, respectively, as compared to non-strictured UC controls; concluding that fibrosis alone was insufficient to explain the stricture [11]. Other studies have described fibrosis along with muscular hypertrophy, including marked submucosal fibrosis in 20 of 28 benign UC strictures [12]. Microscopic examination of benign UC strictures has also revealed expansion of the submucosa by fat, which may be a factor contributing to luminal narrowing [13].

Descriptions of fibrosis in UC outside of the presence of strictures is also lacking [14]. In a study of UC proctocolectomy resections with dysplasia in an American center as compared to a Japanese center, lamina propria fibrosis in non-dysplastic areas was more prominent in American cases as compared to Japanese cases [15]. Interestingly, in control cases without dysplasia, there was no difference in lamina propria fibrosis in the two populations [15]. The authors suggested longer disease duration in the American group and differences in medications as possible explanations for the findings [15]. One very early study from 1949 describes fibrosis in the wall of UC resections in the context of extensive ulceration [16]. This concept was

also discussed in the 1950s and 1960s by Lumb et al. [17, 18]. Indeed, in the wound repair process after epithelial injury, including gut epithelial injury, it is generally accepted that fibrosis is part of the post-inflammatory organization of granulation tissue [19, 20].

We described histologic patterns of lamina propria fibrosis and muscularis mucosae alterations in non-strictured UC resections, and found these histologic features to be correlated with prior medication use and inflammatory activity [21]. The most striking pattern of lamina propria fibrosis is a band of fibrosis between the base of the crypts and the muscularis mucosae, essentially replacing the more typical basal lymphoplasmacytosis (Fig. 11.5). Similar to other studies finding alterations and thickening of the muscularis mucosae in UC, we have also seen altered muscularis mucosae with patterns of splaying, usually with interspersed fibrosis (Fig. 11.6), as well as thickening of the muscularis mucosae, and finally splitting and even duplication of the inner and outer layers of muscularis mucosae (Fig. 11.7).

Regarding submucosal fibrosis in non-strictured UC resections, we observe this phenomenon in ulcerated areas (Fig. 11.8) as well as in non-ulcerated areas. In non-ulcerated areas, fibrous bands within the submucosa typically form adjacent to the muscularis mucosae and muscularis propria and are otherwise perpendicular to the luminal flow (Fig. 11.9). Submucosal fibrosis can be identified in diagnostic H&E

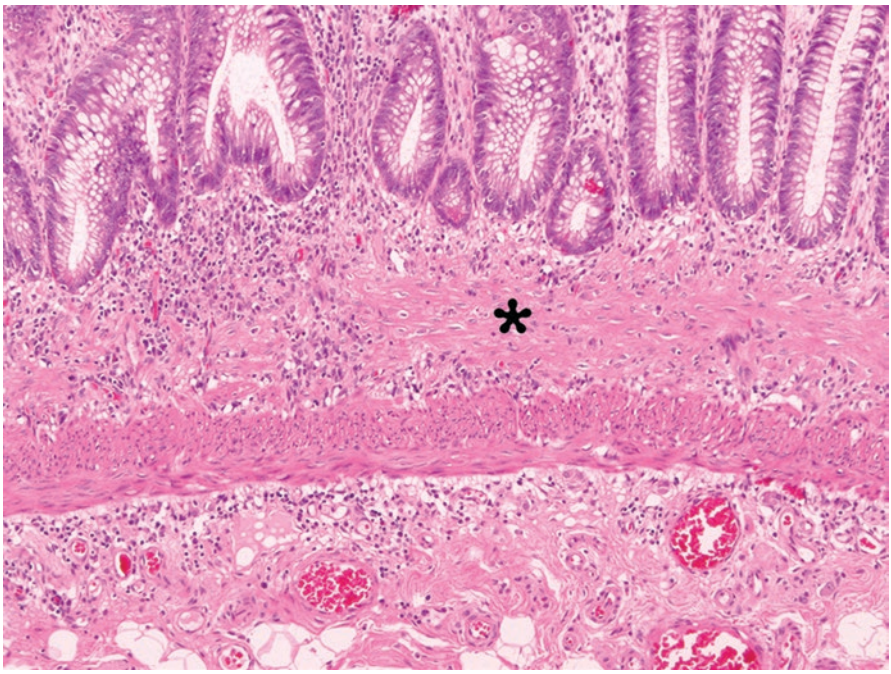

Fig. 11.5 Lamina propria fibrosis in ulcerative colitis. A band of fibrosis (black star) between the base of the crypts and the muscularis mucosae replaces the typical basal lymphoplasmacytosis (Hematoxylin & Eosin, original magnification 10×)

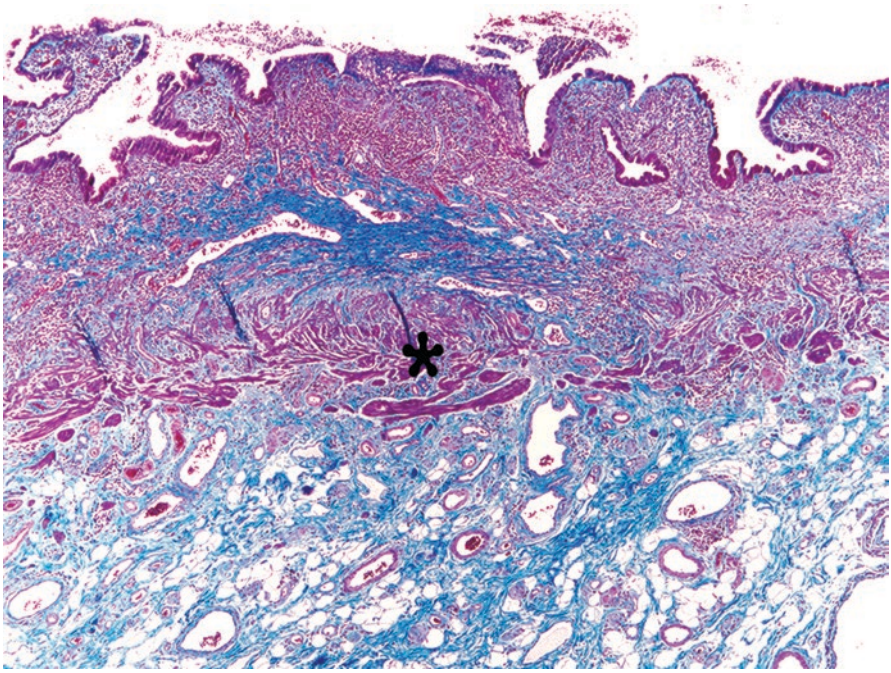

Fig. 11.6 Muscularis mucosae alterations in ulcerative colitis. Hyperplastic and splayed muscularis mucosae (black star) with interspersed fibrosis (blue) (Masson Trichrome, original magnification 4×)

stained sections without the aid of a trichrome stain, and the degree of submucosal fibrosis is associated with the severity of intestinal inflammation [22]. Apart from fibrosis associated with deep mucosal ulceration in fulminant disease, significant changes in the muscularis propria are not typically seen.

11.3 Pathology Fibrosis Scoring Systems in Inflammatory Bowel Disease

Fibrosis scoring systems in inflammatory bowel disease are typically based on imaging and biomarkers [23]. Histologic scoring of fibrosis in Crohn's disease originated from studies in rodents [24, 25], as well as human studies comparing radiographic findings with resection specimen findings [26–29], and early studies examining resection specimen margins to determine factors associated with recurrent disease [30, 31].

The earlier rat scoring system by Theiss et al. (Table 11.1) is progressive and cumulative, and is based on evaluation of sections stained with Masson trichrome and Sirius red [24]. Progression starts from submucosal collagen deposition (score 1), and adds on mucosal collagen deposition (score 2), muscularis mucosae collagen deposition and disorganization (score 3), muscularis propria collagen

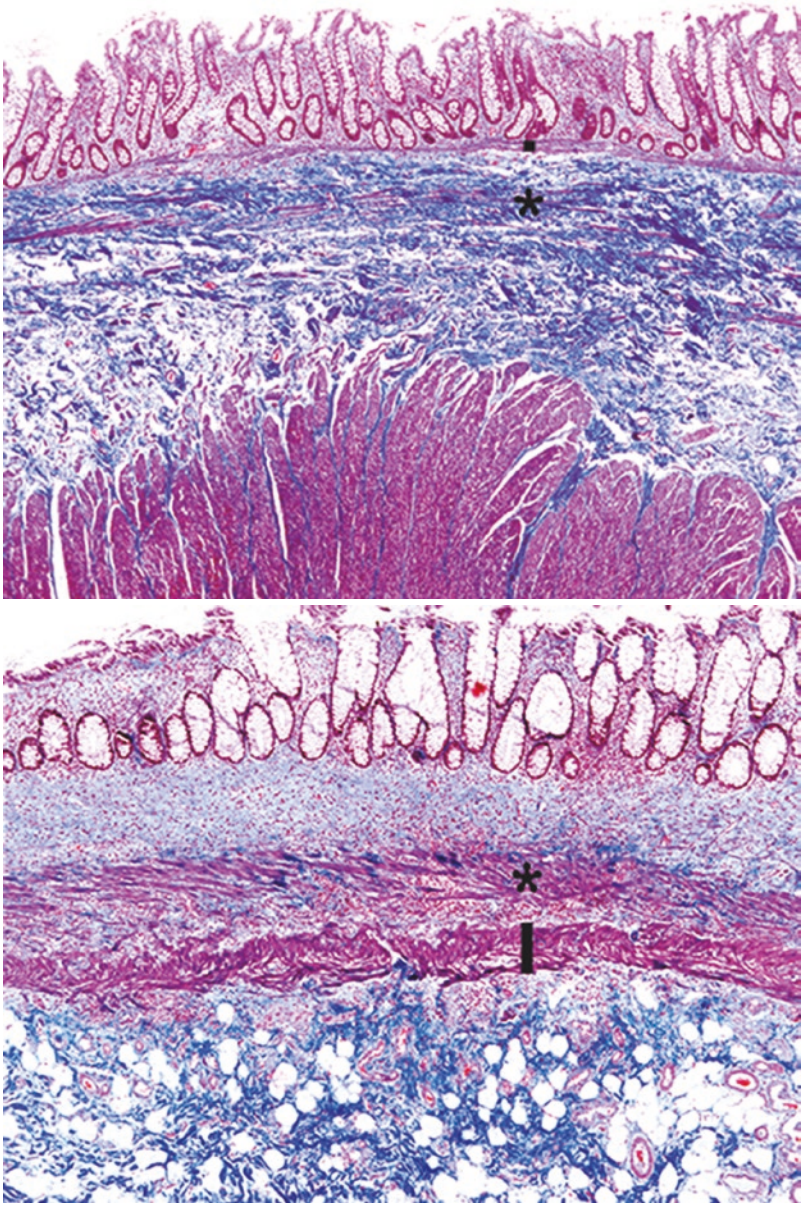

Fig. 11.7 Muscularis mucosae alterations in ulcerative colitis. In these two cases of ulcerative colitis with mucosal healing, contrast the thin and nearly intact muscularis mucosae with splayed muscularis hyperplasia within the superficial submucosa (Top), with the thickened muscularis mucosae with duplication on the mucosal aspect (Bottom). [black bar demarcates the original two layers of muscularis mucosae, black star indicates the alteration] (Masson Trichrome, original magnification 4×)

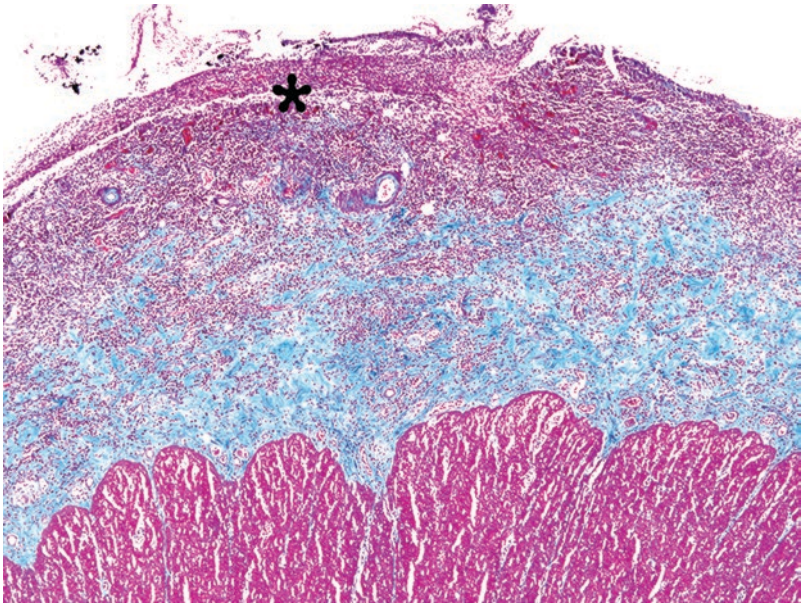

Fig. 11.8 Submucosal fibrosis in ulcerative colitis. Extensive ulceration (black star) is associated with fibrosis (blue) as part of the wound healing process (Masson Trichrome, original magnification 4×)

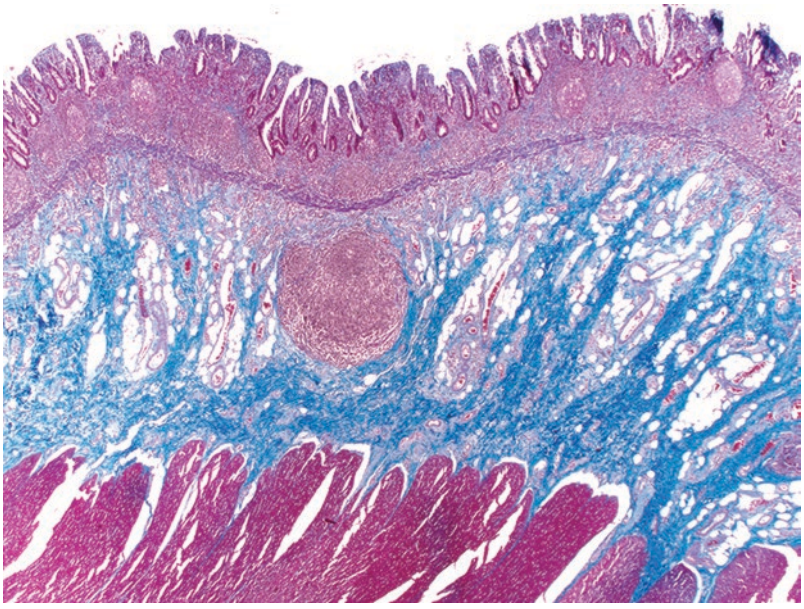

Fig. 11.9 Submucosal fibrosis in ulcerative colitis. Fibrous bands in the submucosa of non-ulcerated areas of ulcerative colitis typically form adjacent to the muscularis mucosae and muscularis propria and are otherwise perpendicular to the luminal flow (Masson Trichrome, original magnification 4×)

Table 11.1 Criteria for histologic fibrosis score of intestine

	Score	Description
Fibrosis	0	No increased collagen deposition
	1	Increased collagen deposition in submucosa
	2	Increased collagen deposition in submucosa and mucosa
	3	Increased collagen deposition in muscularis mucosa, submucosa, and mucosa; thickening, disorganization of the muscularis mucosa
	4	Increased collagen deposition in muscularis propria, muscularis mucosa, submucosa, and mucosa
	5	Increased collagen deposition throughout all layers including serosa
Percent involvement	1	0–25% of section
	2	25–50% of section
	3	50–75% of section
	4	75–100% of section

Note: used in rat model; based on Masson trichrome and Sirius Red stained sections
Reprinted from Theiss et al. [24], with permission

Table 11.2 Criteria for histologic fibrosis score

Score	Description
0	No fibrosis
1	Mild fibrosis (focal mucosal/submucosal collagen deposition without architectural distortion)
2	Moderate fibrosis (significant mucosal/submucosal collagen deposition with modest distortion of mucosal/submucosal architecture but without obscuring of the mucosal/submucosal border)
3	Severe fibrosis (extensive mucosal/submucosal collagen deposition with marked architectural distortion obscuring the mucosal/submucosal border)

Note: used in mouse model; based on Masson trichrome stained sections
Adapted from Higgins et al. [25], with permission

deposition (score 4), and finally all layers including serosa (score 5). Interestingly, there is also consideration of the percent of the section involved by fibrosis (Table 11.1), an important consideration given the segmental, and microscopically patchy, nature of Crohn's disease. The more recent mouse scoring system by Higgins et al. (Table 11.2) utilizes a progressive score considering only fibrosis (collagen deposition) affecting the mucosa and submucosa on Masson trichrome stained sections, with four tiers, from no fibrosis (score 0) to severe fibrosis (score 3). This scoring system is much less detailed than the earlier one, and has also been used on human tissue comparing fibrosis on pathology to ex vivo ultrasound evaluation in patients with inflammatory bowel disease [29].

Histologic scoring of fibrosis in studies comparing to radiographic findings are also somewhat less detailed, and do not include the percent section involvement, presumably due to examining only strictured segments. These studies use human tissue with Hematoxylin & Eosin stained sections. Adler et al. [26] separates fibrosis grade from muscle thickness, specifically thickening of the muscularis propria (Table 11.3). Unlike the earlier rodent scoring systems described above, this scoring

Table 11.3 Histologic evaluation scoring

Fibrosis grades	
0	No fibrosis
1	Minimal fibrosis in submucosa or subserosa
2	Increased submucosal fibrosis, septa into muscularis propria
3	Septa through muscularis propria, increase in subserosal collagen
4	Significant transmural scar, marked subserosal collagen
Muscle	
0	Normal thickness
1	Increased thickness of muscularis propria layer

Note: used in human tissue; based on H&E stained sections
Adapted from Adler et al. [26], with permission

Table 11.4 Scoring system for inflammatory and fibrostenotic features of Crohn's disease lesions

Fibrostenosis (score)	Pathology
None (0)	No or minimal fibrosis limited to submucosa (<25% thickness)
Mild/moderate (1)	Mild stricture (>15 mm) with nondilated lumen Submucosal fibrosis and muscular hyperplasia >25% with preserved layers
Severe (2)	Massive transmural fibrosis; effacement of normal layers; severe stricture

Note: used in human tissue; based on H&E stained sections
Adapted from Chiorean et al. [28], with permission

does not recognize mucosal fibrosis, or muscularis mucosae fibrosis or hyperplasia. Fibrosis is scored progressively, but considers submucosa, muscularis propria, and subserosa in each grade (Table 11.3). Chiorean et al. [28] takes a simplified approach with fewer score options in a three-tiered system (Table 11.4). Focus is again on submucosal fibrosis and on muscularis hyperplasia, though it is not specified whether this is referring to muscularis mucosae or muscularis propria.

Scoring schema from studies looking at resection specimen margins are probably the most generally applicable, as they consider pathologic features of Crohn's disease generally, not just findings from a stricture. Fibrosis of muscularis mucosae and submucosa are the focus, without specific mention of mucosa, muscularis propria, or subserosa, and a three-tiered progressive intensity grade was used [30, 31]. Interestingly, Maconi et al. [27] applied this scoring system, slightly modified to include four-tiers, to a group of Crohn's patients resected for ileal stenosis (stricture), where representative sections along the entire resected segment length were analyzed, and correlated the histologic findings with ultrasound echo patterns.

Neither the mouse nor the human scoring systems described here have been validated. As fibrosis in Crohn's disease is not generally thought to progress from

lumen to serosa, a scoring system that accounts for all layers of the bowel wall simultaneously is preferred. A similar scoring system should be applicable to both mice and human studies. The ideal Crohn's fibrosis scoring system would account for changes in the muscularis mucosae, including both hyperplasia and fibrosis, submucosal fibrosis and muscularization, muscularis propria hyperplasia and fibrosis, and subserosal fibrosis. A four-tiered scoring system (e.g. none, mild, moderate, severe) applied to each site (i.e. mucosa, muscularis mucosae, submucosa, muscularis propria, and subserosa) would be ideal, at least in early studies until clinical correlation could be established, and then perhaps the score could be contracted into three tiers. As Crohn's disease is patchy, multiple sections per specimen should be assessed, and a unified "per specimen" score could also be generated. Again, clinical correlation studies would be needed to best understand whether the unified score would reflect the highest overall score, or a combination of the scores from each section examined.

As the recognition and understanding of fibrosis in ulcerative colitis lags behind that of Crohn's disease, so does the concept of fibrosis scoring schema for ulcerative colitis. Measurement of the bowel wall layers has revealed muscularis mucosae thickening in ulcerative colitis [22], as well as increased fibronectin and collagen I in the mucosa, increased collagen I in the muscularis mucosae, and increased collagen I and III in the muscularis propria [32].

We developed a histologic fibrosis burden score for ulcerative colitis with a three-tiered approach focusing on the percent of submucosal fibrosis, and found moderate interobserver agreement on Hematoxylin & Eosin stained sections, and significant correlation when compared to Masson trichrome stain and Sirius red stain [22]. Bowel wall layer measurements revealed thickening of the muscularis mucosae, and this correlated with the presence of chronic mucosal injury [22].

11.4 Conclusion

In summary, fibrosis is a defining histopathologic feature of Crohn's disease. A variety of histologic scoring systems have been developed to evaluate fibrosis of inflammatory bowel disease, with a focus on Crohn's disease. In ulcerative colitis, recognition of histologic fibrosis has not been part of the diagnostic cadre, but has certainly been recognized and described in the literature. More studies evaluating the clinicopathologic correlation of fibrosis in ulcerative colitis are needed. Current histologic scoring systems reveal a variety of definitions of fibrosis and are incongruent as to the importance of fibrosis and mesenchymal cell hyperplasia in each of the bowel wall layers. Perhaps a unified histologic fibrosis scoring system, which can account for Crohn's disease, ulcerative colitis, strictured and non-strictured areas, and which incorporates the current understanding of the pathophysiology of fibrosis in inflammatory bowel disease, would be needed before widespread use could be established for clinical trials.

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Chapter 12
Clinical, Cellular and Serologic Biomarkers of Intestinal Fibrosis

Antonio Di Sabatino and Paolo Giuffrida

Abstract Intestinal fibrosis, which is due to an exaggerated accumulation of extracellular matrix, is a frequent complication of inflammatory bowel disease (IBD) leading to intestinal obstruction and need for surgery. Currently, there are no biomarkers able to predict the development of intestinal fibrosis in patients with inflammatory bowel disease. Most of the candidate biomarkers, including clinical factors (i.e. smoking, ileal location, early use of steroids), circulating cells (i.e. fibrocytes), serum extracellular matrix components (i.e. collagen, fibronectin) or enzymes (i.e. tissue inhibitor of matrix metalloproteinase-1), serum growth factors (i.e. basic fibroblast growth factor, YKL-40) and serum antimicrobial antibodies (i.e. anti-*Saccharomyces cerevisiae* antibodies ASCA), have been shown to predict a disabling disease course rather than a fibrostenosing phenotype. In this chapter we critically review clinical, cellular and serological biomarkers of intestinal fibrosis in inflammatory bowel disease.

Keywords Anti-microbial antibody · Extracellular matrix · Fibrocyte · Growth factor · Ileal location

Abbreviations

ACCA	Anti-chitobioside carbohydrate antibody
ALCA	Anti-laminaribioside IgG antibody
AMCA	Anti-mannobioside carbohydrate antibody
anti-C	Anti-chitin carbohydrate antibody
anti-CBir1	Anti-bacterial flagellin CBir1 antibody
anti-I2	Anti-*Pseudomonas*-associated sequence I2 antibody

A. Di Sabatino (✉) · P. Giuffrida
Department of Internal Medicine, San Matteo Hospital Foundation, University of Pavia, Pavia, Italy
e-mail: a.disabatino@smatteo.pv.it

F. Rieder (ed.), *Fibrostenotic Inflammatory Bowel Disease*,
https://doi.org/10.1007/978-3-319-90578-5_12

anti-L	Anti-laminarin carbohydrate antibody
anti-OmpC	Anti-*Escherichia coli* outer membrane protein C antibody
ASCA	Anti-*Saccharomyces cerevisiae* antibody
bFGF	Basic fibroblast growth factor
CD	Crohn's disease
ECM	Extracellular matrix
ELF	Enhanced liver fibrosis
FAP	Fibroblast activation protein
IBD	Inflammatory bowel disease
MMP	Matrix metalloproteinase
PDGF	Platelet-derived growth factor
PIIINP	N-terminal propeptide of type III collagen
TGF	Transforming growth factor
TIMP	Tissue inhibitor of matrix metalloproteinases
TNF	Tumor necrosis factor
UC	Ulcerative colitis
VEGF	Vascular endothelial growth factor

12.1 Introduction

Currently, there are no reliable biomarkers useful in clinical practice in predicting the risk of developing intestinal fibrosis with subsequent stricture formation in inflammatory bowel disease (IBD) [1]. Proposed biomarkers, such as clinical factors (i.e. smoking, ileal location, early use of steroids), circulating cells (i.e. fibrocytes), serum extracellular matrix (ECM) components (i.e. collagen, fibronectin) or enzymes [i.e. tissue inhibitor of matrix metalloproteinase (TIMP)-1], serum growth factors [i.e. basic fibroblast growth factor (bFGF), YKL-40] and serum antimicrobial antibodies [i.e. anti-*Saccharomyces cerevisiae* antibodies (ASCA)], have shown contradictory results mostly derived from retrospective studies on relatively heterogeneous patients' cohorts. Moreover, most of the above mentioned factors were reported to be predictive of a disabling disease course rather than of a stricturing phenotype (Fig. 12.1). Biomarkers of intestinal fibrosis are needed as they would be useful for the management of IBD patients at risk of fibrostenotic complications, and for the identification of early phases of the fibrogenic process in order to prevent stricture development [2]. In this chapter we critically revise the diagnostic and prognostic power of fibrogenic biomarkers in IBD.

12.2 Clinical Biomarkers

A number of clinical factors, such as diagnosis before 40 years of age, early need for steroids, thiopurines or anti-tumor necrosis factor (TNF)-α agents, perianal disease, smoking habitus, ileal location, disease activity and deep mucosal ulceration,

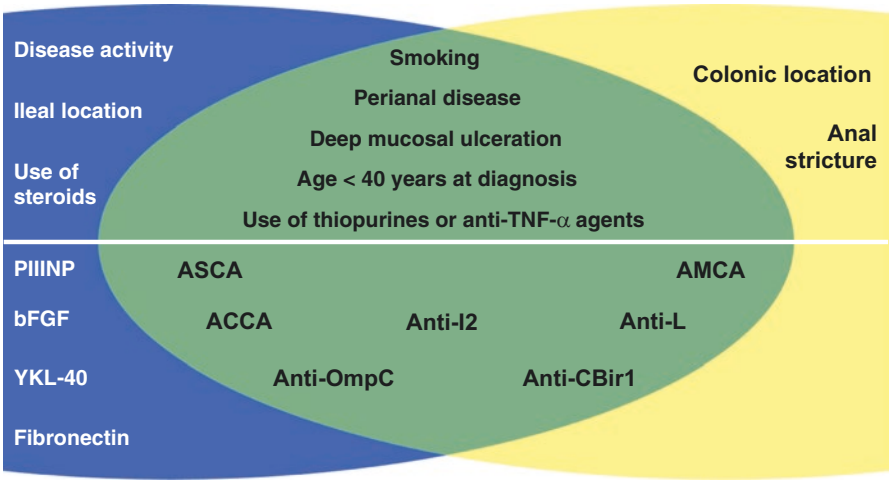

Fig. 12.1 Candidate clinical (upper quadrants) and serologic (lower quadrants) biomarkers of fibrostenosing (blue), disabling (green) and penetrating (yellow) disease. *ACCA* anti-chitobioside carbohydrate antibody, *AMCA* anti-mannobioside carbohydrate antibody, *anti-CBir1* anti-bacterial flagellin CBir1 antibody, *anti-I2* anti-*Pseudomonas*-associated sequence I2 antibody, *anti-L* anti-laminarin carbohydrate antibody, *anti-OmpC* anti-*Escherichia coli* outer membrane protein C antibody, *ASCA* anti-*Saccharomyces cerevisiae* antibody, *bFGF* basic fibroblast growth factor, *PIIINP* N-terminal propeptide of type III collagen, *TIMP* tissue inhibitor of matrix metalloproteinases, *TNF* tumor necrosis factor

have been demonstrated to be predictors of either disabling disease or post-surgical recurrence in IBD patients [3, 4]. However, only ileal location, disease activity and use of steroids were found to be specifically associated with stricture development according to observational data derived from TREAT Registry [5]. Currently, the predictive power of all the above mentioned candidate clinical factors has not been tested in large prospective *ad-hoc* studies, thus their value in clinical practice as fibrogenic predictors is still under debate.

12.3 Cellular Biomarkers

Circulating fibrocytes are mesenchymal progenitor cells derived from bone marrow and considered as marker of fibrosis in idiopathic pulmonary fibrosis [6]. Conversely, no data have been obtained so far on circulating fibrocytes in patients with stricturing CD. However, an increased percentage of fibrocytes has been reported either in the peripheral blood or in the inflamed gut mucosa from CD patients [7]. Of note, fibrocytes isolated from peripheral blood of CD patients produce high amount of collagen type I after *in vitro* stimulation with lipopolysaccharide [7].

12.4 Serologic Biomarkers

Serologic biomarkers include ECM proteins, growth factors and anti-microbial antibodies. As regards ECM components, serum N-terminal propeptide of collagen type III (PIIINP) is increased in stricturing CD prior to intestinal resection in comparison to control subjects, and it decreases 6 months after effective surgery [8]. On the other hand, a previous study reported no significant difference of serum PIIINP between CD patients, whose phenotype was not specified, and controls [9]. Additionally, the same study [9] showed that serum C-terminal propeptide of collagen type I and C-terminal telopeptide of collagen type I were significantly lower and higher, respectively, in CD patients than in controls. Although TIMP-1 was found to be up-regulated in CD intestinal mucosa overlying strictures [10], serum TIMP-1 levels were not increased in stricturing CD patients [11]. The enhanced liver fibrosis (ELF) test, a panel comprising three circulating biomarkers, such as hyaluronic acid, TIMP-1 and PIIINP, and predicting liver fibrosis [12], was shown to be a prognostic marker of liver transplantation-free survival in patients with primary sclerosing cholangitis, most of whom had a concomitant diagnosis of IBD [13]. In our hands, ELF test was able to discriminate patients with stricturing CD from those with other phenotypes [unpublished data]. Serum laminin, a component of basement membrane, is increased in CD patients in comparison to control subjects, and correlates with disease activity but not with the stricturing phenotype [14]. Plasma fibronectin, which is a high-molecular weight ECM glycoprotein, is up-regulated in CD patients with stricturing phenotype [15] and it significantly decreases soon after intestinal resection [16]. Conversely, plasma fibronectin did not predict the development of a post-surgical stricture over 1-year follow-up [16]. A number of serum ECM fragments derived from the cleavage by proteases implicated in tissue remodeling in chronic intestinal inflammation [17], have been associated with liver or skin fibrosis [18–20]. Some of these ECM fragments, such as matrix metalloproteinase (MMP)-2/MMP-8-degraded and citrullinated vimentin, MMP-9-degraded collagen type III and MMP-9-degraded collagen type IV, were found to be abnormally expressed in the serum of IBD patients, however, the results were not stratified according to different CD phenotypes [21, 22].

Growth factors, which have been tested in the serum of CD patients as fibrogenic biomarkers, encompass bFGF, human chitinase 3-like 1 (also known as YKL-40), vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF). Serum levels of bFGF, which promotes wound healing by acting on myofibroblast proliferation, are up-regulated in CD patients [23–25], and are associated with the stricturing behavior and with the increased bowel wall thickness [25]. Serum YKL-40, which is produced by activated macrophages and neutrophils and induces collagen secretion by intestinal myofibroblasts, is increased in patients with CD in comparison to controls [26, 27]. As VEGF has been demonstrated to play a pro-fibrogenic and pro-angiogenic role in liver fibrosis [28], it has been also investigated in CD. In particular, VEGF is not only up-regulated in the serum of patients with CD [24, 25], but also correlates with color Doppler signaling, suggesting that VEGF might be considered a biomarker of angiogenesis

in CD [25]. Likewise, the pro-angiogenic/pro-fibrogenic PDGF, known to increase collagen production by myofibroblasts and smooth muscle cells, is enhanced in the serum of IBD patients [29].

A number of anti-microbial antibodies thought to originate from an abnormal immune response directed to the microflora [30], have been detected in the serum of CD patients and encompass anti-*Escherichia coli* outer membrane protein C antibodies (anti-OmpC), anti-*Pseudomonas*-associated sequence I2 antibodies (anti-I2), anti-bacterial flagellin CBir1 antibodies (anti-CBir1), and anti-glycan antibodies, including ASCA, anti-chitobioside carbohydrate IgA antibodies (ACCA), anti-mannobioside carbohydrate IgG antibodies (AMCA), anti-laminaribioside IgG antibodies (ALCA), anti-laminarin carbohydrate antibodies (anti-L), and anti-chitin carbohydrate antibodies (anti-C) [31–34]. These antibodies are quantitatively and qualitatively associated with a much more complicated clinical course of CD, such as stricturing and fistulizing phenotype, either in adult or in pediatric patients [3]. Conversely, none of the anti-glycan antibodies ASCA, AMCA, ACCA, ALCA, anti-L and anti-C has been recently shown to correlate with disease behavior [35]. ASCA are the most extensively studied antibodies in IBD due to their ability to distinguish CD from UC with a sensitivity of 56% and a specificity of 88% [36]. In addition, CD patients positive for ASCA evolve towards fibrostenosing or penetrating behavior with a sensitivity of 70% and a specificity of 48%, and more often have an ileal or ileo-colonic involvement with a subsequent higher need for intestinal resection [36, 37]. CD patients with serological positivity for ASCA frequently have perianal disease and an early disease onset [37]. As far as the other anti-glycan antibodies are concerned, ACCA are those more often associated with a stricturing or penetrating behavior with lower sensitivity (43%) but higher specificity (75%) than ASCA [36]. Additionally, ASCA, AMCA and anti-L positivity correlates with an increased rate of early occurrence of complications and surgery [38], whereas ASCA, AMCA, ACCA and anti-L positivity predict a more quick evolution into complications or surgery [32]. The risk of developing strictures and/or fistulas is 11-fold higher in CD children with anti-CBir1, anti-OmpC, anti-I2 and ASCA positivity than in seronegative children [39]. A following larger prospective study confirmed these findings on CD children with serological positivity for anti-CBir1, anti-OmpC and ASCA [40]. An Irish study reported a significant association of serum anti-CBir1 positivity with both a complicated disease behavior and ileal location, but not with high need for intestinal resection [41]. ASCA IgG have been shown to be the only antibodies significantly associated with a stricturing/penetrating phenotype in a multivariate analysis in a population-based cohort of CD patients [42]. Recently, a meta-analysis on 11 studies and based on four antibodies-ASCA, anti-OmpC, anti-I2 and antiCBir1, showed that ASCA are the antibodies with the highest sensitivity and anti-OmpC are those with the highest specificity for complications and surgery [43]. The use of at least two anti-microbial antibodies rather than any single one predicted more effectively CD progression towards disabling disease [43]. All these anti-microbial antibodies are suitable for predicting CD complications, but not for differentiating stricturing phenotype from other behaviors. New prospective studies are necessary to establish whether circulating antibodies, alone or together with other biomarkers, are able to predict the clinical course and stricture development in IBD.

12.5 Other Factors

Fibroblast activation protein (FAP), which is a glycoprotein expressed by activated fibroblasts during tissue remodeling, is up-regulated in idiopathic pulmonary fibrosis and liver cirrhosis [44, 45]. Serum FAP has been suggested as an index of liver fibrosis [46], but there are no results on serum FAP as a biomarker of intestinal fibrosis in IBD patients. Likewise, FAP is enhanced in the submucosa and in the muscle layer of CD strictures, whereas the profibrogenic cytokines transforming growth factor (TGF)-β1 and TNF-α increases FAP expression on intestinal myofibroblasts isolated from stricturing CD [47]. The blockade of FAP induces an *ex vivo* reduction of type I collagen and TIMP-1 expression in strictured ileum of CD patients [48].

12.6 Conclusions

Advances in understanding the mechanisms underlying intestinal fibrosis have occurred over the last years. Nevertheless, there is not sufficient indication to support the diagnostic and prognostic power of any fibrogenic biomarker in clinical practice. This is partly due to several limitations, including (1) the long-lasting progression of intestinal fibrosis, that requires studies of long duration and recruiting large patients' cohorts, (2) the frequent overlap of clinical phenotypes along the natural history [49], and (3) the impact of concomitant immunomodulatory drugs on serum biomarker levels. Additionally, the complexity of the pathophysiological mechanisms underlying the fibrogenic process in the gut suggests that a panel of biomarkers would be more accurate than a single factor, as it happens in liver fibrosis for the ELF panel [12]. Serum biomarkers of intestinal fibrosis have been used so far in a phase 1 open label trial on CD patients with inflammatory behavior undergoing an oral treatment with GED-0301, a Smad7 antisense oligonucleotide, whose action restores the anti-inflammatory TGF-β signaling [50]. As TGF-β is also implicated in the fibrogenic process in CD [51], in order to rule out a hypothetical pro-fibrogenic effect of GED-0301, serum bFGF and YKL-40 were measured at baseline and after 6 months [50]. No significant change was identified for both the biomarkers, this in keeping with the absence of bowel thickness modifications evaluated through ultrasonography [50].

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Chapter 13
Imaging in Intestinal Fibrosis. What Is State of the Art?

Jordi Rimola

Abstract Over the past decade, there has been increasing evidence that cross-sectional imaging may be helpful in the evaluation and management of Crohn's disease patients. Multiple studies have analyzed the potentiality of these techniques of detecting bowel wall fibrosis deposition in areas of stricturing disease, even in the setting of superimposed inflammation. Such knowledge may be incorporated in the appropriate medical, endoscopical and surgical algorithm management of stricturing Crohn's disease.

This chapter will review the different imaging modalities for assessing the bowel, published evidence supporting the use of these techniques in Crohn's disease patients, potential roles in clinical practice, and likely challenges and obstacles to future use in clinical practice and in research studies.

Keywords MR enterography · CT enterography · Bowel ultrasound · Elastography · Diffusion weighted imaging · Crohn's disease

13.1 Introduction

Patients with Crohn's disease (CD) often develop intestinal stenosis. Stenosis due to acute inflammation is potentially reversible with medical treatment. However, long-term stenosis due to intestinal fibrosis that accumulates from wound-healing mechanisms in response to transmural injury secondary to segmental bowel inflammation can currently only be treated with surgery. The proportion of patients who develop long-term stenosis increases with the time from the onset of disease, and intestinal stenosis is the main reason for surgery in CD patients [1]. Thus, the accurate determination of the extent of fibrosis accumulation in the bowel is key for the management of CD patients. However, differentiating between inflammation and fibrosis as the causes of stenosis is complex.

J. Rimola
IBD Unit, Radiology Department, Hospital Clínic de Barcelona, University of Barcelona, Barcelona, Catalonia, Spain
e-mail: jrimola@clinic.cat

F. Rieder (ed.), *Fibrostenotic Inflammatory Bowel Disease*,
https://doi.org/10.1007/978-3-319-90578-5_13

Histopathologic analysis of endoscopic biopsy specimens is unreliable for determining the amount of fibrosis in the intestinal wall because they are not transmural and fibrosis is unevenly distributed in stenotic segments and samples are often not representative.

Not only can cross-sectional imaging modalities identify strictures in both the small and large bowel, they can also detect signs of inflammation and fibrosis. Thus, given the transmural nature of the disease, cross-sectional imaging may enable a more objective assessment of bowel injury [2, 3]. In recent years, novel techniques linked to cross-sectional imaging modalities have been used in research and incorporated into radiologists' daily practice with the aim of better characterizing stenosis through quantifying the degree of fibrosis in the bowel.

13.2 Challenges to Assessing Fibrosis by Imaging Techniques

Cross-sectional imaging is highly accurate in detecting inflammatory lesions. One key feature indicating the presence of active disease is mucosal enhancement on a thickened bowel segment. When evidence of mucosal enhancement or hypervascularity is lacking on computed tomography (CT), ultrasound (US), or magnetic resonance imaging (MRI) of the bowel, fibrosis is often assumed. However, fibrosis is closely linked to inflammation, and both components are frequently superimposed in stenotic segments; therefore, standard imaging modalities might be unable to differentiate between them [4–6].

This chapter discusses the potential and the limitations of cross-sectional imaging techniques for assessing bowel fibrosis in patients with CD.

13.3 Bowel Ultrasound

Classically, a stratified bowel echo pattern (identification of different bowel wall layers) in a stenotic segment on bowel ultrasound was associated with collagen deposition, but this association has not been validated [7]. Furthermore, this approach does not allow the degree of fibrosis to be quantified, so additional analyses are required.

Adding contrast-enhanced US has limited value for assessing fibrosis. In a single-center study correlating US findings with histopathologic findings in which stenosis was classified as predominantly inflammatory or predominantly fibrotic, the percentage of bowel enhancement together with Doppler US and presence of a penetrating complication was associated with stenosis with a predominantly inflammatory component but not with stenosis with a predominantly fibrotic component. However, the only US finding associated with fibrosis was a low Doppler signal [8].

Strain elastography can add information to gray-scale US by assessing tissue elasticity. This noninvasive imaging modality assesses tissue mechanical properties

and stiffness by measuring strain (i.e., the degree of compression of a material in response to a force applied to a fixed area). Hard materials or tissues (e.g., fibrosis) exhibit low strain in response to a fixed stress and are commonly described as stiffer. The deposition of extracellular fibrotic matrix together with muscle hypertrophy in the bowel wall contributes to changes in the mechanical properties of fibrostenotic intestinal damage. Table 13.1 summarizes the different studies evaluating US elastography in animal models and in humans.

Initial studies [9] provided evidence that elastography was able to measure the 'hardness' or 'softness' of a tissue in a trinitrobenzenesulfonic acid model of intestinal injury in rats. Bowel segments were stratified in different degrees between early phase inflammation and late phase fibrosis. Using a two-dimensional speckle tracking technique to quantify tissue elasticity, the strain assessment was able to differentiate segments with an inflammatory component from those with a fibrotic component. The same study also evaluated 7 human subjects with CD, finding significant differences in stiffness between stenotic bowel segments and adjacent normal small bowel in (−0.87 vs. −1.99 Kpa; p = 0.0008); moreover, this measurement

Table 13.1 Main studies evaluating US elastography of the bowel as surrogate marker of fibrosis using histopathology as reference standard

Author/publication	Elastography modality	Population	Relevant data
Stidham, Gastroenterology 2011; 141: 819–826	Two-dimensional Speckle tracking technique	Lewis rats after TNBS-induced colitis Human pilot study (n = 7)	Rats: distinguish inflamed from fibrotic inflammatory tissue (2.07 vs. 1.10, p = 0.037) Humans: differences between stenotic bowel segments (−0.87 ± 0.22) vs. normal (−1.99 ± 0.53) (P = 0.0008); correlated with the presence of fibrosis (r = 0.81, p = 0.008)
Dillman, Radiology 2013	Real-time elastography (ARFI)	Lewis rats after TNBS-induced colitis (n = 13)	Distinguish fibrotic and inflammatory intestinal damage with an area under the ROC of 0.971, and a PPV and NPV of 95.0% and 92.9%, respectively
Dillman, Ultrasound 2014	Real-time elastography (ARFI)	Lewis rats after TNBS-induced colitis (n = 17)	Area under receiver operating characteristic curve of 0.91 in distinguishing between bowel segments with low and high fibrosis scores
Baumgart, Radiology 2015	Shear-wave real time elastography	Humans (n = 10)	Good agreement between in vivo real-time elastography and ex vivo mechanical induced elastography
Fraquelli, IBD 2015	Shear-wave real time elastography	Humans (n = 23)	Values for mild-moderate fibrosis overlapped with those obtained in non-stenotic inflammatory segments in a control group

TNBS trinitrobenzenesulfonic acid, *ARFI* acoustic radiation force impulse

correlated well with ex vivo elastometry ($r = -0.81$). However, an important limitation of these initial studies was that the bowel elastography technique was not performed in real time and required extensive image post-processing.

Nowadays, US elastography images of the bowel are acquired in real time using dynamic strain imaging techniques or rapid attenuating shear waves (Fig. 13.1). When focused ultrasound beams from the probe displace tissue posteriorly, the restorative force of the tissue propagates laterally, generating shear waves. Software processes the signals to show the different degrees of strain in a color scale, and the color-coded information about strain is displayed superimposed on the conventional B-mode US image [10]. Real-time bowel elastography measurements acquired in vivo were similar to the measurements obtained using direct mechanical tensiometry in ex vivo specimens [11].

Siemens' acoustic radiation force impulse system allows the stiffness in a specific region of bowel wall to be measured and also allows the absolute value of stiffness to be determined. In a trinitrobenzenesulfonic acid rat model, Dillman et al. [12] found this system distinguished between fibrotic and inflammatory intestinal damage with an area under the receiver operating characteristic curve of 0.971, with a 95.0% positive predictive value and a 92.9% negative predictive value. Interestingly, Baumgart et al. [11] applied this technique before, during, and after surgery in 10 CD patients undergoing intestinal resection. They found that elastography measurements were associated with the degree of muscularis thickness ($p = 0.006$), trichrome stain score (4 vs. 0; $p < 0.001$), and western blot quantification of collagen content (high vs. low) (2.01 vs. 0.87; $p = 0.009$).

However, there are still concerns about the contribution of inflammation to the overall shear wave elastography measurement on the human bowel. In CD, the inflammatory component is usually superimposed on background fibrosis. Shear wave speed is not significantly different between bowel segments with high and low inflammation scores [11, 13]. Although Baumgart et al. [11] reported a modest correlation between shear wave elastography and histologic grade of fibrosis ($r = 0.60$, $p = 0.01$), they found no significant correlation with inflammation.

Nevertheless, some studies obtained significantly greater mean shear wave velocity on segments with high fibrosis score than on segments with low fibrosis score in ex vivo bowel specimens [13]. However, these findings are in conflict with those reported more recently by Fraquelli et al. [14] in a study of 23 human subjects, where the strain ratio (strain of stenotic segment normalized by the strain of mesenteric fat within subjects) determined in vivo on the terminal ileum was significantly different between mild-moderate fibrosis and severe fibrosis, but where values for mild-moderate fibrosis overlapped with those obtained in non-stenotic inflammatory segments in a control group.

Despite the promising results published recently, further evidence is needed before US elastography can be incorporated into routine clinical care for patients with CD. Unresolved issues include establishing reproducibility across vendors, defining the dynamic range, and determining the technique's ability to quantify intermediate grades of bowel fibrosis that could identify the progression or improvement of fibrosis and ultimately predict the natural history and clinical outcomes of CD.

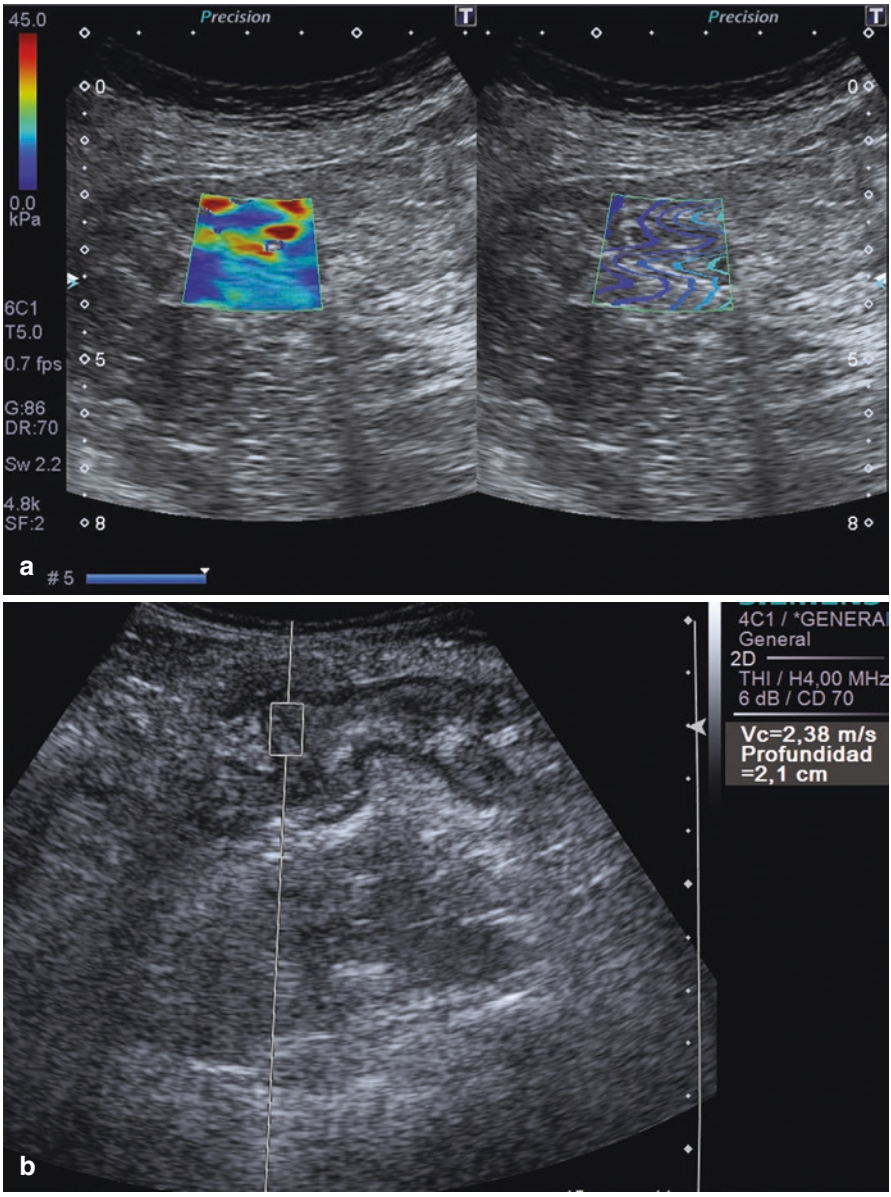

Fig. 13.1 Two examples of real time ultrasound elastography. (**a**) Corresponds to an image of stenotic bowel wall in a longstanding CD patient. The color scale (from red—highest KPa to blue—lowest KPa) shows the distribution of the elasticity within the region (box) of interest overlaid on grayscale images. (**b**) Corresponds to a 31-year old male patient with CD with stenotic segment on the terminal ileum evaluated by Acoustic Radiation Force Image (ARFI). The ARFI, or push pulse, displaces targeted tissue at a specified depth and estimates the shear wave velocity on the area delineated by the box

13.4 Computed Tomography Enterography

To assess the accuracy of CT enterography in differentiating inflammatory from fibrostenotic lesions, Chiorean et al. [15] correlated CT enterography findings with those of histopathologic evaluation of surgical specimens. Although this study showed that the presence of fibrosis was associated with stenotic lesions, it did not provide any information about how to quantify inflammation and fibrosis in stenotic lesions. Nevertheless, the idea persists that signs of active inflammation on CT enterography predict the presence of inflammation and that, by contraposition, the lack of these signs in a stenotic segment is associated with predominantly fibrotic disease. However, Adler et al. [6] recently demonstrated that this line of argument was fallacious. Comparing preoperative CT enterography findings against histopathology of the surgical specimens showed that although signs of inflammation (e.g., increased mucosal enhancement, perienteric hypervascularity, fat stranding, and bowel wall thickening) correlated with the histological degree of inflammation, the absence of these signs on CT enterography did not predict the presence of fibrosis.

Furthermore, the risk of cumulative radiation exposure associated with CT enterography, especially in young patients, makes the development of a reliable diagnostic technique that does not use ionizing radiation a necessity.

13.5 Magnetic Resonance Imaging

T2-weighted MRI has advantages over other imaging modalities for reflecting edema on the bowel wall. Some studies have indicated that low T2 signal on the bowel wall is associated with fibrosis [16] and with the amount of fibrotic matrix and fibroblasts in the submucosal and muscularis propria layers. However, the usual superposition of active inflammation on fibrotic tissue means that fibrosis can be masked on imaging.

MRI can potentially provide crucial information for the detection of fibrosis in CD lesions. However, the characteristics of fibrosis on MRI have yet to be defined, as initial studies have reported conflicting data. In 2009, Punwani et al. [16] found that only stratification of mural signal enhancement following gadolinium administration was associated with fibrosis, whereas Zappa et al. [5] found that mural thickness and edema were associated with fibrosis.

13.5.1 Perfusion MRI as Surrogate Marker of Fibrosis

Some data indicate that bowel segments with some degree of fibrotic tissue have a layered pattern of enhancement after gadolinium injection (mucosal enhancement with no or mild enhancement of the submucosa), whereas segments without fibrosis

show homogeneous enhancement across the bowel wall [17]. These observations suggest that the representation of tissue perfusion on imaging may be affected by the presence of a dense and compact fibrotic matrix, and this idea is also supported by the identification of differences in the slope of initial enhancement between fibrotic and non-fibrotic segments ($p = 0.02$) [17]. Rimola et al. [4] concluded that dynamic assessment of gadolinium contrast uptake is more accurate than anatomic assessments; early saturation with contrast material is seen in predominantly inflammatory lesions, and delayed progressive enhancement of deep intestinal layers over a 7-min observation period separates low and high fibrosis, independent of the degree of inflammation.

13.5.2 Functional MR Imaging Techniques

Regulatory agencies have recently recommended the suspension of marketing authorizations of linear gadolinium contrast agents due to evidence that repeated use leads to small amounts of gadolinium being deposited in the brain. Avoiding gadolinium would make MRI more acceptable and less expensive, so alternative sequences such as diffusion-weighted imaging (DWI) and magnetization transfer MRI (MT-MRI) are being researched.

Two small studies comparing DWI with histology have shown this technique might be able to detect fibrosis [17, 18]. DWI reflects the Brownian motion of water molecules in biologic tissues, which is restricted by cells (inflammation) and collagen (fibrosis); restricted Brownian motion can be quantified by means of apparent diffusion coefficient (ADC) maps. Significant differences in ADC values were identified between different degrees of fibrosis on the bowel ($p = 0.023$), as well as between normal and stenotic segments and between normal and inflamed non-stenotic and stenotic segments ($p = 0.0052$). However, since ADC is also a biomarker associated with inflammation in CD and inflammation and fibrosis are often superimposed in stenotic segments, it remains difficult to determine the contribution of each component to the overall ADC value.

More recently, MT-MRI, a promising MRI technique has shown potential in assessing the collagen content of the bowel wall based on the use of the inherent tissue biophysical contrast. MT-MRI detects the exchange of protons (magnetization) between fixed macromolecules and surrounding free water within a tissue. In an animal model of trinitrobenzenesulfonic acid-induced colitis, MT-MRI quantified fibrosis with a 92% positive predictive value and an 83% negative predictive value, and correlated strongly with collagen quantification ($r = 0.72$) [19]. MT-MRI is feasible in humans, yielding sufficient image quality, and may help identify fibrotic scarring in patients with CD [20]. Again, further prospective studies are needed to validate this MRI modality in CD patients and to investigate whether fibrotic strictures can still be identified and characterized when active inflammation is superimposed. Figure 13.2 shows an example of different imaging modalities for assessing fibrosis deposition on the bowel.

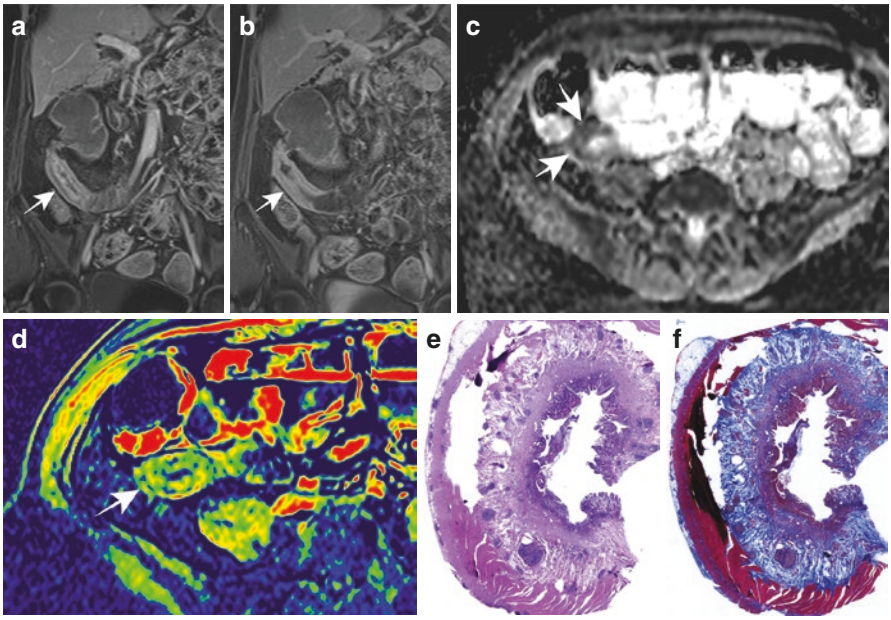

Fig. 13.2 Example of a surgically resected bowel segment with overlap moderate-to-severe fibrotic deposition and inflammation evaluated by Magnetic Resonance Enterography (MRE). MRE T1 coronal images with fat saturation show a stenotic neo-terminal ileum with mucosal enhancement at T1 acquired 70 s after gadolinium injection (arrow in (**a**)) that progresses to diffuse enhancement of the deep layers of the bowel after 7 min of injection (arrow in (**b**)). The irregular mucosal surface of this segment corresponds to mucosal ulcerations. The ADC map on axial plane of the same segments shows a marked hyposignal intensity (arrows in (**c**)) associated with high fibrosis and/or inflammation. MTR map shows moderate magnetization transfer in the terminal ileal wall (arrow in (**d**)) similar to that is seen in the psoas muscle. Microscopic examination ((**e**) hematoxylin & eosin—H&E; and (**f**) Masson's trichrome stain) shows the overlap of fibrosis and inflammation. Fibrosis is enhanced in blue in the Masson's thrichome stain, and the inflammation and ulcerations on mucosa surface are better seen in the H&E stain

13.5.3 Hybrid Imaging Techniques

In recent years, hybrid imaging combining MRI and positron emission tomography (PET) has been introduced for clinical use. The main advantage of PET/MRI over MRI alone is its ability to combine the metabolic information from fludeoxyglucose (FDG) uptake detected by PET with the information obtained by MRI. Catalano et al. [21] compared different PET/MRI descriptors including SUV_{max}, ADC, signal intensity on T2, and combinations of these variables for discriminating between predominantly fibrosis and predominantly active inflammation; they found that the combination of ADC and SUV_{max} best discriminated between the two classifications, with a cutoff of 3000 associated to fibrosis with an overall accuracy of 70%.

Again, this study's dichotomic approach differentiating between predominantly inflammation or predominantly fibrosis limits its applicability, because inflammation and fibrosis usually coexist to varying degrees. Thus, further studies focusing on predicting the presence and the degree of fibrosis are necessary.

13.6 Conclusion

Although the evidence on the utility of these imaging modalities is still weak, in the near future they may be able to provide critical information for planning the best management approach. Further studies, in particular in ultrasound elastography and MT-MRI, are required to determine the reliability and accuracy of these techniques for detecting and grading fibrosis in vivo in subjects with CD.

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Chapter 14
The Future of Intestinal Fibrosis Imaging

Ryan W. Stidham and Mahmoud Al-Hawary

Abstract Though the capabilities of available imaging technologies to assess intestinal damage have substantially improved, emerging alternative non-invasive imaging methods may offer advancements in detecting and quantifying intestinal fibrosis in the inflammatory bowel diseases. Both the immediate clinical need to measure fibrosis for therapeutic decision-making and the near-future need for tools to assess pipeline anti-fibrotic medications highlight the demand for better non-invasive biomarkers of fibrosis in Crohn's disease. Developing imaging platforms assessing tissue mechanical properties, perfusion characteristics, and structural protein content provide new perspectives and possibilities for approaching intestinal fibrosis quantitation. In this chapter, we will discuss existing, emerging, and experimental imaging methods using ultrasound elastography, novel MRI sequences, and photoacoustic imaging to measure fibrosis in Crohn's disease.

Keywords Intestinal fibrosis · Fibrosis · Crohn's disease · Ultrasound elastography Stiffness imaging · Shear wave · Photoacoustic imaging · Magnetization transfer MRI · Fibrostenotic disease · Stricturing disease

Intestinal fibrosis is recognized as a dominating pathologic contributor to the most feared complications of inflammatory bowel disease, including stricturing and penetrating phenotypes. Very often therapeutic failure, at least in Crohn's disease, is more a function of the degree of accumulated irreversible bowel wall fibrosis with its resultant complications and less commonly an inability to control disease-related inflammation. Complicating management further, many patients are unaware of

R. W. Stidham (✉)
Division of Gastroenterology and Hepatology, Department of Internal Medicine, University of Michigan, Ann Arbor, MI, USA
e-mail: ryanstid@med.umich.edu

M. Al-Hawary
Department of Radiology, University of Michigan, Ann Arbor, MI, USA
e-mail: alhawary@med.umich.edu

F. Rieder (ed.), *Fibrostenotic Inflammatory Bowel Disease*,
https://doi.org/10.1007/978-3-319-90578-5_14

underlying intestinal disease until significant intestinal damage has already occurred. In 2018, the available therapeutic armentarium, as well as candidate treatments undergoing formal clinical trials, address various mechanisms driving inflammation. As a result, the bulk of diagnostic, prognostic, and measurement attention continues to focus on inflammatory aspects of disease. Given the majority of so-called 'fibrotic' changes occur in the deep submucosa and muscularis propria, imaging is better suited than endoluminal evaluation to investigate these contributors to bowel injury. In the previous chapter, Jordi Rimola outlined how current technologies can be used or adapted for the detection and quantification of intestinal fibrosis. Perhaps more importantly, he provided his guidance on best-practices to discriminate between fibrotic and inflammatory contributions of chronic bowel damage. Moving forward, fundamentally new, purpose-built technologies are needed to improve fibrosis detection and measurement. In this chapter, we will explore emerging methods for measuring fibrosis in IBD.

Because of the inability of ileocolonoscopy with biopsy to obtain full thickness core samples of the bowel wall to interrogate transmural injury, cross-sectional imaging methods, including ultrasound (US), computed tomography enterography (CTE), and magnetic resonance enterography (MRE), have become essential companions to endoscopy [1, 2]. Excellent image-based disease activity assessments, are strongly correlated with endoscopic activity, but principally reflect findings related to tissue inflammation [3]. The concept that high or low degrees of bowel wall contrast enhancement features are characteristic of predominantly inflammatory or fibrotic intestinal disease are being called into question. Adler and colleagues, along with other groups, showed that that the radiologist global impression of whether a stricture was "active" or "inactive" was not associated with the presence or degree of fibrosis [4, 5]. The Lémann index represents an international effort to more comprehensively describe total bowel damage accounting for length of diseased bowel, stricturing, and penetrating disease using imaging [6]. However, obvious strictures represent late stage macroscopic events, when the window for medical intervention is nearing closure. Further, claims that bowel dilation is associated with the degree of intestinal fibrosis remain controversial [7]. One could make a pragmatic argument that treating to an endpoint of objective resolution of inflammation to halt the progression of existing fibrosis is sufficient. However, emerging data raises the possibility that while inflammation may initiate fibrogenesis, for reasons poorly understood fibrosis can auto-propagate even after inflammation is effectively treated [8]. Taken together, these points capture the need to objectively measure intestinal fibrosis as an independent marker of the disease status. Given our inability to safely and routinely obtain full thickness intestinal samples, several methods are being explored to provide a "virtual biopsy," relying on changes in tissue mechanics, perfusion, and macromolecular composition as surrogate biomarkers of fibrosis.

14.1 Using Tissue Mechanical Properties to Measure Intestinal Fibrosis

Differences in the mechanical properties between what is histologically classified as predominately fibrotic vs. inflamed intestinal tissue offers a method for classifying and quantifying these two phenotypes [9]. Elasticity imaging has emerged as a useful method to non-invasively detect and quantify hepatic fibrosis, potentially distinguish benign from malignant tissue, and may have potential for assessing intestinal fibrosis in inflammatory bowel disease [10, 11]. Stenotic intestine has been observed to be stiffer, harder, and less flexible than predominantly inflamed tissue in both animal models and ex-vivo human samples of Crohn's disease [12]. The increased stiffness observed between fibrotic and inflammatory tissue results from the accumulation and cross-linking of extracellular macromolecules including collagen, smooth muscle hypertrophy, and architectural distortion which limits the distensibility of the intestine [13]. Ultrasound-based elastography technologies may have the potential for measuring fibrosis and predicting fibrosis-related clinical outcomes through tissue stiffness assessments.

Though often considered synonyms, understanding the differences between mechanical property descriptors is relevant in appreciating the capabilities of each method of stiffness measurement. Elasticity describes a material's resistance to deformation due to an applied force. There are several related and dependent descriptors of elasticity including the elastic modulus (Young's modulus) and the shear modulus. The elastic modulus is the ratio of stress to strain for tissue. Stress is the deformation force applied perpendicular to the object, divided by the area over which the force is applied. Strain is the ratio of new length divided by the original length along the same axis as the applied force. Typically, the elastic modulus is measuring deformation due to compressive stress in medical applications. The shear modulus is a related measure of material elasticity, describing the resistance to deformation due to forces parallel to an object. Elasticity is measured in pascals (Newtons/area) as strain is a unit-less measurement. Tissue 'stiffness' is a measure of elasticity, but is additionally dependent on object shape, volume, and material distribution characteristics.

US elastography has revolutionized the assessment of hepatic fibrosis and cirrhosis. Transabdominal transient elastography (TE) of the liver offers a non-invasive, no risk, convenient and low cost method for serial assessment of hepatic fibrosis [14, 15]. Several commercial US-elastography devices are now available, with the majority utilizing a 1-dimensional (1D) shear wave for stiffness assessment. The ultrasound transducer is placed on the skin and a low frequency vibration pulse is directed towards the liver. The vibration wave induces liver tissue oscillation where the reflective wave velocity is proportional to liver stiffness. Using a half-duplex pulse-echo (send-receive) sequence, return vibration velocity is repeatedly sampled

and converted into an elastic modulus ($E = 3\rho v^2$), where ρ is a tissue density constant and v is shear velocity. Return wave velocity increases with increasing tissue stiffness. Beyond the convenience and safety of this method, 1D-TE has an additional benefit of measuring a relatively large volume of hepatic parenchyma, 1 × 4 cm within the scan field, addressing sampling error limitations that hinder histologic assessment [16].

Dozens of studies have demonstrated an excellent correlation between non-invasive liver stiffness and traditional histologic fibrosis assessment by liver biopsy [10, 17, 18]. Despite its success in grading fibrosis in liver and other solid organs, 1D-TE is unreliable when applied to the intestine. One-dimensional shear wave velocity measurement methods, such as those used to evaluate liver fibrosis, are subject to boundary effect [19]. While 1D-TE shear waves pass reliably through solids of differing stiffness, the fluid and air within the bowel lumen result in variability in the speed and vector of the shear wave. Boundary effect therefore has limited the application of the widely available 1D-TE methods used in liver disease to bowel wall assessments.

14.1.1 Ultrasound Stiffness Imaging

Ultrasound has been studied for decades as a method for non-invasive tissue stiffness assessment. The real-time dynamic image capture provided by US offers the capability to assess the deformation of intestine from an applied force [20]. Strain measurement using two-dimensional speckle tracking of B-mode ultrasound data has been examined in animal models and small sets of humans with CD [12]. Speckle tracking quantifies the relative movements of groups of B-mode ultrasound pixel clusters (kernels) as pressure is applied using the transducer to measure accumulated tissue strain in the horizontal and vertical spatial planes [21, 22]. In the rat-TNBS model of CD, strain measurement by ultrasound stiffness imaging (USI) distinguished predominately fibrotic from inflammatory intestinal damage (2.07 vs. 1.10, $p = 0.037$, Fig. 14.1). In a small group of human subjects with CD (N = 7), diseased fibrostenotic bowel demonstrated a mean normalized strain of 0.87 ± 0.22, compared to 1.99 ± 0.53 in adjacent ultrasonographically normal bowel ($p = 0.0008$). Direct elastic modulus measurements of fibrostenotic (4.14 ± 1.88 kPa) and normal (0.96 ± 0.25 kPa) full-thickness intestinal samples found an excellent correlation with strain imaging assessment, $r = 0.81$. This pilot work established the feasibility of USI as a measure of intestinal fibrosis in humans.

The development of pressure sensitive ultrasound transducers has improved the standardization of applied force measurement and overall strain-imaging performance. Baumgart and colleagues used a commercial real-time ultrasound elastography platform to measure the elastic modulus of intestine and found tissue strain to be associated with tissue collagen content ($p < 0.001$), muscularis thickness ($p = 0.012$), and intestinal thickness ($p = 0.011$); all surrogate components of

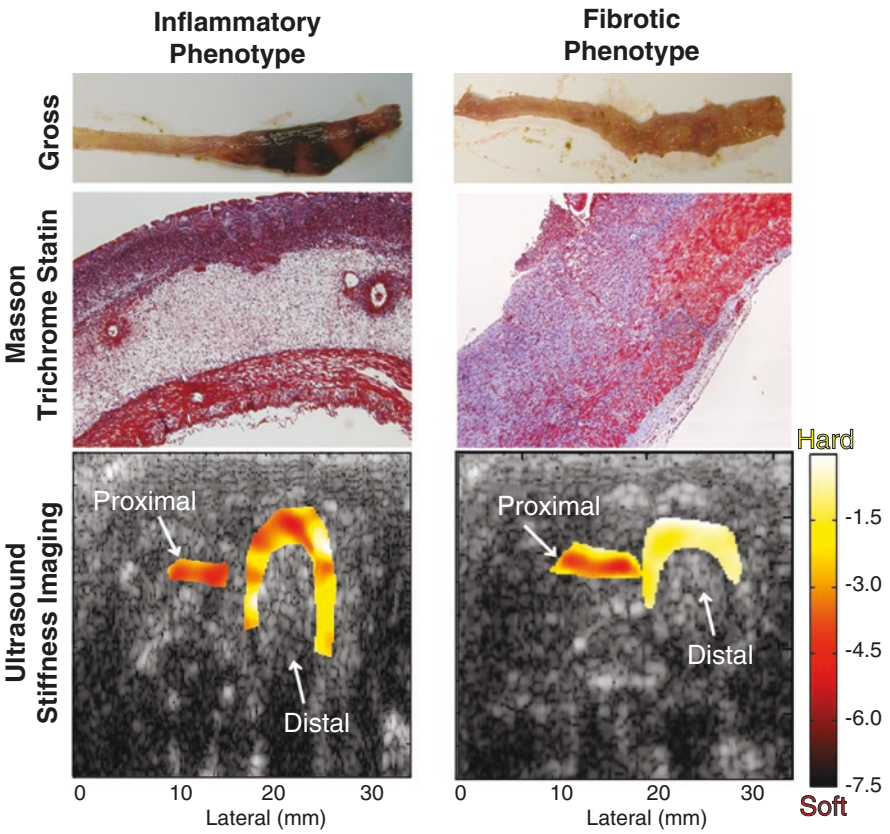

Fig. 14.1 Demonstration of ultrasound stiffness imaging using strain imaging. Here, stress-strain quantitation is used for assessment of tissue stiffness. The rat TNBS model of intestinal damage was used to generate predominantly inflammatory and fibrotic phenotypes of intestinal damage. Gross ex-vivo intestine sections and representative tissue stained with Masson's trichrome demonstrate inflammatory and fibrotic changes. Representative stiffness imaging reveals lower strain (stiffer or harder tissue) within the bowel wall of fibrotic compared to inflamed intestine (adapted from Stidham et al. [12])

intestinal fibrosis [23]. Several other groups have examined strain elastography in CD [24, 25]. Notably, Fraquelli et al. studied 23 CD subjects and showed tissue strain to be strongly correlated with the severity of histologic fibrosis scores (AUROC 0.917, 95% CI 0.788, 1.000), though it was unable to separate mild and moderate histologic fibrosis from predominantly inflammatory disease [26]. These studies offer encouraging evidence of non-invasive strain assessment that may have the capacity to detect and quantify intestinal fibrosis in CD. Remaining opportunities to improve the clinical utility of USI include more accuracy of region of interest selection, reducing the technical expertise needed for bowel ultrasound, and generating data demonstrating prognostic performance for predicting treatment response in CD.

14.1.2 Shear Wave Elastography

Appreciation of the potential of elastography for the assessment of intestinal fibrosis resulted in exploration of other elastographic methods. Shear wave elastography (SWE) has undergone rapid maturation as a clinical tool for directed tissue elastography with increasing study and application in CD. Similar to TE platforms, SWE relies on a vibration pulse, by way of acoustic radiation force impulse to generate shear waves within the tissue. SWE differs in that it measures the velocity of the shear waves propagating orthogonal to the tissue of interest with full duplex send-receive, or continuous listening (Fig. 14.2). SWE reduces the impact of

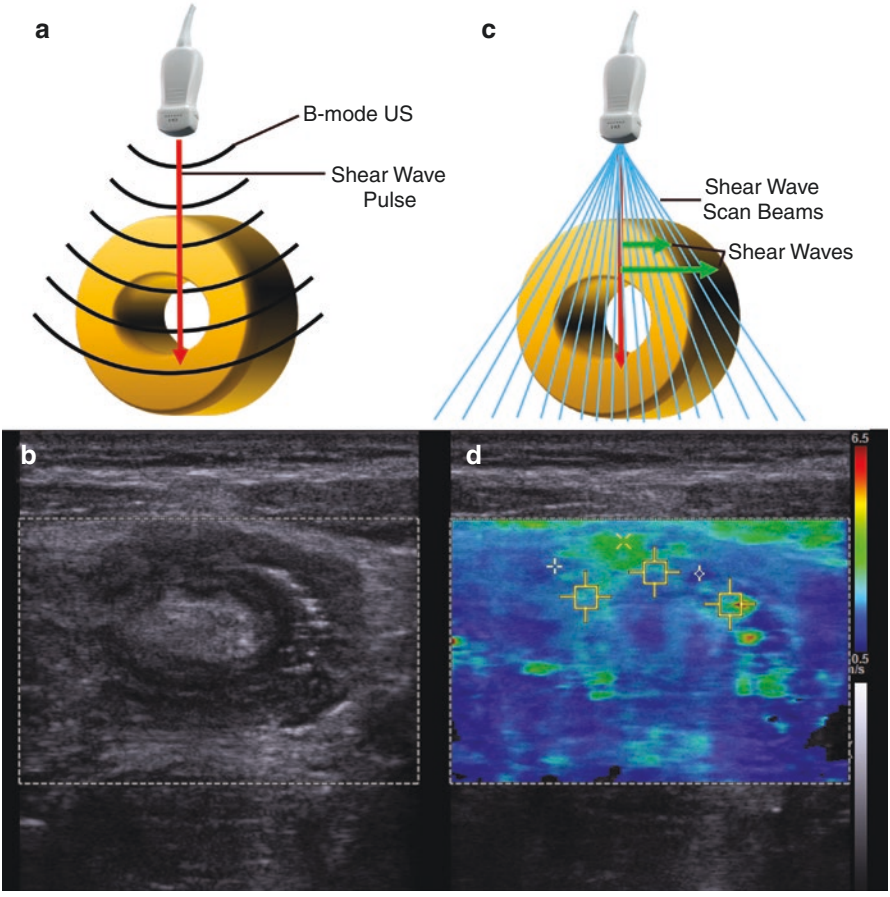

Fig. 14.2 Shear wave imaging for assessing Crohn's disease. Several commercial platforms are now available for shear wave imaging. (**a**) Traditional B-mode ultrasound imaging is performed, with the addition of a shear wave pulse directed in the scan field; (**b**) conventional small bowel imaging is performed. (**c**) Shear waves are generated orthogonal to the vibration pulse (*green arrows*) and their velocity is calculated by the time to passage through a second scan beam field. (**d**) An overlay of shear wave velocity (m/s) is generated with the capability for point measurement within specific regions of interest, here within the bowel wall

boundary effect and geometric acoustic distribution, and improves targeting of bowel wall for elastography.

SWE has demonstrated an ability to distinguish fibrotic and inflammatory intestinal damage in animal models of CD with an AUROC of 0.971, exhibiting a PPV and NPV of 95.0% and 92.9% [27]. Dillman et al. measured shear wave velocity in resected intestine specimens from 17 CD subjects, comparing fibrostenotic full thickness sections to grossly normal intestine from the same individual. Shear wave velocity modestly correlates with the histologic grade of fibrosis ($\rho = 0.60$, $p = 0.01$), but not inflammation ($\rho = 0.24$, $p = 0.36$), in *ex-vivo* human intestinal specimens with an AUROC of 0.91 for distinguishing low from high grade fibrosis [28]. Lu et al. examined the predictive potential of SWE in ileal Crohn's disease, performing US with SWE and contrast enhanced ultrasound (CE-US) in 105 consecutive patients [29]. During the follow up period the 15 subjects undergoing bowel resection for stricturing disease had significantly greater SW velocities (less elastic, more stiff) compared to those avoiding surgery (2.8 ± 0.7 vs. 2.2 ± 0.8 m/s, $p < 0.01$). Interestingly, CEUS peak enhancement values in the most diseased segments were inversely correlated with shear wave velocity, $r = -0.61$, $p = 0.03$, suggesting reduced perfusion and tissue stiffness are related and dependent features. While promising, much more study is needed prior to incorporating elastography into routine clinical care, including establishing reproducibility, dynamic range, ability to quantify not only extremes, but intermediate gradations of fibrosis, and ultimately predict natural history and clinical outcomes in CD.

14.2 Perfusion and Metabolic Methods for Discriminiating Fibrosis and Inflammation in Crohn's Disease

14.2.1 Tissue Perfusion Characteristics for Assessing Intestinal Fibrosis

Methods to measure neovascularization and changes in vascular auto-regulation observed in intestine affected by Crohn's disease have been used as surrogate markers of histologic inflammation [30]. Fibrotic histologic changes are hypothesized to retard, or at least alter, blood flow into the corresponding region of diseased intestine. Potential differential perfusion characteristics between inflammatory and fibrotic tissue may offer an opportunity to improve assessments of deep intestinal damage. Careful quantitation of perfusion dynamics using MR-based methods have demonstrated encouraging data supporting its use as a marker of fibrosis. Diffusion weighted imaging [31] and study of delayed contrast enhancement [32, 33] using MRI are discussed comprehensively in other chapters of this volume.

Tissue perfusion characteristics can also be assessed by ultrasound. Contrast-enhanced ultrasound (CE-US) uses intravenously administered microbubbles

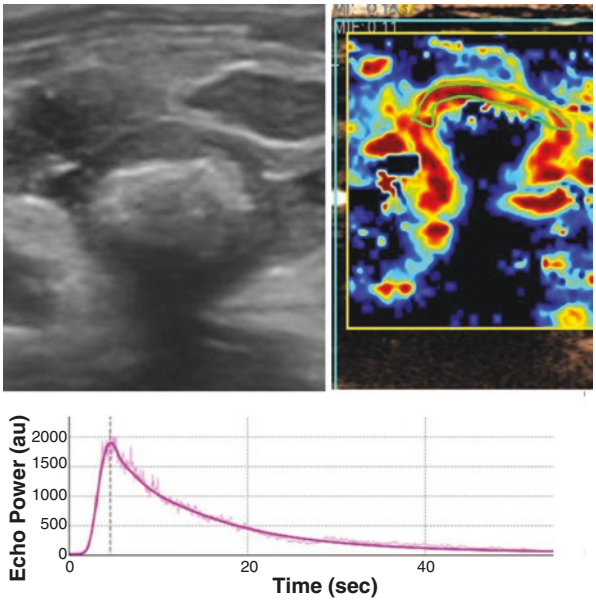

Fig. 14.3 Contrast enhanced ultrasound (CE-US) for Differentiating Inflammation from Fibrosis. Perfusion kinetics, including inflow, outflow, and flow rate, can all be assessed within an area of interest and may be useful for fibrosis detection and differentiation from inflammation. Representative images show typically thickened intestinal wall in a rat-TNBS model of intestinal damage (upper left), with a CE-US overlay provided (upper right) to demonstrate perfusion characteristics. Signal kinetics and be quantified, including peak signal and rate of signal wash out (adapted from Dillman et al. [35])

which can be quantified and standardized using image analysis platforms (Fig. 14.3) [34, 35]. Readouts can provide not only peak contrast values, but also tissue perfusion kinetics; this is a more objective and quantitative method than traditional Doppler US. A 14 patient study of CE-US showed that delayed contrast wash-in ($p = 0.02$) and wash-out ($p = 0.008$) were associated with therapeutic non-response, though no full thickness histology was available to assess bowel wall composition [36]. A 39-subject study where patients were dichotomized as being predominantly fibrotic (required elective surgery for refractory stricturing disease), or inflammatory (no strictures but required steroid or anti-TNF), found those judged to be fibrotic had a slower rate of perfusion compared to the inflammatory group (22.6 vs. 45.3 ml/min/100 ml, $p = 0.003$) [37]. The same group found that the ratio of intestinal tissue perfusion to bowel wall thickness (cutoff of 0.56 ml/min/mm) predicted surgical vs. medically managed disease with an AUROC of 0.92 and sensitivity and specificity 82% and 94%, respectively. Similar results have been shown by other groups [38], substantiating the potential of CE-US as a measure of inflammation and fibrosis. These CE-US studies presented are subject to error stemming from using clinical decisions, outcomes, and unverified standards as surrogates of fibrosis. It is also possible that the findings of imaging stud-

ies influenced clinical decision making. Though longitudinal studies and more histopathology correlates are needed, tissue perfusion characteristics quantified by dynamic contrast-enhanced MRI (DCE-MRI) and CE-US appear to correlate with intestinal fibrosis.

14.2.2 Tissue Metabolic Imaging for Assessing Intestinal Fibrosis

Positron emission tomography (PET), commonplace in the follow-up of hypermetabolic malignant tissues, has been investigated as a method to differentiate predominately inflammatory from fibrostenotic bowel damage. Conventional PET uses fludeoxyglucose (FDG), a labeled glucose analogue, for identification of regions with increased metabolism relative to surrounding tissues with CT (or more recently MR) overlay for anatomic localization. Based on the premise that predominately inflammatory intestine has increased metabolism relative to fibrotic tissue, a supposition which is questioned, PET-CT and PET-MR have been explored in CD. Small pilot studies to date have demonstrated mixed, generally discouraging, results for PET-imaging to distinguish inflammation from fibrosis. Jacene et al. performed FDG PET-CT in 17 CD patients prior to planned bowel resection. They reported greater standardized uptake values (SUL_{max}) in chronically inflamed compared to predominant fibrostenotic disease, though SUL_{max} was unable to classify the degree of fibrosis [39]. Lenze and colleagues prospectively evaluated PET-CT, MR-enteroclysis, and B-mode ultrasound for identifying stricturing disease and predicting therapeutic outcomes [40]. While non-significant trends in their data suggested some ability of all modalities to predict medical response vs. surgical management within 6 months, no single technique was superior. A recent study showed that PET-MR in 19 patients undergoing elective bowel resection was able to predict the histology of resected segments (fibrosis alone vs. a mixed fibro-inflammatory disease), albeit with underwhelming accuracy (sensitivity 0.67, specificity 0.73) [41]. Considering the cost, radiation exposure, and marginal performance, enthusiasm for fibrosis detection using FDG-PET based imaging has declined relative to other modalities.

14.3 Detection of Collagen and Other Structural Proteins in Crohn's Disease

Assessing intestinal mechanical, perfusion, and metabolic characteristics are useful features, but nonetheless are surrogate measures for fibrosis. Ideal biomarkers would directly measure histological or biochemical components of fibrotic intestine. Several emerging techniques in development provide a direct measure of

structural protein content within tissues. These methods designed specifically to differentiate inflammatory from fibrotic tissue damage are under development.

14.3.1 Magnetization Transfer MR-Imaging for Assessing Intestinal Fibrosis

Magnetization transfer MRI (MT-MRI) is an alternative non-invasive endogenous contrast technique that detects fibrosis by measuring the exchange of protons (magnetization spin transfer) between fixed macromolecules and surrounding free water within a tissue [42]. In this way, MT-MRI can detect collagen and other large macromolecules in the extracellular matrix by measuring the MT signal intensity (SI) on two sequences; one sequence obtained without and one with the application of an off-resonance radio-frequency (RF) pulse. These pulse sequences are intended to saturate the SI from spins related to the macromolecule in question which then undergo cross relaxation with the free water molecules causing indirect saturation of the water signal and hence the "magnetization transfer." An MT ratio (MTR) is then calculated as [$MTR = 100 \times (1 - MT_{sat}/MT_0)$], where MT_0 is the measured SI before the RF pulse and MT_{sat} is the SI measured with the off-resonance RF pulse. MT-MRI sequence can be added to conventional MR-enterography protocols and is available on most existing commercial MR scanning platforms. The MT ratio increases with increasing tissue collagen content and can be localized to any region of interest, including the intestinal wall.

Adler and colleagues first showed that MT-MRI was able to quantify intestinal fibrosis in the PG-PS rat model of intestinal damage [43]. MT ratios were significantly correlated with the histologic grade of fibrosis ($\rho = 0.74$) and tissue collagen content ($\rho = 0.72$). MT-MRI exhibited positive and negative predictive values for moderate-severe fibrosis of 92% and 83%, respectively, in the PG-PS model of intestinal fibrosis. Building on this work, Dillman et al. found that the addition of T2-weighted signal intensity (a measure of water content and surrogate measure of inflammation-related edema) to MTR improved discrimination of inflammation and fibrosis in the rat TNBS model of intestinal damage [44]. The T2-weighted SI to the MTR ratio (T2-WSI/MTR) resulted in an AUROC of 0.98 for distinguishing moderate from severe intestinal fibrosis (Fig. 14.4). The value of this work is in distinguishing between grades of intestinal fibrosis with a non-invasive measurement tool. Human pilots of MT-MRI clinical utility are underway. Translating these findings to human study, a single pilot of 31 CD patients showed MT-ratios significantly differed between fibrotic and acutely inflamed intestine (35.3 vs 22.9, $p < 0.0001$) [45]. In line with the limitations of fibrosis research, these human pilots use surrogate endpoints themselves (radiologist definition of fibrotic vs. inflammatory) and give some pause when considering the strength of this evidence for MT-MRI integration into clinical practice. Though more work is needed, these preliminary results, technology feasibility and low incremental cost substantiate continued work in MT-MRI study and development in prospective trials with tissue accrual for pathologic correlation.

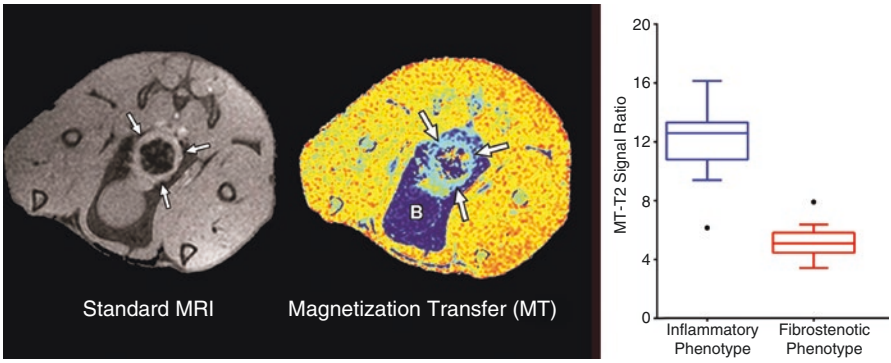

Fig. 14.4 Magnetization transfer (MT) MRI for Quantifying Intestinal Fibrosis. MT-MRI detects heavy macromolecules within a tissue, including collagen. In the TNBS-rat model of intestinal inflammation and fibrosis, a parametric map of calculated MT-ratios shows the bowel (*arrows*) having less MT effect than surrounding muscle, but more than fat and fluid in the bladder. Adjusting for the T2-weighted signal, which quantifies inflammatory disease, T2-WSI-MTR ratios provide very good quantitative discrimination between inflammatory and fibrostenotic phenotypes (adapted from Dillman et al. [44])

14.3.2 Photoacoustic Imaging for Detecting Intestinal Fibrosis

Spectral absorption is a physical property that has been used for material identification and quantitation in material sciences, forensics, and astronomy and may also be applied to tissue fibrosis quantitation. Photoacoustic imaging (PAI) is a non-invasive imaging method for quantifying specific molecular and macromolecular content of a tissue. PAI uses pulsed laser light to penetrate tissues at variable depths. The resulting molecular vibrations produced in the megahertz range can be detected by ultrasound transducers, capturing both macromolecular signatures and producing two- or three-dimensional images [46]. Exploiting differences in collagen, water, and hemoglobin optical absorption spectra, PAI is being explored as a method for fibrosis quantitation in CD [47]. Additionally, PAI has a penetration depth capability of 7 cm at good resolution making this a potentially practical method for non-invasive bowel assessment.

Exploratory animal investigations of PAI were first performed by Lei et al., using the rat-TNBS model of intestinal injury for differentiating inflammation from fibrosis. *Ex-vivo* intestine assessment by PAI demonstrated a 2.9-fold increase in pixel intensity at 1310 nm wavelengths (collagen) within fibro-inflammatory bowel segments relative to acutely inflamed segments, $p < 0.0001$ (Fig. 14.5) [48]. Other groups are exploring the potential of photoacoustics, specifically for inflammation assessment. Knieling et al. examined transabdominal PAI in 108 CD patients with spectral detection tuned to the 700–900 nm for detection of hemoglobin to quantify local intestinal inflammation [49]. They found that signal intensity was correlated with endoscopic scoring (SES-CD) of the intestinal segment studied. Though promising, several technical challenges for PAI clinical development remain, including adjusting for the optical absorption of water (near 1300 nm) and the need for an

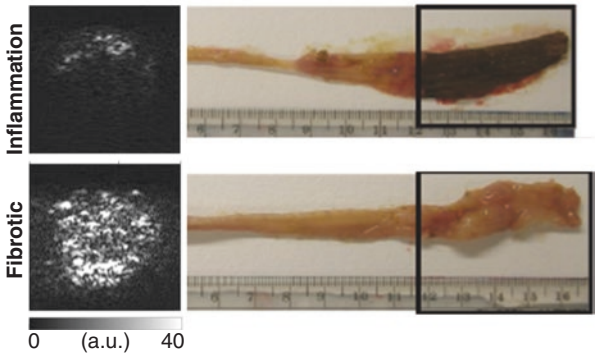

Fig. 14.5 Photoacoustic imaging (PAI) for non-invasive tissue collagen quantitation. Relying on the optical absorption spectra differences between collagen, hemoglobin, water, and fats, PAI may separate inflammation from fibrosis. Shown (left) are gross resected intestine specimens from rats treated with TNBS to induce inflammatory or fibrotic intestinal damage. *In vivo* ultrasound photoacoustic signal from the bowel at 1310 nm (collagen signal) is higher in mixed fibro-inflammatory compared to inflammation alone (adapted from Lei et al. [48])

efficient means of light delivery with tandem ultrasound. Nonetheless, mounting evidence supports the potential for photoacoustics as tool to measure both intestinal inflammation and fibrosis assessment using a non-invasive, radiation-free technique.

14.4 Emerging Technologies with Potential Applications in Fibrosis Imaging

14.4.1 Endoscopic Confocal Microscopy in Crohn's Disease

Endomicroscopy with molecular tagging is exciting imaging method with potential applications in CD. While direct applications in CD fibrosis have yet to be performed, successful molecular tagging of therapeutic and malignant targets in gastrointestinal tissues offers a platform for localization of emerging fibrotic proteins and tissue-based markers. Atreya and colleagues have demonstrated the capability for *in vivo* membrane-bound TNF measurement using florescent antibodies and real-time endoscopic confocal laser microscopy. In a pilot of 25 CD subjects, anti-TNF responders had a mean of 30 TNF+ cells per field compared to 11 TNF+ cells in non-responders ($p = 0.0004$). In a separate pilot study, a fluorescein isothiocyanate (FITC) labeled version of vedolizumab was administered to 5 CD patients refractory to anti-TNF therapy. FITC signal detected by confocal laser microscopy directed to diseased segments identified the 2/5 patients who would go on to experience therapeutic clinical response but was absent in the 3/5 vedolizumab clinical non-responders [50]. Of course, fibrostenotic changes often occur deep to the mucosa and submucosa and

successful imaging of fibrotic features will require deep bowel penetration. Molecular tagging and detection in deep bowel layers has been accomplished in IBD. Apoptosis was assessed in animal models and 14 humans using IV administration of Technetium-99-Annexin-V followed by CT-scintigraphy prior to anti-TNF treatment [51]. Subjects with anti-TNF response exhibited 98.7% bowel uptake of ^{99m}TC-annexin V in affected segments compared to 15.2% in non-responders. Radiolabeling techniques could conceivably be applied to constituents of intestinal fibrosis.

14.4.2 Opportunities for Using Nanoparticles to Detect Intestinal Fibrosis

Emerging nanotechnology is providing additional tools for intestinal assessment. Nanoparticles can be engineered for high-affinity tissue or molecular binding, can be detected by multiple non-ionizing methods, and are stable enough for both intravenous and oral administration [52]. Nanoparticles, once targeted to specific cells (intestinal myofibroblasts, smooth muscle, dendritic cells) or macromolecules (intestinal collagen, fibrin, alpha-smooth muscle actin, myosin) can be manipulated for maximal detection by several imaging modalities with enhanced signal-to-noise ratios. Orally administered nanoparticles have been used as a photoacoustic contrast agent to increase intestinal tissue contrast without ionizing radiation for assessing bowel motility [53]. Opportunities are now ripe for customizing nanoparticle platforms for intestinal imaging applications in IBD.

14.5 Conclusion

Our conceptions of Crohn's disease activity have evolved from non-descript unspecified disease activity assessment, towards contemporary efforts to quantify inflammatory and structural disease. Ultimately, new efforts aim to develop objective biomarkers of fibrosis. Accurate and sufficiently dynamic measures of intestinal fibrosis will have an immediate impact in our understanding of the natural history of CD and an individual's prognosis. Objective measures of fibrosis will improve clinical management by guiding decisions on the necessary intensity of anti-inflammatory therapy needed to prevent irreversible fibrotic complications and choice of medical vs. surgical treatment in the patient presenting with obstructive disease. The bowel imaging techniques presented may develop the basis for evaluating the efficacy of candidate anti-fibrotic therapies in CD, as well as improving our ability to provide patients with an accurate prognosis. Independent measurements of inflammation and fibrosis together will provide a more comprehensive description of intestinal damage for increasingly precise and individualized treatment of Crohn's disease.

Disclosures No potential conflicts of interest relevant to this manuscript are present.

Writing Assistance No writing assistance was provided.

Acknowledgment NIH K23DK101687 (Stidham).

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Chapter 15
Medical Therapy in Stricturing Inflammatory Bowel Diseases

Damien Soudan and Yoram Bouhnik

Abstract Both, Crohn's Disease (CD) and Ulcerative Colitis (UC) may be complicated by the occurrence of strictures. They appear in 50% of patients after 20 years of CD evolution, but are less common in UC. The management of stricturing inflammatory bowel diseases has long been based on surgery and steroid therapy. In recent years and due to the advent of biologics, medical therapy has been increasingly used. Based on their clinical experience, physicians should be able to determine stricture features and patient characteristics to make the best tailored therapeutic decision. Anti-tumor necrosis factor (TNF) antibodies are currently the most effective drugs available in specific cases of stricturing CD.

Keywords Fibrostenoting IBD · Anti-TNF · Infliximab · Adalimumab · Prevention · Cancer · Crohn's disease · Ulcerative colitis

Abbreviations

CD	Crohn's Disease
CDAI	Crohn's Disease Activity Index
CDEIS	Crohn's Disease Endoscopic Index Score
ECM	Extracellular Matrix
HBI	Harvey Bradshaw Index
IBD	Inflammatory Bowel Diseases
OR	Odds Ratio
SES-CD	Simple Endoscopic Score in Crohn's Disease
TNF	Tumor Necrosis Factor
UC	Ulcerative Colitis
UCEIS	Ulcerative colitis endoscopic index of severity

D. Soudan · Y. Bouhnik (✉)
Gastroentérologie, MICI et Assistance Nutritive, PMAD—DHU UNITY—CRI UMR 1149 Inserm—Labex Inflamex, Université Paris Diderot Hôpital Beaujon, APHP, Clichy, France
e-mail: yoram.bouhnik@aphp.com

F. Rieder (ed.), *Fibrostenotic Inflammatory Bowel Disease*,
https://doi.org/10.1007/978-3-319-90578-5_15

15.1 Introduction

Inflammatory bowel diseases (IBD) are chronic relapsing disorders resulting in structural bowel damage over time. Both Crohn's disease (CD) and ulcerative colitis (UC) may be complicated by chronic inflammatory mechanisms triggering excessive extracellular matrix (ECM) production [1, 2]. The accumulation of collagen-rich ECM (fibrosis) in the intestinal wall leads to a narrowing of the gut lumen diameter and results in stenosis up to the point of occlusion [3]. Strictures are more common in CD, with an incidence of about 50% after 20 years of disease evolution [4]. Stricturing UC is less common, with a frequency ranging between 1.5% and 11.2% [5]. A prevalence of colonic strictures of 2.4% has been reported in IBD in a large retrospective study and colonic strictures appeared to be an independent risk factor for adenocarcinoma in the IBD population (OR = 8.42; $CI_{95\%}$ [3.85–16.79]) [6]. In this study, 80% of adenocarcinomas where located in the stricture site, a meticulous pathological assessment of the entire colonic mucosa is therefore essential, especially in stricturing IBD. The management of fibrostricturing IBD has for long been empirically based on surgery or endoscopy, and medical therapy was limited to steroids and bowel rest. Since the advent of biologics, the place of medical therapy has evolved in this clinical scenario.

15.2 Stricturing IBD: A Multifaceted Disease for Clinicians

15.2.1 Stricturing IBD

In clinical practice, the term "stricturing IBD" includes various diseases. There is no consensual definition for this condition and clinical study criteria, mainly used for the small bowel, vary from localized luminal narrowing to luminal narrowing and bowel wall thickening with pre-stricture dilation and the presence of obstructive symptoms [7–9]. A dilation of the upstream tract seems to be the most rigorous definition but it remains limited.

15.2.2 Stricturing CD

European guidelines define stricturing CD as a localized, persistent narrowing, whose functional effects may be apparent from prestenotic dilation, and include obstructive symptoms (EL5) [10]. In the Montreal classification, the B2 phenotype corresponds to intestinal strictures. It is important to keep in mind that the B3 phenotype corresponding to fistulizing intestinal disease is associated with intestinal strictures in more than 80% of cases so that stricturing CD is the most common complication of CD [11, 12]. Ileal stricture is the most common location due to

possibly due to location of inflammation and its narrow luminal diameter; 20% of fibrostenotic CD only affect the colon, and about 10% affect the upper tract [13, 14]. The prevalence of multifocal small bowel strictures has been estimated at 28.8% in a multicentric prospective cohort study conducted in B2 patients [15].

Stricturing CD may be diagnosed during an *endoscopic* procedure and is defined by a luminal narrowing, impossible or difficult to pass with an adult endoscope assessed using the CDEIS [16]. The SES-CD describes 3 groups of stricturing lesions with increasing significance: single passable narrowing (grade 1), multiple passable narrowing (grade 2) or impossible to pass (grade 3) [17]. Ileo-colonoscopy is recommended for the detection of colonic or ileal strictures.

Cross-sectional imaging (Magnetic resonance enterography (MRE) or CT enterography) is required in all cases of passable or non-passable strictures to assess their features and associated lesions. One study has suggested the superiority of enteroclysis in the diagnosis of low-grade stenosis [18]. The Lemann Index [19] allows defining stricturing lesions into three groups as a wall thickening <3 mm or segmental enhancement without prestenotic dilation (grade 1) or a wall thickening ≥3 mm or mural stratification without prestenotic dilation (grade 2) or a stricture with prestenotic dilation (grade 3). MRE is helpful to use another usual classification based on the discrimination between inflammatory, fibrotic or mixed narrowing (cf. Chap. 14) [20]. Predominantly inflammatory strictures are more likely to resolve through the use of anti-inflammatory drugs via edema resorption [21] but the distinction between inflammatory and fibrotic strictures, based on imaging criteria, is more theoretical than reflecting reality. A pathological study has shown that CD patients who undergo surgery for obstructive symptoms have mixed histopathological findings of inflammation, fibrosis and muscle hypertrophy [22]. It has also been shown and confirmed that inflammation was positively correlated with fibrosis in stenotic CD [23, 24].

Another definition of stricturing CD is a *capsule endoscopic retention* event. That is why in patients with small bowel CD capsule examination should be preceded by patency capsule [25]. The definition of retention is the capsule staying in the small bowel for longer than 2 weeks after ingestion, which may require endoscopic or surgical removal. It has been reported to occur from 0 to 13% of all examinations [26, 27]. Thus, patency capsule is required in patient with suspected intestinal stricture. If there is no impediment of patency progression, patency capsule should have passed out of the body within 30 h or observed in the colon on a radiograph or CT scan at least 30 h after being swallowed. All other cases are not considered patent and capsule endoscopy is contraindicated. Patency capsule retention is not specific for stricturing IBD, many other disease can cause retention such as tumors, larges polyps, radiation therapy, long-term use of nonsteroidal anti-inflammatory drugs (NSAIDs) [28]. In a CD population, capsule endoscopy retention occurs in 13%, while it occurs in 1.6% in a suspected CD population [27]. The sensitivity of patency capsule for detecting significant small bowel stricture is superior to other examinations [29, 30]. Positive predictive value of the patency capsule examination for detection of severe intestinal strictures vary from 44 to 62% [31, 32].

Clinically, symptoms of stricturing CD may range from highly symptomatic (Konïg syndrome caused by incomplete obstruction of small bowel, includes abdominal pain related to meal, constipation alternating with diarrhea, meteorism, gurgling sounds (hyper-peristalsis) on auscultation (especially in the right iliac fossa), and abdominal distension [33] to mildly symptomatic or asymptomatic. In case of asymptomatic strictures, it is essential to ensure that patients are on a normal diet, due to adaption of their diet based on stricture symptoms. In our practice, it is uncommon since patients usually are on a low-fiber diet, except in case of long-lasting strictures. Patients that adapt nutritional habits such as a low-residue or low-fiber diet, become secondarily asymptomatic. Because of its larger bowel diameter, a colonic stricture may remain asymptomatic, whereas a duodenal or ileal stricture may early be sub occlusive.

It is of note that there is a poor correlation between stricture symptoms and severity. Indeed, in a retrospective study including patients with long colonic CD strictures (6-cm length, $Q_{25–75\%}$ [4–10 cm]) who underwent surgery, 27% of patients were asymptomatic [34]. The clinical scores commonly used in CD based on stool frequency such as the Crohn's disease activity index (CDAI) [35] or Harvey Bradshaw Index (HBI) [36] are not adapted to monitor patients with stricturing CD. A small bowel narrowing may lead to sub occlusion with reduced stool number while a colonic stricture may lead to chronic diarrhea in addition to the inflammatory activity of the disease. There is no validated score to monitor symptomatic stricturing disease. The use of a specific score referred to as CDOS (Crohn's Disease Obstructive Score) based on symptoms related to bowel strictures (obstructive pain, nausea, vomiting, dietary restriction and occlusion) developed empirically in the CREOLE cohort study [15] has been suggested, but this score has not yet been validated.

Finally, before considering medical therapy in stricturing CD, stricture location, diameter, length (±5 cm [37]) and shape (a major angulation make endoscopic dilatation difficult or impossible) should be investigated. Other factors should be considered, including a distinct pathogenesis of anastomotic strictures that may be explained by a locally reduced vascular flow, high luminal pressure and bacterial stasis [38]. These parameters may impact the efficacy of medical therapy. Abscess and fistula may be more likely to occur in areas with high pressure, upstream of a stricture. The positive value of fistula to predict stricture has previously been shown to be 86.2% [39].

15.2.3 Stricturing UC

Stricturing UC should be separately considered, because of the high rate of dysplasia- or cancer-related stenosis. Patients with stricturing UC have a longer disease duration than those with non-stricturing UC [40]. ECCO guidelines recommend to perform a careful pathological assessment, complete colonoscopy or if impossible CT or MRI colonography [41].

The definition of stricturing UC has not been clearly characterized. A fixed narrowing of the colonic lumen excluding an obvious polypoid lesion is the most accepted definition in the literature. Both the UCEIS and endoscopic Mayo score do not allow grading strictures as a severity indicator [42, 43]. In most cases, colonic stricture is pauci- or asymptomatic. Symptomatic strictures are more likely to be malignant according to former studies based on mixed stricture definitions (colonoscopy or Barium Enema) [28, 29].

The prevalence of strictures in UC is underestimated and only old data are available. It varies from 0.4% [44] to 11.2% [45] depending on the definition. A large retrospective study including 1156 consecutive UC patients has shown that 59 (5%) patients had a stricturing disease and 9 (0.7%) patients had multiple strictures [5]. Among the 70 strictures, 17 (24%) were malignant. Three features were found to be associated with the presence of a malignant stricture: disease duration >20 years (61% risk of malignancy *versus* 0% if the disease duration was <10 years), location proximal to the splenic flexure (86% risk of malignancy *versus* 47 and 10% in the sigmoid and rectum) and obstructive symptoms. Among all strictures reported in the literature, 70–100% appear to be benign [5, 30, 31]. The rectal location seems to be the most common, and proximal strictures appear to be more often malignant.

It should be mentioned that there is an increased risk of dysplasia or cancer in case of colonic stricturing IBD. In a large retrospective study, an incidence of dysplasia or cancer in IBD colonic strictures (after surgery and dysplasia-free preoperative biopsies) of 3.5% has been reported (2.4% for CD and 10% for UC) [34].

15.3 Towards a Tailored Strategy

In IBD, one of the major challenges is to identify predictors for medical or non-medical therapy failure. To clearly determine the place of medical therapy in stricturing CD, it is important to define the clinical situations that can obviously not be medically treated: complete occlusion despite IV steroid course, bowel rest, IV fluids, and nasogastric tube require intestinal resection. A prestenotic dilation or local complications (abscess, fistula or peritonitis) are also usual surgical indications. A recent retrospective study including 221 subjects aimed to identify factors that predicted surgery within 2 years of hospitalization for CD, to guide medical versus surgical management decisions[46]. Multivariate modeling demonstrated small bowel dilation >35 mm (hazard ratio, 2.92; 95% confidence interval, 1.73–4.94) and a platelet: albumin ratio ≥ 125 (hazard ratio, 2.13; 95% confidence interval, 1.15–3.95) to predict surgery. The complications of surgical resection mainly include postoperative morbidity, the risk of transient stoma up to one third of patients and the high rate of postoperative surgical recurrence (44% at 10 years) [47]. Both British, American and European guidelines recommend endoscopic dilation (ED) in case of short (≤4 cm) symptomatic strictures, but surgery in case of longer strictures in addition to optimal medical systemic therapy [10, 12, 48, 49].

Table 15.1 Parameters to be considered for stricturing IBD management

Type of stricturing IBD	Strictures that should be considered for medical therapy	Strictures that should be considered for surgical therapy
Stricturing Crohn's disease	*Clinical features and patient characteristics* – Previous resection/short bowel syndrome – Current smoking – Naive to anti-TNF – Severe nutritional impairment – Short history of obstructive symptoms *Morphological features* – Multifocal strictures – Very long stricture (>40 cm) – Presence of inflammation (late contrast enhancement) – Limited dilation of the upstream tract (≤35 mm) – Absence of complex fistula *Histological features* Absence of dysplasia or adenocarcinoma	*Clinical features and patient characteristics* – No risk of short bowel syndrome – Previous failure of anti-TNF – Long history of obstructive symptoms – Low risk of postoperative recurrence *Morphological features* – Single stricture – Limited stricture (<40 cm) – Predominant fibrotic stricture – Large dilation of the upstream tract (>35 mm) – Presence of complex fistula, abscess *Histological features* Presence of dysplasia or adenocarcinoma
Stricturing ulcerative colitis	*Clinical features and patient characteristics* – Asymptomatic – Disease duration <20 years – Naive to anti-TNF *Morphological features* – Complete colonoscopy with careful biopsies throughout the colon – Single lesion – Absence of polypoid lesion – Rectal or sigmoid stricture *Histological features* – Absence of dysplasia or cancer	*Clinical features and patient characteristics* – Disease duration >20 years – Symptomatic – Previous failure of anti-TNF *Morphological features* – Proximal (before splenic flexure) – Non-passable, incomplete colonoscopy – Multifocal lesion *Histological features* – Presence of dysplasia or cancer

In other cases, the decision may be more difficult and clinicians need to be aware of the specific context. Previous resection, current smoking and penetrating disease are independent risk factors for postoperative recurrence, and should lead to initiation of medical therapy. A recent prospective cohort has shown that administering an anti-TNF therapy during the last 6 months before ileocecal resection increased the risk of postoperative morbidity [50]. These data stress the importance of implementing a tailored strategy (Table 15.1).

15.4 Medical Therapy in Fibrostricturing IBD

No specific anti-fibrotic drugs are currently available for treating the digestive tract [2]. Clinical trials assessing the efficacy of medical therapy on fibrosis-related UC strictures are lacking. Most publications are focused on CD.

15.4.1 Steroids

The effects of corticosteroids on fibrosis are unclear. Indeed, in in vitro studies, they have been shown to induce procollagen expression in human intestinal myofibroblasts [51]. On the other hand, a decreased procollagen expression has been reported with dexamethasone administration in animal models [45]. The indications of systemic steroid therapy are limited to brief (60 mg/day methylprednisolone for 5–7 days) IV bolus in symptomatic inflammatory strictures as a "therapeutic test" to induce clinical remission [52]. A former study has provided data on steroid infusions during occlusion due to small bowel strictures in 26 CD patients. Occlusion resolved within 72 h in 96% of cases, but 75% of patients experienced re-occlusion [53]. Corticosteroids have been shown to be an independent risk factor for postoperative morbidity as well as severe nutritional impairment and perforation [54, 55]. Using corticosteroids has been shown to be associated with intestinal stricture, or obstructive symptoms in the TREAT registry, as well as an ileal location and disease duration [56]. The long-term use of steroids should be avoided due to serious adverse events, and their known inability to induce mucosal healing, a condition necessary to prevent evolution to a fibrostricturing phenotype [57]. Intralesional injections of steroids did not seem to have a major impact on endoscopic dilation outcomes despite promising results in small case reports [58].

15.4.2 5-ASA

There is no evidence to support the use of 5-aminosalycilates (mesalamine) as a therapeutic agent or in the prevention of transmural stricturing CD [10]. Overall, in a meta-analysis, Hanauer and Strömberg have shown in CD that mesalamine may slightly reduce the CDAI with no clinical significance [59].

15.4.3 Purine Analogs

A prospective randomized study including 72 sub occlusive patients with ileal CD stricture, responding to IV steroids has compared the efficacy of mesalamine *versus* azathioprine 2–3 mg/kg [60]. The rate of rehospitalization-free survival was significantly higher in the azathioprine groups than in the mesalamine group, especially at 1 year. Among the 36 patients with azathioprine, the mean time to rehospitalization was 27 ± 10.4 months (*vs.* 18 ± 10.7 with mesalamine), 38.9% were admitted for occlusion and 22.2% for intestinal resection. There are not enough data available to support the use of purine analogs alone in stricturing CD.

Using azathioprine or 6-mercaptopurine has been shown to induce complete or partial mucosal healing of inflammatory ileitis following resection [61] and to delay clinical and endoscopic fibrostricturing recurrence after surgery [62]. Unexpectedly, the early prescription of thiopurines in naive patients did not change the rate of stricture occurrence or the frequency of surgical interventions [63]. More recently,

the POCER study has provided information about the post-operative medical strategy to be used to prevent CD recurrence. In the population with a high risk of recurrence (i.e. current smokers, previous resection and penetrating disease), purine analogs were prescribed in addition to metronidazole in the first 3 months after resection, and in case of intolerance, adalimumab was initiated. After 6 months, the most advanced forms of the disease (i3, i4 including stricture) were found in 8% of patients in the thiopurine group *vs.* 4% in the adalimumab group. Purine analogs did not appear to be the best medical therapy to prevent fibrostenoting CD.

15.4.4 Methotrexate

There is no specific data on the efficacy of methotrexate in fibrostricturing CD used either as a therapeutic or preventive agent. What is known is that mucosal healing is less frequently achieved with methotrexate than with thiopurines or infliximab in CD [64].

15.4.5 Anti-TNFs

The last two decades have seen the advent of anti-TNFs in severe IBD. Does stricturing CD benefit from anti-TNF therapy? In the early twenty-first century, retrospective studies have reported a potentially increased risk of complete bowel obstruction when using infliximab in stricturing CD [65, 66]. In 2006, Lichtenstein et al. have reported that the use of infliximab was not associated with stricture occurrence [67]. Between 2003 and 2011, three uncontrolled studies have confirmed the finding that using anti-TNFα (infliximab, n = 3) is safe and effective in inflammatory stricturing CD. Most of these studies included small bowel strictures, and stricture definition was heterogeneous [7, 54, 56]. A randomized controlled trial has stressed the preventive effect of infliximab intravenous injection for anastomotic stricture relapse (0% *vs.* 30% for Rutgeerts i4 at 1 year) [68].

The CREOLE study, a large prospective interventional cohort study, has recently provided more information about the safety and efficacy of adalimumab in CD patients with symptomatic small bowel stricture [15]. Stricture was defined as a constant luminal narrowing associated with upstream dilation or obstructive symptoms. They were defined using a score specifically built for this trial, the CDOS, to show that all patients had a severe clinical obstruction. After week 24, the treatment was successful in 62/97 (64%) patients. Thirty-five patients failed to achieve success for the following reasons: 14 needed corticosteroids after week 8, two patients were switched to infliximab, 8 patients underwent an intestinal resection, 2 patients had an endoscopic dilation, 10 patients had a severe adverse effect leading to adalimumab discontinuation, 2 patients interrupted adalimumab treatment and 5 patients withdrew from the study (four were lost to follow-up, one withdrew consent). In 8 cases, the failure was due to multiple reasons. After a long follow-up (3.8 ± 0.1 years), 29% of patients were still under adalimumab with no need for surgery or endoscopic dilation. Among patients in whom treatment was successful at week 24, 21

underwent subsequent intestinal resection and 64.9 ± 6.6% of patients did not need surgery 4 years after inclusion. The predictive factors for treatment success were analyzed using a multivariate analysis showing that the use of immunosuppressive agents at the time of adalimumab initiation, the presence of obstructive symptoms for <5 weeks and a CDOS >4, a small bowel stricture length <12 cm, a maximum small bowel diameter proximal to stricture(s) of 18–29 mm, a marked improvement in the delayed phase and the absence of fistula had an independent predictive value for adalimumab success (Table 15.2). In other words, combotherapy (immunosuppressant + adalimumab) seems to be more effective on short, symptomatic, inflammatory strictures with a short period of evolution. The median time to intestinal resection in the whole cohort was 3.8 years (Fig. 15.1). Because of the need to better

Table 15.2 Prognostic factors associated with a high success rate according to the CREOLE study

Factor/group with a high success rate	Coefficient estimate ± SE	Odds ratio estimate	p	Number of points
Immunosuppressant/yes	1.23 ± 0.62	3.42	0.040	1
CDOS/>4[a]	1.25 ± 0.65	3.48	0.046	1
Duration of obstructive symptoms (week)/<5	1.79 ± 0.81	6.00	0.016	1
Length of stricture (cm)/<12	1.80 ± 0.67	6.04	0.0042	1
Maximum proximal diameter (mm)/[18–29]	1.99 ± 0.68	7.32	0.0013	1
T1 delayed enhancement intensity/severe	1.78 ± 0.66	5.92	0.0034	1
Fistula/no	1.55 ± 0.76	4.72	0.035	1

[a]CDOS >4 is defined by daily mild to moderate obstructive pain with more than 3 days of associated nausea-vomiting, or severe obstructive pain during 1–7 days for the previous 8 weeks

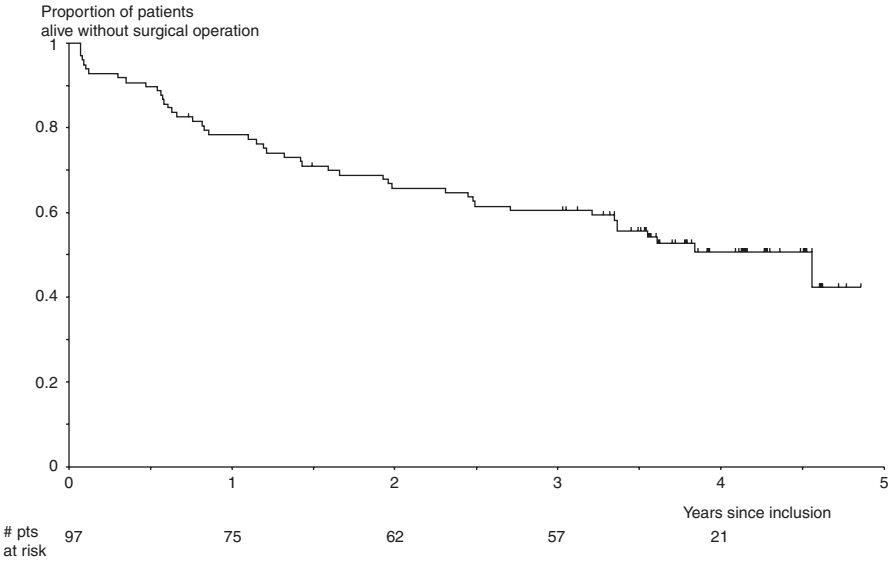

Fig. 15.1 Time to intestinal resection from inclusion in the 97 Crohn's disease patients with symptomatic small bowel stricture treated with anti-TNF (median follow-up ± SE, 3.8 ± 0.1 years, 46 resections were needed) [15]

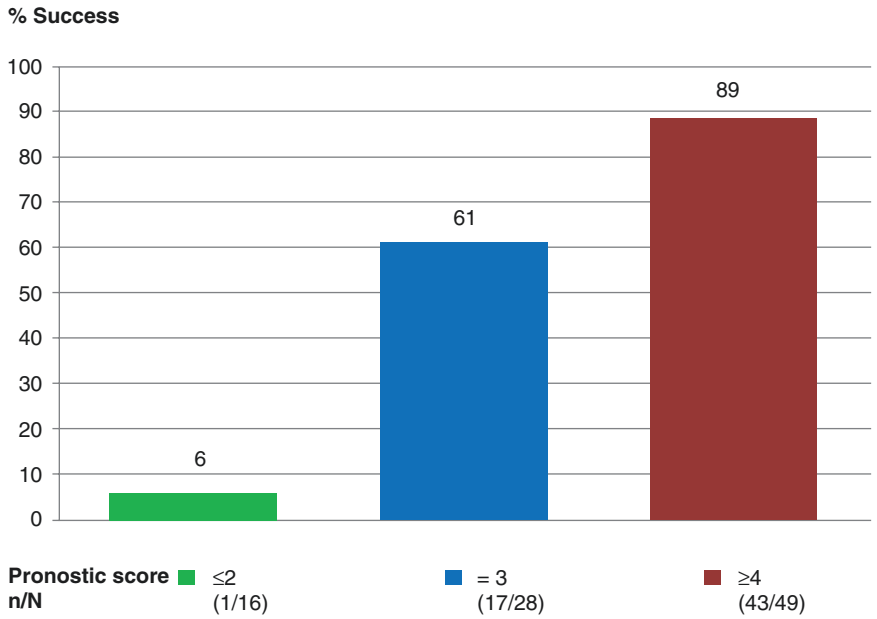

Fig. 15.2 Probability of success at week 24 in the 93 Crohn's disease patients withsymptomatic small bowel stricture(s) according to the clinicoradiological prognostic score [15]

determine in which patients' medical therapy will be successful, the authors have developed a prognostic score based on these parameters. Patients with a score of less than 3 points had a treatment success rate of 6% and in those with a score of at least 4 points, the treatment success rate was of 89% (Fig. 15.2).

15.4.6 Other Biologics

There are no specific data on the use of ustekinumab in stricturing CD. Symptomatic stricturing CD has been excluded from the UNITI studies [69]. The VICTORY consortium assessing vedolizumab use in a real-life setting in a CD population, included 118/212 (55.7%) patients with stricturing or penetrating CD, and after 12 months, resection was needed in 3 colonic and 2 small bowel strictures [70]. Mongersen (SMAD 7 antisense oligonucleotides) may restore TGFβ1 activity, leading to the inhibition of inflammatory pathways, and the resolution of enteritis in CD patients. TGFβ1 has also been shown to have profibrotic properties through stromal cell collagen stimulation [71]. In a phase I study, patients were closely monitored for the development of small bowel strictures by imaging and quantification of a fibrosis serological marker and no significant change was observed [72]. More data are

needed on the safety and efficacy of mongersen in stricturing IBD. However, phase 3 trials evaluating this drug in active CD patients were prematurely interrupted for lack of efficacy. No specific data are currently available on other biologics, such as janus kinase inhibitors, and fibrostricturing CD.

15.5 Other Measures

As in all chronic diseases, the medical treatment of stricturing IBD is not limited to pharmacological treatments. Medical therapy should always be based on a multimodal approach. ECCO guidelines recommend to treat patients with obstructive symptoms in the context of a multidisciplinary team (EL5) [73]. IBD are nutritional debilitating diseases and the intestinal narrowing worsens the nutritional impairment by increasing painful symptoms related to the alimentary bolus passage. In 2014, the British Dietetic Association has established guidelines for stricturing CD [74]. Oral or enteral nutritional supplementation may be required, especially in case of weight loss. Nutritional components that may cause a mechanical obstruction [e.g. fibrous parts of fruits and vegetables (skins, seeds, woody stalks), whole grains, nuts and seeds, gristle on meat, skin on meat or fish, edible fish bones] or food leading to excess gas production driving prestricturing pain should be excluded from the diet. Pre- or probiotics have been assessed in several studies but no obvious efficacy in maintaining remission or preventing or relieving stricturing IBD has been shown [66, 67, 75–77]. Current smoking worsens the clinical course and induces stricture occurrence in CD, reduces the therapeutic response, and is associated with post-surgical recurrence [78]. ECCO guidelines state that smoking is a risk factor for postoperative recurrence after resection or stricturoplasty for fibrostricturing CD (EL4) [73]. Therefore, all smoking patients with CD should be referred to a smoking cessation program (EL1) [10].

15.6 Conclusion

Medical therapy for stricturing IBD has recently become available. Anti-TNFs are currently the best molecules to be used in this context. Their administration implies the use of strict selection criteria to identify the best candidates and to balance the benefit-risk ratio with surgery. Further studies are needed to define the potential effect of other biologics in stricturing IBD. A major challenge for the coming years will be to identify a specific intestinal anti-fibrotic agent like those that are already available in skin healing [79], interstitial renal fibrosis [80], and systemic sclerosis [81].

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Chapter 16
Endoscopic Therapy of Intestinal Strictures: What Is State of the Art?

Talat Bessissow and Gert Van Assche

Abstract Symptomatic intestinal strictures develop in more than one third of patients with Crohn's disease during their lifetime. Strictures can be inflammatory, fibrotic or mixed. Fibrosis occurs as a result of excessive deposition of extracellular matrix protein. It can lead to severe symptoms affecting patients' quality of life. As a result, patients will often need to undergo surgery to improve their symptoms. Endoscopic balloon dilatation appears to be a safe and effective alternative therapeutic procedure to replace or postpone surgery. It is less invasive and can be performed during a regular colonoscopy. Non-complex strictures that are ≤5 cm can be dilated endoscopically. Up to 80% of patients will have immediate relief of symptoms and it can prevent surgery in up to 70% of patients after a 3-year follow up. Serious complications are rare and occur in less than 3% of procedures.

Keywords Endoscopic balloon dilatation · Stricture · Crohn's disease

16.1 Introduction

The natural history and phenotype of Crohn's disease (CD) is highly variable [1]. Even though most patients will present with purely uncomplicated inflammation, approximately 70% will develop either strictures or fistulae within 10 years of disease [2–5]. Whereas the location of disease remains stable over time, changes in disease behavior may occur. Approximately 30–50% of patients with fibrostenotic disease present as such and many others will develop a stricture over the course of their life [2, 3, 5, 6]. Intestinal strictures will occur in at least one-third of CD patients and can be fibrotic, inflammatory or mixed leading to luminal narrowing resulting in symptomatic obstruction, pre-stenotic fistulizing disease and potentially

T. Bessissow
Division of Gastroenterology, McGill University Health Center, Montreal, QC, Canada
e-mail: Talat.bessissow@mcgill.ca

G. Van Assche (✉)
Division of Gastroenterology and Hepatology, University Hospitals Leuven, Leuven, Belgium
e-mail: gert.vanassche@uzleuven.be

F. Rieder (ed.), *Fibrostenotic Inflammatory Bowel Disease*,
https://doi.org/10.1007/978-3-319-90578-5_16

harbor malignant lesions. It is currently one of the main indications for surgical treatment of CD [7, 8]. In fact, 75% of affected individuals will undergo surgery in their lifetime [2]. Disease recurrence at the site of anastomosis is common which may lead to recurrence of luminal strictures [9].

The development of fibrosis is caused by excessive deposition of extracellular matrix protein produced by activated myofibroblasts as a consequence of chronic uncontrolled localized inflammation [10, 11]. Given the transmural nature of CD, all bowel layers are involved by fibrosis and will present features of histomorphological thickening. Despite recent advances in the understanding of the pathophysiology of CD, the exact mechanism responsible for luminal fibrosis remains to be elucidated. In addition, the incidence of intestinal strictures has not changed over time despite the introduction of novel therapeutic options [12–16]. Although biologics and immunosuppressants may delay the onset of complicated disease, they have not been shown to prevent it. Currently, specific anti-fibrotic therapy is not commercially available [17].

Significant bowel strictures will often lead to a varying degree of severity of obstructive symptoms that negatively impact on patients' quality of life [8]. As a result, patients will often need to undergo repeated surgical resections of the affected segments which exposes them to the risk of immediate and long term postoperative complications such as short bowel syndrome, loss of gut functionality, and high risk of stricture recurrence (up to 50%) [8, 18]. In light of this information, endoscopic balloon dilatation (EBD) has emerged as an attractive alternative therapeutic procedure [19]. Given that most strictures are located in the colon or ileum [3], they are accessible by using through-the-scope colonoscope or balloon-assisted enteroscope [20–22]. To improve outcomes of EBD, injection of medical therapy has been attempted.

In this chapter, we will describe the data on endoscopic balloon dilation as well as presenting the short and long term outcomes and complications associated with it. In addition, we will provide a practical description on how to perform the procedure.

16.2 Efficacy of Endoscopic Balloon Dilatation

In the absence of medical therapy targeted at treating intestinal fibrosis and with the failure of medical therapy to relieve obstructive symptoms, endoscopic balloon dilatation is a very good alternative to conserve bowel length. EBD has become an accepted modality for the treatment of bowel strictures in patients with CD. It is mainly applicable in short strictures (≤5 cm) and in locations that easily accessible by endoscopy [23]. The most common location tends to be at the ileocolonic anastomosis in a patient who underwent bowel resection [17, 19, 24]. EBD can also be performed anywhere in the colon using a colonoscope, in the upper GI tract using a gastroscope or in the small intestine when reachable with an enteroscope.

Most of the published data on outcomes of EBD is observational with its inherent limitations. However, the data clearly confirm the role of EBD with excellent short

term efficacy and moderate long term efficacy for EBD in the management of CD strictures. The immediate success rate is generally very high ranging between 71% and 100% [23, 24]. In a recent pooled analysis of 33 retrospective studies including 1463 CD patients treated with 3213 EBD procedures, the immediate intra-procedure success rate was 89% [23]. The median stricture length was 2 cm and the treated lesions were mainly post-operative strictures. More recently, the Cleveland Clinic group also showed that in their cohort of patients with post-operative anastomotic strictures, the immediate success rate was 91.3% [25]. Although the technical success is important, it needs to translate into a clinical symptomatic improvement. In the same pooled analysis, 80.8% of patients had relief of clinical symptoms or clinical efficacy [23]. Long term clinical efficacy defined as being free of surgery with a median follow up period of 40.1 months was achieved in 69.2% of patients. However, at 24 months, 73.5% of patients required repeat dilatation. Interestingly, the technical success for dilating a post-operative stricture was lower than that of native strictures (odds ratio (OR) = 2.3, $P < 0.001$) but the long-term outcomes were similar [23]. This finding is contradictory to common experience as most endoscopists find it technically easier to dilate a post-operative stenosis provided the anastomosis is not too angulated. On the other hand, these findings were not corroborated in the Cleveland Clinic cohort where the success rate was much lower with 52% of patients requiring surgery over the follow up period post EBD [25].

Factors that have been associated with favorable short term dilatation outcomes include greater maximal dilatation diameter (OR = 1.4, $P < 0.001$), 'de novo' or native strictures (OR = 2.3, $P < 0.001$, not confirmed), technically successful dilatation, stricture ≤5 cm, and absence of ulcers in the stricture [26–28]. Clinical efficacy was neither associated with location of stricture nor was it dependent on the type of stricture (native vs post-operative). In addition, no factors were identified as a predictor of long term outcomes or of the need for repeat dilatation [17]. Neither CRP, endoscopic disease activity, or medical treatment after dilation influenced the subsequent disease course [29]. As for factors predicting need for surgery, every increase by 1 cm in stricture length resulted in an increased risk for surgery by 8% ($P < 0.005$). A stricture length of ≤5 cm was associated with a surgery-free outcome (hazard ratio (HR) = 2.5, 95% confidence interval (CI) = 1.4–4.4). Strictures located in the duodenum compared with those located in the jejunum/ileum or colon were associated with a nearly 5 times increased hazard for shorter time to surgery (HR 4.7, $P < 0.038$; HR 5.6, $P < 0.03$, respectively). None of the other investigated factors was linked to need for earlier surgery [17].

16.3 Safety of Endoscopic Therapy

In general, EBD is considered as a safe procedure. However, when mechanically dilating the bowel, perforation is a valid concern. In the above mentioned systematic review, major complications defined as hospitalization, bleeding or perforation was observed in 2.7% of procedures [17]. In the Cleveland clinic cohort, the perforation

rate was only 1.1% which is very reassuring when compared to postoperative complications which occurred in 8.8% of patients and consisted mainly of intra-abdominal abscesses and enterocutaneous fistula [25]. No death related to EBD has ever been recorded and none of the factors evaluated in the systematic review was associated with a higher risk of complications. Although it is a rare occurrence, small bowel adenocarcinoma could be fatal if overlooked [30]. Therefore, it is recommended to take biopsies of the stricture prior to EBD, particularly when it is irregular or displays other features suspicious of malignancy. There has been no evidence to suggest that mucosal biopsy prior to EBD increases the risk of perforation. It noteworthy to mention that EBD is contraindicated in a stenosis associated with an abscess, a phlegmon, fistula, high-grade dysplasia or malignancy [31].

16.4 Concomitant Injection of Pharmacological Agents

The use of intra-lesion injection of steroids has been shown to be effective in the management of several types of gastrointestinal strictures such as peptic, post-radiotherapy, and corrosive strictures but is still controversial in CD-associated strictures [32–35]. Most of these studies have used triamcinolone as it is considered an appropriate agent given its long local effect which can last up to 3–4 weeks [36]. Much of the data available on the use of steroids in the management of CD-related strictures is retrospective and uncontrolled. In a systematic review, the use of steroids injection in addition to EBD did not show an additive effect [23]. However, in a small randomized controlled trial of 29 pediatric CD patients, combination of EBD and intra-lesional triamcinolone was shown to reduce the time to re-dilatation and surgery when compared to the placebo group [37]. On the contrary, a small prospective study including 13 adult patients was terminated prematurely when initial results showed that patients who received steroid injection required earlier re-dilatation compared to placebo [38]. However, this study was limited to longstanding post-operative strictures and the methodology might harbor significant biases. Meanwhile, intra-lesional injection of anti-tumor necrosis factor alpha was only assessed in small, uncontrolled cases reports and series but the preliminary results are promising [39, 40]. Immunization to biologics is of course a concern when local injection is performed.

16.5 How to Perform an EBD

Commonly, EBD is performed using the through-the-scope balloons (TTS) and pneumatic dilatation is applied to the stricture. However, the available reports are very heterogeneous with respect to the balloon size used, inflation time, endpoints achieved, and follow-up intervals.

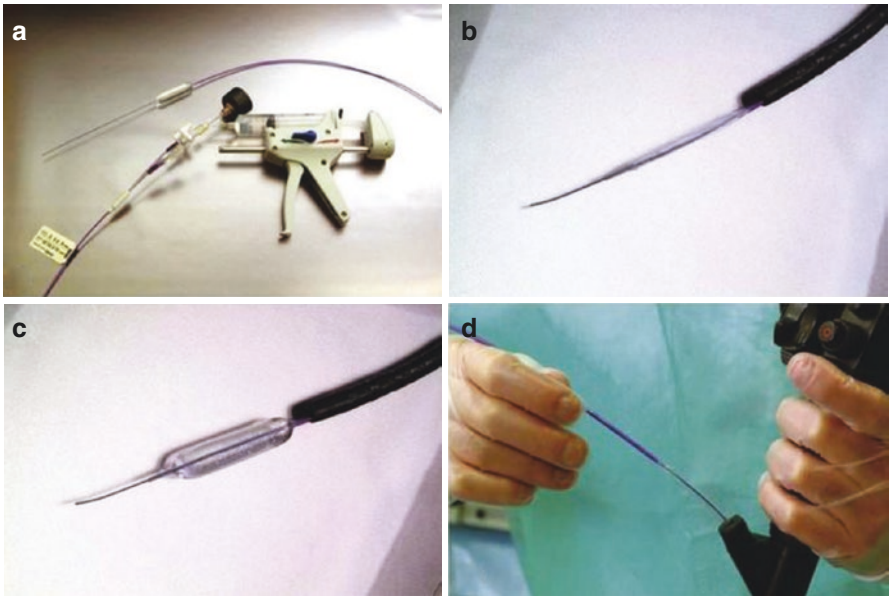

Fig. 16.1 Balloon dilation. Syringe gun, manometer and balloon (**a**). Through the scope (TTS) balloon (**b**), and following inflation (**c**). TTS balloon is inserted into the operating channel (**d**)

Radially expanding balloon dilators are available commercially in several calibers and lengths (Fig. 16.1). Balloon dilators are made of low-compliance inflatable thermoplastic polymers which will allow to have a reproducible and uniform expansion of the balloon to its desired maximal size. Dilator diameter is measured in millimeters or French (Size in millimeters can be converted to French at a ratio of 1:3, e.g. 10 mm = 30 F). The balloons usually range in size from 6 to 20 mm diameter. Most balloons allow for sequential expansion and they are marked as single-use. The balloon is expanded by pressure injection of liquid, mainly water but in some instances with radiopaque contrast, by using a handle accessory device. The hydraulic pressure of the balloon is monitored manometrically to gauge the radial expansion force [41].

Before attempting to perform a dilatation, it is very important to know the length and complexity of the stricture. If this information can be obtained during the endoscopy i.e. the stenosis is very short, can easily see through it and the length can be estimated than no other investigation is required. If this is not the case, further imaging with either a CT enterography or MR enterography is required to gather all the required information for a safe procedure. This is preferably done prior to dilation. In some centers, luminal contrast assisted radioscopy is performed during the procedure using the balloon catheter to inject contrast fluid. As discussed earlier, strictures $\leq$5 cm without any of the mentioned contraindications is amenable for dilatation.

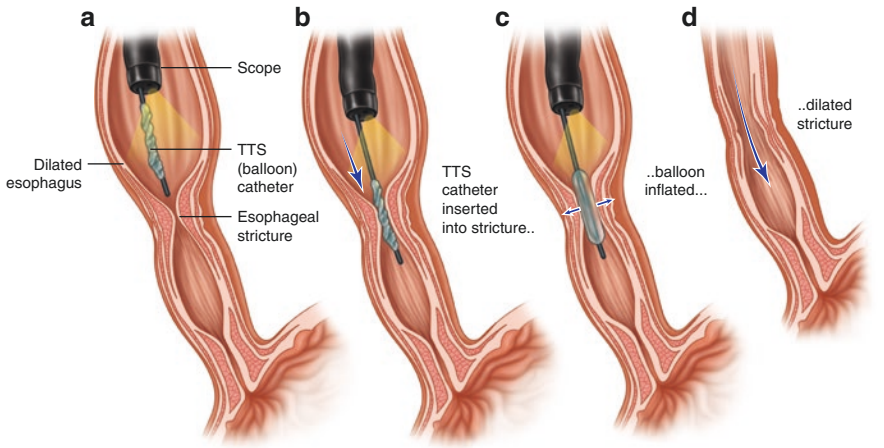

Fig. 16.2 Balloon dilatation. Through the scope (TTS) balloon inserted into the endoscopic lumen (**a**). TTS balloon passed through the stricture (**b**). Insufflation of balloon to dilate stricture (**c**). Dilated stricture (**d**)

Once the endoscope is passed to the stenosis site, initial selection of the dilator size is based on an estimation of the diameter of the stenosis (Fig. 16.2). The balloon is then passed through the scope accessory channel with or without a guidewire which allows direct visualization during the procedure. If a guidewire is used, it should be first advanced through the stenosis and the balloon is advanced over the wire. The balloon is placed across the obstruction and inflated under direct vision and the guidewire is retracted. If the guidewire is not used, the balloon is directly advanced through the stricture and placed across. The balloon is then inflated with a pressure or volume-controlled handles to the desired pressure, representing the chosen balloon diameter. After removal of the balloon, the dilated stricture is usually examined endoscopically [41]. A three-step inflation is preferred as it is considered to induce more controlled dilation. The diameter of the balloon will increase with every step of increased pressure. The diameter corresponding with every step is clearly depicted on the balloon catheter. Most centers dilate to a maximum of 18–20 mm. Repeated dilation, with intermittent deflation, during the same procedure can be employed if the first dilation is judged to be suboptimal.

16.6 Conclusion

In non-complex strictures that are ≤5 cm in length, endoscopic balloon dilatation is a safe and effective alternative procedure to surgery. The short-term outcomes are excellent and it can prevent or delay surgery in most patients.

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Chapter 17
Resectional Surgery for Intestinal Strictures: What Is State of the Art?

Karin A. T. G. M. Wasmann, Christianne J. Buskens, Pieter J. Tanis, and Willem A. Bemelman

Abstract Novel scientific insights and our progressing experience have enlarged the armamentarium of surgical treatment options for Crohn's disease. It has become daily clinical practice to be able to choose between medical or surgical treatment. The natural course of Crohn's disease often results in fibrosis and strictures. Despite the improvement of medical therapy for Crohn's disease, to date no medical therapy for strictures exist. Those patients are destined to dilatation for short strictures but otherwise undergo stricturoplasty or resectional surgery. This chapter will focus on the technical aspects of resections for stricturing disease, discussing indications for surgery, the surgical approach and results of surgery.

Keywords Fibro-inflammatory strictures · Pure stenotic strictures · Crohn's disease · Ileocolic resection · Single port · Multi port · Anastomosis · Radicality Mesenteric

17.1 Introduction

The course of Crohn's disease is described as starting with luminal inflammation, which can progress to irreversible damage of the bowel wall resulting in fibrotic ileal strictures and/or fistulas [1–5]. According to the natural history of Crohn's disease, the majority of patients present with pure inflammatory disease at diagnosis (77%) (Montreal B1) [1]. Others already present with stricturing disease (Montreal B2), or penetrating disease i.e. fistula or abscess (Montreal B3) [6, 7]. In population-based cohorts up to 36% have complications at diagnosis, including strictures or penetrating disease [8–10]. Approximately half of the patients with terminal ileitis will develop a stricture [3, 6]. Although, this could be underestimated as traditionally Montreal and Vienna classification systems score patients according to the highest level of disease, consequently a stricture is only scored in the absence of

Karin A. T. G. M. Wasmann · C. J. Buskens · P. J. Tanis · W. A. Bemelman (✉)
Department of Surgery, Academic Medical Center, Amsterdam, The Netherlands
e-mail: k.a.wasmann@amc.nl; c.j.buskens@amc.nl; p.j.tanis@amc.nl; w.a.bemelman@amc.nl

F. Rieder (ed.), *Fibrostenotic Inflammatory Bowel Disease*,
https://doi.org/10.1007/978-3-319-90578-5_17

penetrating disease [6, 7]. Whereas the anatomical location of Crohn's disease is mostly stable, behaviour of Crohn's disease varies substantially during the course of the disease. As an example, almost a fifth of patients clinically evolve to a more advanced phenotype within 90 days [8]. The above described traditional concept with inflammation, complicated by strictures, fistulas, and abscesses might be too simple and rigid, since the natural history of Crohn's disease is a continuum of gradual progression towards complicated disease. Within the modern concept of Crohn's disease of the terminal ileum emphasizing the progressive nature, the stages of disease can be divided into fibro-inflammatory disease, pure fibrotic disease, fistulising disease, and fistulising disease with abscess formation. To identify these phenotypes enterography combined with magnetic-resonance imaging (MRE) is the preferred choice for preoperative imaging [11–13]. Subclassification into these four phenotypes can be helpful in standardising timing of surgery, preoperative optimisation, staged surgery, expected morbidity and stoma rates, and prophylactic medical treatment postoperatively. So far, for this staging system only little evidence exists, but it appears helpful in daily clinical practice [14, 15]. This chapter will address surgical therapy for stricturing Crohn's disease, with the focus on the terminal ileum, as its preferred location.

17.2 Indication for Surgery

Formerly, up to 90% of patients underwent a surgical resection within 15 years after diagnosis, mainly due to complicated Crohn's disease of the terminal ileum [1, 16–18]. Surgery is generally considered an invasive and last resort option once all medical therapies have failed. With the introduction of biologics (in particular anti-tumour necrosis factor alpha (anti-TNF)) in 1999, the treatment of Crohn's disease shifted away from surgery to medical therapy, resulting in a decline in surgical resections up to 30% over the last decade [19, 20]. The incidence of stricture formation and surgery for strictures did not seem to decrease in the past decade [21]. Whether a patient with Crohn's disease of the terminal ileum should have medical or surgical therapy depends on many factors: extent of the disease, co-existing other localisations of Crohn's disease, prior medical treatment, the fibrotic component of the disease with or without the presence of prestenotic dilatation. In order to achieve a tailored treatment approach for each patient, decisions should be made in multidisciplinary teams. The radiology input is vital for decision making, as it can identify the phenotype correlating with the surgical outcome [22].

17.2.1 Fibro-Inflammatory Phenotype

Worldwide, the standard treatment of terminal ileitis in Crohn's disease is a step-up medical protocol with the start of anti-inflammatory drugs and immunomodulators, followed by biologics and/or experimental medication. Medical therapy is most

effective when the disease is still in its inflammatory stage of disease [21]. In fibro-inflammatory disease, defined as predominant inflammatory disease evident by bowel wall thickening, established indications for surgery include therapy refractory disease, and unacceptable medical therapy side-effects. If patients continue to have symptoms or persistent inflammation under properly dosed anti-TNF, discontinuation of medical treatment and decision to undergo surgery is warranted. The indication for surgery is also dependent on the extent of the disease: generally, surgery is advised in limited disease (<40 cm), because resection of more than 40 cm can result in bile acid diarrhea [23]. Furthermore, additional locations of the disease should also be taken into account when discussing these patients in the multidisciplinary team (MDT) meeting. If patients have synchronous Crohn's disease lesions, e.g. colonic involvement or perineal fistula, this would be an argument for (continuation of) medical therapy, as biologics can treat all locations at the same time. Surgery is effective in all stages but might seem less attractive in the inflammatory stage of the disease, mainly because of the risk of complications like anastomotic leakage and/or (temporary) ileostomy. However, there are no data, whether the affected bowel segment, even after mucosal healing was achieved with medical therapy, still functions normally [24]. Currently, only one head to head comparison of laparoscopic ileocolic resection versus anti-TNF has been performed in patients with limited terminal ileitis (<40 cm) not responding to immunomodulators, concluding that surgery is a good alternative to anti-TNF. The randomized control trial (RCT) showed non inferiority of surgery compared to anti-TNF with regard to disease specific quality of life and endoscopic remission rates, and superiority of surgery for general quality of life [25].

17.2.2 Fibrotic Phenotype

Secretion of extracellular matrix (ECM) components, such as collagens and fibronectins, is intended to close defects of the bowel wall. So far, it is reported that an excessive production of ECM driven by the misbalanced inflammatory mechanism of Crohn's disease causes fibrotic stenosis [26, 27]. Independently of inflammation, fibrosis can progress, and once tissue stiffness increases, it can serve as an activator itself [28]. Medication is less effective when stenosis or perforation has occurred, and endoscopic balloon dilatation is only applicable for short, isolated strictures (<4 cm) in reach of standard colonoscopy [29]. The absence of inflammation on MRE reliably predicts a pure fibrotic stricture [30, 31]. However, the great difficulty is the fibro-inflammatory stricture. With the current diagnostic tools, we are not able to quantify the relative contribution of fibrosis to the fibro-inflammatory stricture and to the clinical symptoms of the patient. For this reason, a trial of medical therapy is generally started. If the inflammation is refractory or the symptoms persist despite mucosal healing, the patient is sent to the surgeon. This is a pragmatic but suboptimal strategy, because trial and error consequently cause a delay in appropriate treatment resulting in more extensive disease, loss off quality of life and increased costs. Prestenotic dilatation is generally considered as a sign of severe

fibrosis requiring surgical management. Pure fibrotic disease is an established indication for surgery. Also for pure fibrotic disease the extent of disease should be taken into account, as it is still not preferable to resect more than 40 cm of intestine. In those cases, stricturoplasty is the preferred treatment.

17.2.3 Fistulising Disease

Symptomatic fistulising disease is an indication for surgery. Clear examples are enterovesical, enterovaginal and enterocutaneous fistula. The phenotype of the disease is likely to reflect surgical outcome e.g., fistulising disease associated with abscesses correlate with higher rates of ileostomy, postoperative complications, and longer hospital stay [32]. Sequential application of new medical therapies has broadened the spectrum to treat patients with good short-term results, but with the caveat that surgery might be postponed [33, 34]. Prolonged, partially ineffective medical therapy might result in healthy nearby organs being included by the inflammatory mass resulting in more extensive resections [35]. Furthermore, corticosteroids, immunomodulators and anti-TNF use within 3 months of surgery could worsen postoperative morbidity [36–38].

17.2.4 Abscess

Intra-abdominal abscesses are caused by perforating disease. Most patients will require resection of the perforated segment particularly in the presence of a distal stricture. Other factors indicative for surgery are the development of an abscess under anti-TNF, involvement of a short segment, and absence of other locations of Crohn's disease. The presence of an abscess, anaemia, the use of immunosuppressive medications within 3 months of surgery, and perioperative hypalbuminaemia are associated with increased post-operative complications [32]. Therefore, patients must be optimised prior to surgery, by improvement of the nutritional status, stopping of the immunomodulating drugs, and starting treatment by percutaneous drainage of the abscess in combination with antibiotics. Also, patients can be planned for a staged surgery, meaning a resection with stoma in the first stage and secondly restoration of continuity with an anastomosis or firstly a stoma only and secondly resection with anastomosis.

Less often strictures present in the upper gastrointestinal tract, the colon, and the rectum (see Chap. 20). Crohn's strictures of the colon are best treated with surgical resection, because of the high risk for the presence, or development of cancer [39, 40]. Strictures in the stomach or duodenum are uncommon. Duodenal strictures and strictures of the antrum are preferably treated with stricturoplasty or bypass surgery, because resection requires extensive surgery [41].

17.3 Stricturoplasty or Resection

Once surgery is indicated in stricturing Crohn's disease of the small bowel, the treatment options are either limited to bowel resection or stricturoplasty [42]. Stricturoplasty can be done in pure fibrotic strictures as well as in fibro-inflammatory strictures. The principle of stricturoplasty is stricture lysis and widening, adapted from the pyloroplasty procedure. Remarkably, despite leaving the affected bowel in situ, it has been demonstrated that the mucosa can heal after stricturoplasty, and recurrences are generally in-between the stricturoplasties rather than at the site of the stricturoplasty [26, 43, 44]. Accordingly, even though the location of Crohn's disease is considered to be stable (see Sect. 17.1), the recurrence is more likely to develop at a different site [45]. Strong indications for stricturoplasty are (multiple) short strictures (<10 cm), patients at risk for short-bowel syndrome due to large strictures (>40 cm), prior resectional surgery of more than 100 cm, recurrent strictures at the ileocolic anastomotic sites, and strictures within 1 year from previous surgery [40]. Contraindications are strictures associated with perforation, including abscess and peritonitis, phlegmon and suspicion of carcinoma in the stricture. Whereas short strictures are easily treated with the Heineke-Mikulicz or Finney stricturoplasty [46], the longer strictures demand technically more challenging stricturoplasties. With the Michelassi stricturoplasty, long segments up to 80–100 cm can be treated, resulting in a 40–50 cm stricturoplasty [47]. When stricturoplasty was introduced, concerns were potential increase in septic complications because of preservation of diseased bowel and suture line through macroscopic disease. The main drawbacks of surgical therapy are surgical morbidity and recurrence of disease. However, recent data on long-term outcome of ileocolic resections showed an anastomotic leakage rate of only 3% and a surgical recurrence rate (recurrence requiring surgery) of less than 20% within 10 years [48]. As stricturoplasty and resection for terminal ileum strictures have different indications, no randomised control trial exists to compare outcomes of both techniques. However, it is hypothesized that a stricturoplasty over the valve would have the same therapeutic effect as a surgical resection, with an upcoming RCT to test this hypothesis (see Chap. 20). So far, a systematic review on stricturoplasties concluded that stricturoplasty is safe and effective, even in the presence of active disease [45, 49]. The incidence of overall postoperative complications, septic complications, and surgical complications e.g. anastomotic leak, fistula, and abscess are comparable to resection [46, 50]. Eight studies compared recurrence rates after stricturoplasty and resection for strictures in the terminal ileum (Table 17.1) [39, 51–57]. Three of these studies found no significant difference in the recurrence rate after stricturoplasty and resection [39, 51, 52]. Four of these studies, including patients with multiple lesions, found no significant difference after stricturoplasty versus stricturoplasty and resection [54–57]. One study reported significant superiority of stricturoplasty versus resection, although this data was not corrected for primary and recurrent disease [53]. One retrospective study with over 500 patients reported more surgical recurrences

Table 17.1 Stricturoplasty versus resection for strictures in the terminal ileum

			5-year recurrence rate	
Authors (years)	No. of patients	Median follow-up (months)	*Stricturoplasty* (%)	*Resection* (%)
Sayfan et al. (1989) [51]	82	60	26	26
Broering et al. (2001) [52]	56	86	50	37
Tonelli et al. (2010) [39]	28	120	36	24
Fichera et al. (2006) [53][a]	79	41	45	70
			Stricturoplasty (%)	*Stricturoplasty & resection* (%)
Stebbing et al. (1995) [54]	52	50	35	44
Ozuner et al. (1996) [55]	162	42	31	27
Yamamoto et al. (1999) [56]	111	107	47	32
Tonelli and Ficari (2000) [57]	Not available	48	46	35

[a]Studies reporting significant different recurrence rates

at the site of the anastomoses after resection compared to the site of stricturoplasty, (18% vs 7%, P < 0.01) [58]. Conversly, another systematic review concluded that surgical recurrence after stricturoplasty was more likely than after resection [59]. Additionally, patients undergoing resection had a significantly longer recurrence-free survival than those undergoing stricturoplasty alone. Disease regression in the post-stricturoplasty sites has been evaluated based on cytokine production in biopsies, radiologic -, endoscopic-, and histopathologic regression. One study evaluated cytokine production 1 year after stricturoplasty and reported the same concentration at the post-stricturoplasty site compared to normal mucosa [44]. The hypothesis is that stricturoplasty, resolving the obstruction and releasing the pressure, results in a downregulation of the immune system. An important argument for stricturoplasty is to spare the bowel, and prevent short bowel syndrome. In this respect, it must be stressed that the most important cause of short bowel is the inadvertent resection of unaffected small bowel loops during surgery for complications after surgery for Crohn's disease. Rarely, the disease is so extensive that it requires long segments of resection. The disadvantage of stricturoplasty is the risk of developing cancer as the inflamed segment remains in situ, 5 of 1616 patients developed and died of adenocarcinoma in the small bowel arising at the site after a stricturoplasty [47].

17.4 Approach

Over the past two decades, the implementation of laparoscopic surgery and enhanced recovery programs are the most important achievements in surgery for IBD. In daily clinical practice, several techniques exist to perform an ileocolic resection, comprising of open surgery, hand assisted laparoscopic surgery, and straight laparoscopic surgery via multi-port or single port. The extent of what is done laparoscopically

can also vary between facilitated laparoscopic, laparoscopic assisted, and total laparoscopic approaches. Facilitated laparoscopy includes the combination of laparoscopic and open approach: only mobilisation of the bowel is done laparoscopically. Subsequently, the vascular ligation, bowel transection, and anastomosis creation is done in an open fashion. In the laparoscopic assisted approach mobilisation and vascular division is done laparoscopically, and anastomosis creation is done outside the body. Finally, in the total laparoscopic approach all phases of the operation are done laparoscopically.

17.4.1 Open

Multiple factors are linked to using the open approach. One hard indication of open surgery is multiple prior open operations with extensive adhesions. However, the benefits of laparoscopic surgery for IBD with regards to short- and long-term morbidity, including safety, efficacy, reduced postoperative complication, earlier recovery, and superior body image and cosmetics, are extensively reported and are now established [60–63]. Therefore, laparoscopic ileocolic resection is considered the preferred approach for strictures, as long as appropriate expertise is available. Ultimately, a good open operation is better than a bad laparoscopic one.

17.4.2 Handassisted

Indication for handassisted surgery are fistulas of the terminal ileum to bladder, vagina and sigmoid. Using a handport located in a Pfannenstiel position, the fistulas can safely be disconnected, followed by a handport mobilisation of the right colon. Furthermore, extraction of a more sizable specimens and inflammatory masses is possible with handassistance, without converting to an open approach. In this way, cosmetics and incisional hernia rates can be compromised [64].

17.4.3 Multi-Port

Straight laparoscopic surgery via multi-port for Crohn's disease was first reported in 1992 [65]. For multi-port ileocolic resections, a camera port is introduced at the site of the umbilicus. Additionally, 2 or 3 ports are placed. Mobilisation of the right colon and the terminal ileum is performed from lateral to medial or medial to lateral. The ileocolic anastomosis can be done extra- or intracorporeally. The specimen can be extracted through the umbilical incision by extending the umbilical port access or via a Pfannenstiel suprapubic incision, in case of a larger inflammatory mass.

17.4.4 Single-Port

The single-port technique was first described in 2008 to further reduce the invasiveness of the procedure by avoiding additional port sites [66, 67]. Single port ileocolic resections are performed using a port positioned at the umbilicus, suprapubically, or at the planned stoma site. This site also functions as the extraction site. Regular straight laparoscopic instruments can be used. Mobilisation of the ascending colon can be performed either from lateral to medial or the other way around, according to the surgeon's preference. Again, the ileocolic anastomosis can be done extra- or intracorporeally. Some surgeons put the port suprapubically avoiding an incision in the umbilicus. The prerequisite of single port surgery is that the size of the specimen is relatively small in order to keep the skin incision within the limits of the umbilical folds. Technically, this can be done by a close bowel transection of the mesentery reducing the size of the specimen.

17.4.5 Single Port versus Multi-Port

Single port technique decreases the abdominal trauma by reducing the number of incision sites. Patients undergoing a single port report less postoperative pain with a reduction of the need for analgesics (including opiates) compared to multi-port patients [68]. Regarding postoperative complication, conversion rates to open surgery, and need of stoma creation, no differences between single port and multi-port are recorded. The main advantage of single port ileocolic resection over multi-port is the superior cosmesis limiting the number of scars to one at the umbilicus (Fig. 17.1). Therefore, it represents the ideal surgical approach in predominantly

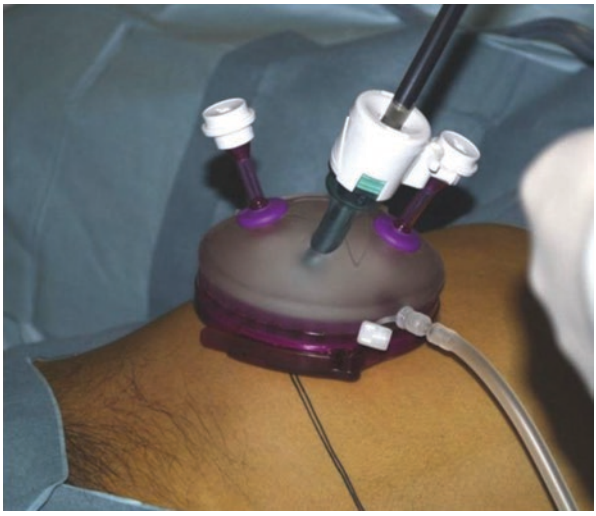

Fig. 17.1 Single port at umbilicus

young CD patients with strong consideration to preserve the body image. Obviously, fewer incisional hernias will occur since single port surgery does not require additional trocar sites. When the single port is placed in the future stoma site, an increase in parastomal hernias has been described, due to enlargement of the stoma site to facilitate the single port and the specimen extraction [69]. It must be stressed, that if single port is difficult, there is no argument against the later insertion of one or two additional trocars or a conversion to multi-port.

17.4.6 Decision Making

The approach and type of surgery depends on disease characteristics (e.g. size of the inflammatory mass), multiple locations, patient characteristics (e.g. prior surgeries), and surgical characteristics (e.g. surgeon's preference and expertise). Preoperative imaging of the inflammatory process must guide the surgeon towards either open surgery or laparoscopic surgery with specimen extraction via umbilicus or Pfannenstiel incision. Sizable masses or fistula towards pelvic organs are best approached via a laparoscopic procedure with a Pfannenstiel incision as the extraction site, whereas the umbilical up and down incision is more suitable for limited masses. It is generally accepted that single-port surgery is more difficult to perform, due to a decrease in the range of motion for the surgeon. In case preoperative imaging was not informative to assist in deciding the best approach, it's recommended to start with a diagnostic laparoscopy via the umbilicus and decide the most optimal approach during surgery [70] (Fig. 17.2).

17.5 Anastomosis

For ileocolic resections four different types of anastomostic configurations are used: side-to-side isoperistaltic anastomosis, side-to-side anisoperistaltic anastomosis, end-to-side anastomosis, and end-to-end anastomosis (Figs. 17.3, 17.4, 17.5, 17.6, and 17.7). Side-to-side (S-S) anastomosis can either be performed by the use of a stapler or handsewn, and end-to-side (E-S) and end-to-end (E-E) only by handsewn. Handsewn anastomosis can be performed as a single-layer or a two-layer anastomosis, depending on the preference of the surgeon. S-S anastomosis consist of isoperistaltic and anisoperistaltic anastomosis. In isoperistaltic anastomosis the fecal stream remains in the original direction (functional end-to-end anastomosis), whereas anisoperistaltic anastomosis interfere with the fecal stream. Grossly, the isoperistaltic stapled S-S is done by close stapling of the resection margins, followed by resection of a corner of the resection line to pass the stapler and construct a S-S anastomosis (Fig. 17.3). Anisoperistaltic stapled S-S is done by enterotomies of the resection margins, next the stapler is passed through both enterotomies to create a S-S anastomosis (Fig. 17.4). The observation that recurrence almost

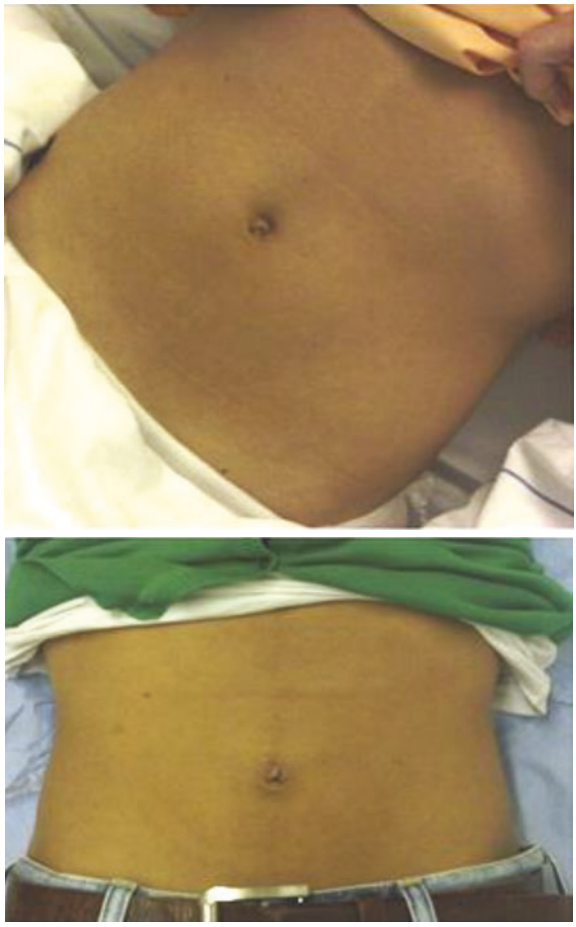

Fig. 17.2 Patient before and after single port ileocolic surgery

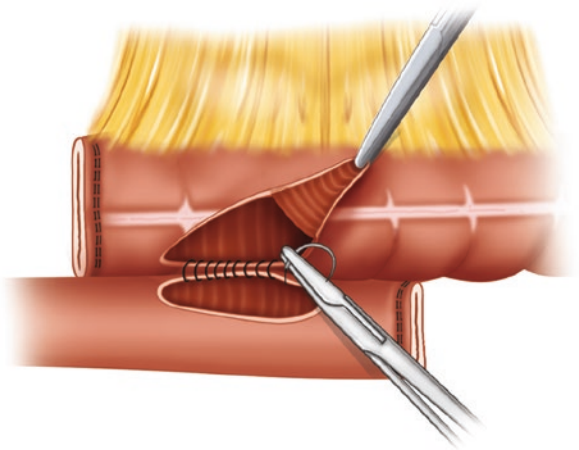

Fig. 17.3 Isoperistaltic S-S handsewn [71]

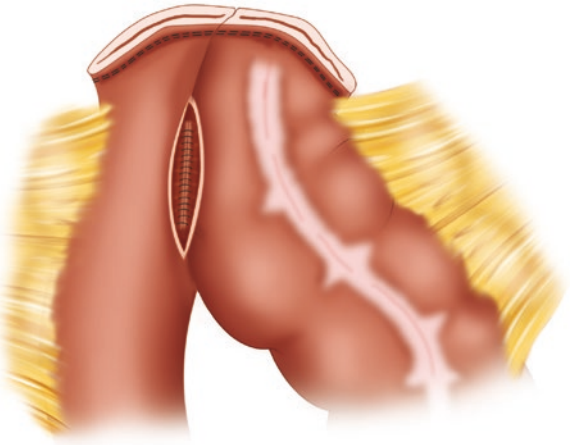

Fig. 17.4 Anisoperistaltic S-S handsewn

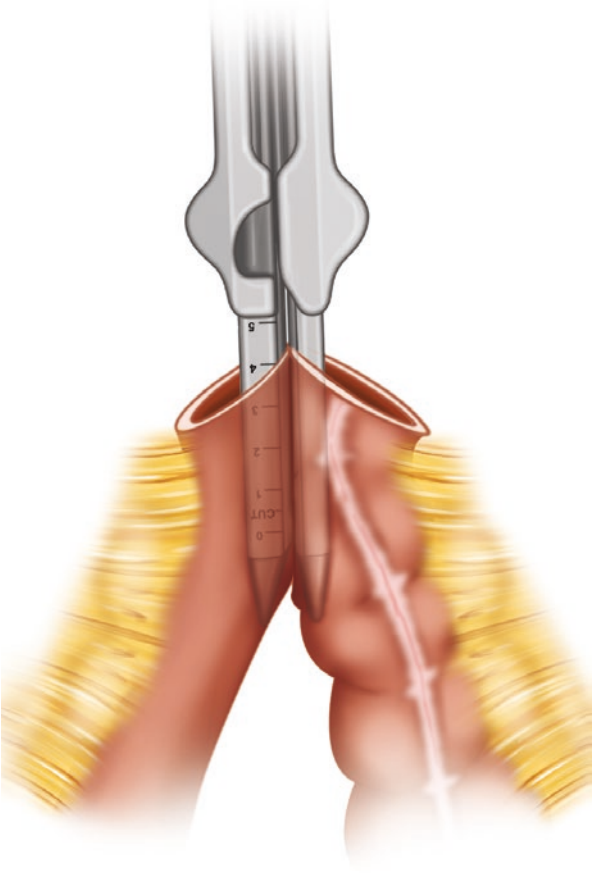

Fig. 17.5 Anisoperistaltic S-S stapled

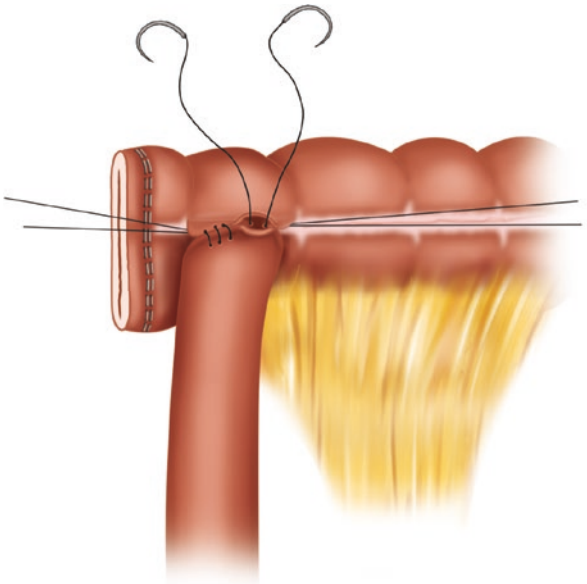

Fig. 17.6 S-E handsewn

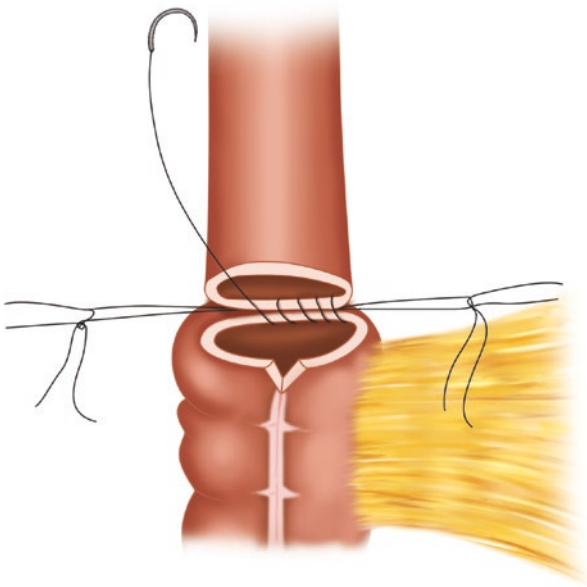

Fig. 17.7 E-E handsewn

invariably appears at the anastomotic site, has led to the assumption that the type of anastomosis could matter. In the literature, predominantly data comparing S-S versus E-E exist. Two studies, including one RCT, showed superiority of stapled anastomosis for anastomotic leakage. For surgical recurrence only a trend of delayed

surgical recurrence compared to handsewn anastomoses was observed [72, 73]. Usually, S-S is a wider anastomosis compared to an E-E anastomosis, less resistance suggests S-S would lead to less complication and postoperative recurrence. This hypotheses was supported for short term outcomes in four meta-analyses, as S-S anastomosis reduced overall postoperative complications and anastomotic leakage rate, compared to handsewn E-E anastomosis [58, 73–75]. One of the meta-analyses specifically compared stapled isoperistaltic S-S versus handsewn E-E and reported superiority of the S-S anastomosis for short term-outcomes [75]. This has not been confirmed in RCT's, reporting similar surgical outcomes, e.g. anastomotic leakage rates, for stapled S-S anastomosis and handsewn E-E anastomosis [76, 77]. Concerning long-term effects, it is hypothesized that a higher resistance to the fecal stream is associated with a higher recurrence rate. Probably all four techniques have a different resistance to the fecal stream, either due to the width of the anastomosis or due to the anti- or isoperistaltic direction. Therefore, the existing evidence directs the surgeon towards using a wide anastomosis of which the aniso- and isoperistaltic anastomosis are the best in this respect [74, 75]. The disadvantages of anisoperitaltic anastomoses are difficult intubation by colonoscopy, and of isoperistaltic anastomoses the creation of a blind loop on both sides of the anastomosis. Taken these studies together with the practical consideration, aniso- or isoperistaltic stapled S-S anastomosis are the preferred types of anastomosis.

17.6 Extent of Resection

Considering the relapsing entity of CD and the risk of short bowel syndrome caused by multiple resections, guidelines advise limited ileocolic resections of macroscopically involved bowel [78]. However, conflicting findings regarding the clinical relevance of inflammation-free resection margins, and new insights into the possible role of the mesentery in recurrent disease and the extent of an ileocolic resection with respect to transection of bowel and mesentery is currently a topic of great interest.

17.6.1 Level of Bowel Transection

Guidelines are based on studies showing no prognostic value of inflamed resection margins, and a RCT demonstrating no difference in extensive vs. limited ileocolic resection [79–81]. So far microscopic positive section margins are accepted as of no importance concerning recurrence. A recent cohort study identified disease activity at resection margins as an independent risk factor for CD recurrence [48]. But, it was difficult to draw clinical conclusions, as no uniform pathological definitions were used. Recently, a study was performed that microscopically analyzed the proximal (ileum) and distal (colon) resection margin separately. Inflammation at the colonic resection margin, and not the ileal site, was associated to increase clinical

recurrence (K. A. Wasmann et al., submitted). This could explain previous discrepancy, as until now the discussion was focussed on the proximal resection margin. However, further research and validation studies are necessary to finally elucidate the clinical consequence of a positive ileum or colon resection margin.

17.6.2 Extent of Resection of Mesentery

In benign IBD surgery the mesentery is spared and typically taken close to the bowel wall. Increasing evidence shows that the mesentery plays an active role in the pathophysiology of Crohn's disease (it is hypothesized that it is an participant in the pathogenesis of fibro-inflammatory and pure fibrotic stenosis) [82–84]. In ulcerative colitis close rectal dissection with preservation of the mesorectum versus total mesorectal excision in ileal pouch-anal anastomosis reduces anastomotic leakage and improves quality of life [85]. In contrast, a study comparing postoperative outcomes in close rectal dissection versus total mesorectal excision proctectomy for therapy-refractory Crohn's disease, leaving the mesorectum in situ, resulted in more perianal complications and decreased perianal healing (E. de Groof et al., submitted) [86]. Moreover, analyzing the mesorectum after close rectal dissection, ongoing pro-inflammatory characteristics could be demonstrated. Although not established yet in clinical studies, it could be hypothesized that a more extended resection of the mesentery reduces postoperative recurrence. The question, if extended 'oncological' mesenteric resection, could reduce recurrence is currently addressed in two RCT's (NCT02542904, and A. Lightner, 2017, open for inclusion).

17.7 Conclusion

In conclusion, clear indications for surgery of Crohn's disease of the terminal ileum are patients with pure fibrotic strictures, patients with symptomatic fistulising disease and most of the patients with abscesses. Patients not responding to anti-inflammatory therapy and immunomodulators, and having a combination of inflammation and fibrotic stricture should be counselled for both medical (anti-TNF) and surgical therapy. This decision making must be done together with the patient (shared decision making). Furthermore, with the validation of a classification of Crohn's disease in the terminal ileum per phenotype (fibro-inflammatory disease, pure fibrotic disease, fistulising disease, and fistulising disease with abscess formation) standardized care per phenotype could be achieved. An RCT is recommended to determine the role of stricturoplasty over the valve compared to surgical resection for Crohn's disease in the terminal ileum. For ileocolic resection, eligible patients should undergo single port surgery with and stapled S-S anastomosis. As stated before, surgery is not a cure for stricturing Crohn's disease.

The 5-year clinical recurrence of surgery for fibro-inflammatory, pure fibrotic, fistulizing, and fistulizing with abscess disease, is up to 45%, and the 10-year surgical recurrence is approximately 20% [1, 48, 87]. Recurrence rate per phenotype have not been described yet. After ileocolic resection for all four phenotypes, postoperative recurrence at the anastomosis is the most common site. Conventional risk factors for postoperative recurrence are smoking, prior intestinal surgery, and absence of prophylactic treatment [78]. Studies analysing risk factors in resection margins and mesentery could determine the most effective extent of resection in Crohn's disease.

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Chapter 18
Management of Ileal Pouch Strictures and Anal Stricturing Disease: A Clinical Challenge

Jean H. Ashburn and Tracy L. Hull

Abstract Restorative proctocolectomy with an ileal pouch-anal anastomosis (IPAA) has been an ideal surgical option for patients with chronic ulcerative colitis (UC), familial adenomatous polyposis, and selected patients with colorectal cancer and Crohn's disease for nearly four decades. In most cases, patients enjoy excellent quality of life with a durable surgical and functional result, avoiding the need for a permanent conventional ileostomy.

Despite great success, patients with IPAA may suffer from several pouch-related complications that are a challenge for the patient and clinician. IPAA-associated fibrotic stricturing disease is one such challenging complication that requires thoughtful judgment for successful management. Treatment of fibrotic strictures of the IPAA requires a multidisciplinary approach involving medical, endoscopic and surgical input for accurate diagnosis, effective treatment, and improvement of quality of life.

The focus of this review is to provide a structured approach to the challenges that the clinician encounters when faced with a patient with IPAA-associated fibrosis and stricturing disease and to discuss the surgical options that alleviate the morbidity caused by ileal pouch fibrosis when medical treatments fail.

Keywords Ileal pouch · Surgery for IPAA stricture · Ileostomy · Multidisciplinary ileal pouch team · Ileal pouch failure · Pouch disorders · Pouch stricture
Ileal pouch fibrosis

J. H. Ashburn · T. L. Hull (✉)
Department of Colorectal Surgery, Digestive Diseases and Surgery Institute, Cleveland Clinic Foundation, Cleveland, OH, USA
e-mail: jashburn@wakehealth.edu; HULLT@ccf.org

F. Rieder (ed.), *Fibrostenotic Inflammatory Bowel Disease*,
https://doi.org/10.1007/978-3-319-90578-5_18

18.1 Introduction

Restorative proctocolectomy with ileal pouch-anal anastomosis (IPAA) has been an ideal surgical option for patients with ulcerative colitis and familial adenomatous polyposis, and very selected patient with colorectal cancer and Crohn's disease, for over three decades [1, 2]. In most cases, patients report excellent quality of life with a durable surgical and functional result, and are able to avoid a lifelong ileostomy [3]. IPAA has undergone several modifications in its approach since it was popularized in the early 1980s. Over this time, innovative approaches have been applied to IPAA surgery, functional outcomes have improved, and pouch survival has remained high when performed in high-volume centers with surgeons experienced in these types of surgery [4, 5].

When surgery goes according to plan and recovery proceeds without event, patients enjoy excellent quality of life with manageable bowel function and are without major lifestyle limitations [3]. However, circumstances may occur in which patients suffer from immediate or eventual IPAA dysfunction with compromised bowel function and quality of life [1, 6, 7]. One cause of a poorly functioning IPAA that poses great challenges to the patient and clinician alike is development of fibrotic stricturing disease in or adjacent to the IPAA. A proposed etiology, diagnostic approach, and management strategies often employed to address this challenge will be discussed at length in the following text.

18.2 Construction of the Ileoanal Pouch

IPAA surgery consists of removal of the colorectum and creation of an ileal reservoir, which is constructed from the distal ileum (Fig. 18.1). The reservoir is joined, using varying methods, to the anorectal ring to restore intestinal continuity. In patients with severe fulminant colitis or who have poor health, the procedure is performed over an extended time period in multiple stages. This usually involves performing a colectomy with end ileostomy, followed by proctectomy with diverted IPAA when health is restored, usually after a waiting period >6 months. In very carefully selected patients who are otherwise fit and have no risk factors for poor healing, a single-stage IPAA may be a safe option, but this should be a rare occurrence [8].

The first reports of IPAA decades ago described construction of an S-shaped ileal pouch that was secured to the anal canal using a hand-sewn anastomosis [9]. A variety of configurations have been considered over time, including the S, J, W and H configurations (Fig. 18.2) [10]. The J pouch is the most popular configuration presently, as it is the easiest and most expeditious to construct and its construction may be assisted by stapling devices [11]. The S and W pouches necessitate a lengthier segment of distal ileum and typically require a hand-sewn approach to construct the

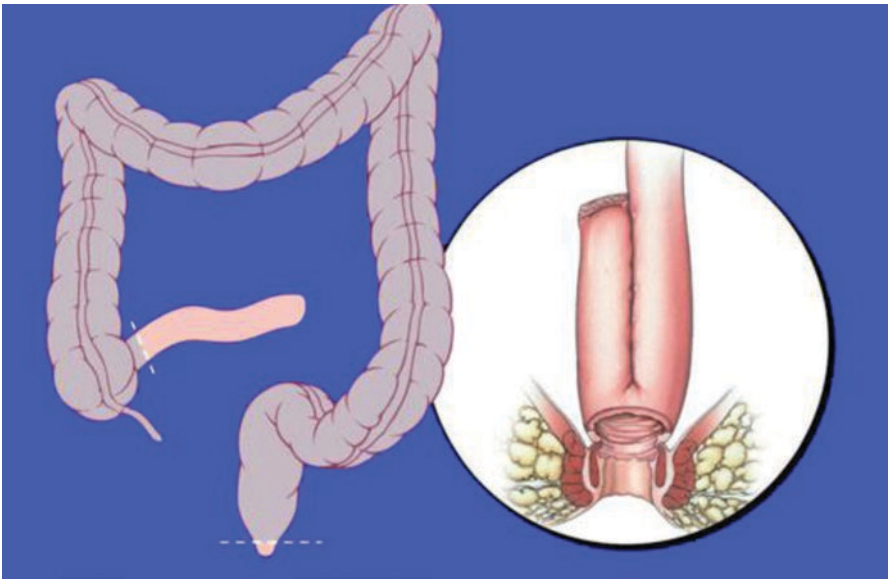

Fig. 18.1 Restorative proctocolectomy with ileal pouch-anal anastomosis (IPAA)

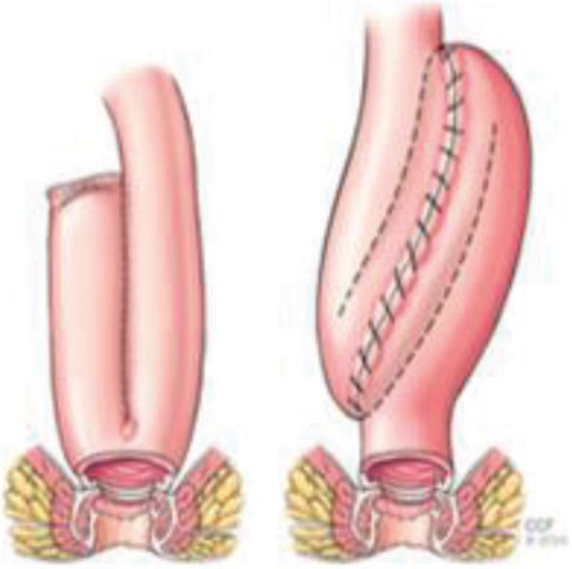

Fig. 18.2 Ileal J pouch (left) and S pouch (right)

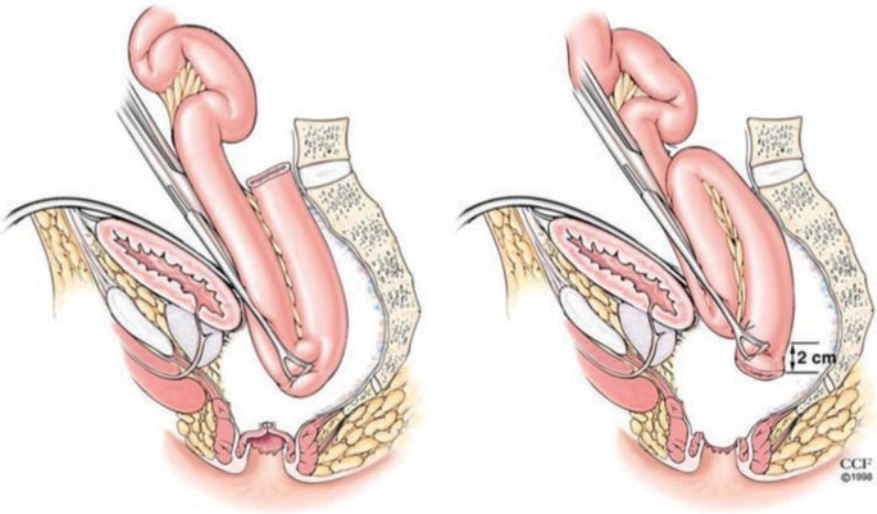

Fig. 18.3 Mesenteric reach with J (left) and S (right) pouch configurations

actual pouch, and thus are more time-consuming and technically challenging to create. The J pouch configuration is most commonly used unless adequate mesenteric length is not available, as creating a tension-free pouch-anal anastomosis is the most critical step to successful pouch surgery. In the case where a J pouch will not reach without tension, an S pouch may be helpful as its configuration allows for a longer reach (2–4 cm longer than J pouch) into the pelvis (Fig. 18.3). A pouch-anal anastomosis created under tension is destined to result in anastomotic leak and pelvic sepsis in the short term, and leads to pelvic fibrosis or chronic pouch ischemia with poor pouch function over time [1].

The ideal method of constructing the pouch-anal anastomosis has long been debated, with the stapled IPAA as the preferred method over hand-sewn IPAA in most instances. The introduction of stapling devices several decades ago made it possible for the stapled IPAA to be less-time consuming and associated with better outcomes than hand-sewn IPAA [12]. In addition, patients with UC undergoing a stapled IPAA rarely develop cancer in the preserved anal transition zone (ATZ) [13]. The stapled IPAA is carried out with either a single or double-stapled approach and the IPAA is joined to the ATZ, thus preserving anal sensory epithelium (Fig. 18.4). Conversely, a hand-sewn IPAA is performed by first removing all anorectal mucosa from the dentate line cranially to the anorectal transection (Fig. 18.5). The IPAA is then delivered into the pelvis and sutured to the internal sphincter at the neo dentate line in a radial fashion. If properly performed, the anal sensory epithelium and all rectal mucosa is removed in this method. However, this method is more likely to exhibit stricture formation at the anastomosis.

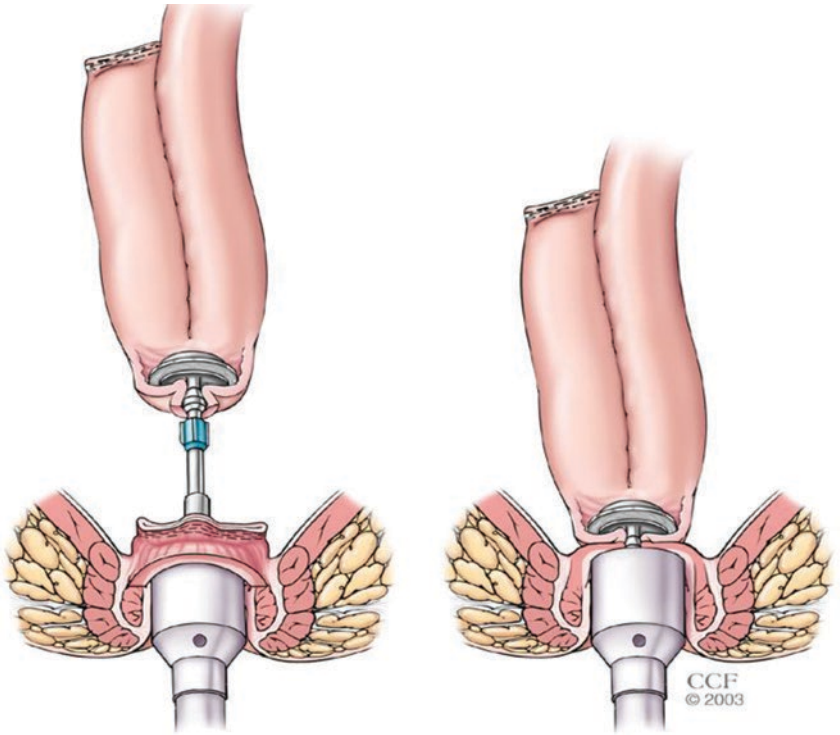

Fig. 18.4 Stapled IPAA

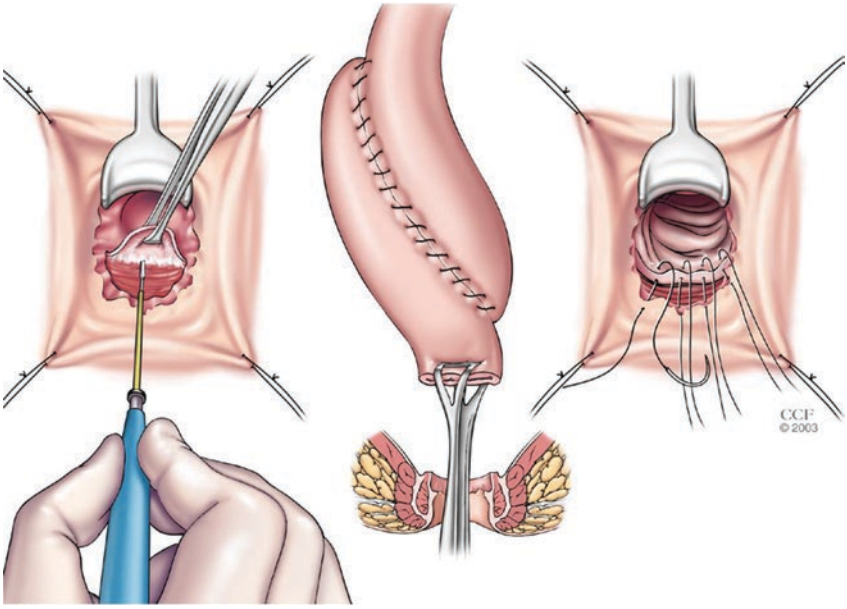

Fig. 18.5 Mucosectomy with hand-sewn IPAA

18.3 Etiology of Fibrotic IPAA Dysfunction

Although many factors may underlie stricture formation, patients who undergo pelvic pouch surgery most commonly develop fibrosis due to the presence of chronic pelvic sepsis. This persistent inflammation in the pelvis and/or anoperineum, if not controlled early, leads to fibrotic changes in the pre-pouch small bowel (afferent limb), pouch body, pouch outlet (efferent limb) or anoperineum [14, 15]. Chronic pelvic sepsis that develops in the months following IPAA surgery is likely the result of technical complications leading to pouch-anal anastomotic leak. Conversely, pelvic sepsis which develops many months to years after IPAA surgery is more likely to be untoward sequelae of Crohn's disease. Regardless of etiology, all pouch-related sepsis necessitates expeditious diagnosis and drainage in order to reduce the risk of stricture development.

Other etiologies have been proposed as causes of IPAA fibrosis and stricture, including weight gain and increased abdominal girth after pouch surgery resulting in excessive mesenteric tension and chronic pouch ischemia [16–18]. In addition, pelvic radiation in the setting of IPAA surgery is associated with pouch fibrosis and subsequent high risk for failure [19].

Regardless of etiology, clinical symptoms from IPAA-related fibrosis depends upon location and severity of inflammation. Fibrotic strictures upstream of the IPAA in the pre-pouch ileum (afferent limb) cause patients to suffer from obstructive symptoms like abdominal pain, cramping, and limited dietary intake of fibrous foods. Bowel motions may be primarily watery or loose, as more bulky components of stool do not pass easily and are detained upstream of the stricture. Fibrosis around or involving the pouch body restricts the ability of the pouch to accommodate and distend, thus reducing its volume and leads to frequent bowel motions. Strictures of the efferent limb (rectal cuff) or anal canal may make pouch emptying difficult, leading to excessive straining, feelings of incomplete emptying, chronic pouch dilation and stretch, and overflow incontinence [20]. Often, a careful and meticulous history can elicit these telltale symptoms from the patient, allowing the clinician to predict the location of stricture even before radiographic or endoscopic evaluation is complete.

An additional site of concern after IPAA surgery is the ileostomy closure site, which may develop stricturing disease due to a subclinical anastomotic leak or ischemia at the time of ileostomy closure, or excessive scar formation after closure (Fig. 18.6). This site must always be interrogated and considered as a part of the

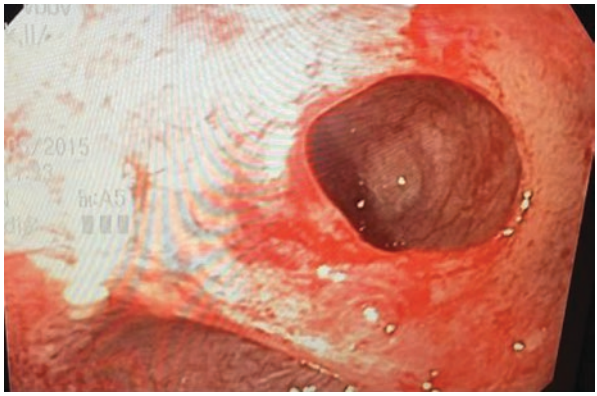

Fig. 18.6 Fibrotic stricture at stapled ileostomy closure site (reuse by permission only JA CCF)

evaluation for IPAA dysfunction as it may mimic obstructive symptoms similar to that of a strictured afferent limb. This would be particularly important in the patient with an endoscopically healthy IPAA but with ongoing obstructive symptoms.

18.4 Evaluation of the Dysfunctional Pelvic Pouch

18.4.1 Initial Evaluation

First and foremost, patients referred with a diagnosis of IPAA-associated fibrosis or stricture should undergo a comprehensive and standardized evaluation for IPAA dysfunction. A complete history should be obtained including a full review of the patient's symptoms, treatments that have been attempted prior to the surgical evaluation, and response to each treatment. Commonly, patients report obstructive symptoms as a result from fibrotic disease of the small bowel and IPAA, with specific complaints dependent on the location of the stricture and fibrosis. They often relay difficulty with abdominal cramping and bloating after meals, intolerance of fibrous foods, and challenges related to storage and emptying of stool from the IPAA.

Operative reports should be obtained and reviewed, with specifics of surgery and convalescence noted. Any indication of technical difficulty must be thoroughly explored, as a technical complication of the initial pouch surgery that may lead to fibrosis development may be easily missed. One should pay particular attention to the condition of the patient at the time of pouch creation and the use of temporary fecal diversion. Large doses of immunosuppression negatively affect pouch healing and anastomotic complications may result in occult sinus tracts, anastomotic leaks, chronic inflammation, and subsequent IPAA-associated fibrosis [21–23]. Also important is a review of the patients weight history, with notations regarding weight and body habitus at the time of IPAA surgery and subsequent changes since this time. Weight gain, specifically growth in abdominal adiposity and girth may put undue tension on the small bowel mesentery, resulting in a relative chronic ischemia leading to fibrotic stricture of the pouch-anal anastomosis [16–18].

A thorough physical exam is necessary during evaluation for several reasons. Fibrotic strictures causing obstruction in or around the IPAA may manifest as chronic abdominal distention and tympany on exam. A contracted, fibrotic IPAA with limited reservoir capacity causes increased bowel frequency and severe perineal excoriation from excessive wiping. An anal exam and anoscopy conducted in the clinic setting may reveal a distal stricture, but is often limited by patient discomfort unless sedation is administered.

Selective use of cross-sectional and fluoroscopic imaging studies help to further characterize symptomatic fibrotic disease. CT enterography gives information regarding stricturing disease in the upper gastrointestinal tract and more proximal small bowel that is not reachable by endoscopy. Distal contrast enema with adequate administration of transanal contrast is helpful to identify fibrotic strictures in or around the pouch, reveal the distensibility of the pouch, and identify strictures

upstream at the ileostomy closure site, all of which may cause or contribute to patient symptoms. MRI of the pelvis demonstrates the presence of pouch-anal sinus and fistula tracts that cause inflammation and stricture of the efferent pouch limb or contracture of the pouch body itself, compromising its function.

Pouchoscopy is an effective diagnostic tool and allows for therapeutic intervention in some situations. The exam is best performed under sedation for optimal patient comfort and minimal disturbance if intervention is performed, or in the operating room as discussed later in this section. This study allows one to locate and characterize the severity of the stricture, identify additional contributory pathology, and allows intervention with endoscopic dilation, therapeutic maneuvers such as needle-knife therapy, and tattooing for eventual surgical localization and therapy [24].

A comprehensive exam performed as outlined above allows the clinician to characterize the location (pre-pouch/afferent limb, pouch body, or pouch outlet/efferent limb) and severity of IPAA-associated fibrosis so the appropriate patient-centered treatment strategy may be formulated. In addition, one must assess and consider the patient's health status and quality of life in the decision-making process, even if the etiology of fibrotic pouch dysfunction is still unclear. Patients are often evaluated after years of suffering that have left them malnourished, decompensated, and mentally exhausted. These individuals may benefit from temporary fecal diversion to alleviate symptoms of fibrotic strictures even though a definitive plan for the primary disease has not yet been established.

Finally, it is important for the clinician to have an honest and straightforward discussion with the patient regarding expectations of treatment for IPAA-related fibrotic disease. It must be emphasized that interventions, whether medical, endoscopic or surgical, may mitigate symptoms and improve quality of life, but also risk damaging the pouch, small bowel, or anoperineum. Inadvertent injuries may require repair or temporary fecal diversion, or even lead to pouch excision and permanent conventional ileostomy. Expectations must be discussed and agreed upon prior to embarking on these interventions.

18.4.2 Multidisciplinary Approach to Diagnosis

When a patient presents with symptoms concerning for IPAA-related fibrosis, the authors often use a multidisciplinary approach to evaluate the IPAA. After preoperative evaluation with history, physical, and radiographic testing as outlined above, an evaluation with an anoperineal exam under anesthesia with pouchoscopy is performed as a team by the colorectal surgeon and gastroenterologist. The anoperineum, pouch-anal anastomosis, pouch body, and afferent limb (complete to the ileostomy closure site) are examined with members of both specialties in the operating room, offering both perspectives of expertise. Any clinical signs of IPAA complications are noted (anastomotic sinus or fistula, stricture, pouch prolapse, Crohn's disease, etc.), many of which may cause similar symptoms [25]. Biopsies are obtained for pathologic review. At the completion of the exam, the findings are

discussed with the patient and family member along with a patient-centered treatment strategy. This multidisciplinary team approach is ideal for the patient as he/she is presented an immediate plan for treatment with opportunity for discussion with members of both specialties. The strategy can always be tailored at a later time as pathology results or recommendations from our Multidisciplinary Inflammatory Bowel Disease Conference are available.

18.5 Treatment Strategies for IPAA-Related Fibrosis

Any IPAA-related intervention must be preceded by a frank discussion with the patient regarding the possibility of injury to the pelvic pouch or associated small bowel requiring urgent laparotomy, attempt at repair, and need for fecal diversion. Unfortunately, some situations may result in irreparable injury requiring pouch excision, and the patient must understand and accept this risk prior to embarking on endoscopic or surgical management. Ideally, the patient, endoscopist and surgeon together make coordinated decisions in a multidisciplinary and patient-centered fashion, with a contingency plan present and rehearsed in case surgical exploration is required to address complications.

18.5.1 Pre-IPAA (Afferent Limb/Ileostomy Closure Site)

Choosing the best treatment option for strictures in the pre-IPAA bowel begins with assessment of severity and etiology of disease. Asymptomatic strictures found incidentally on routine endoscopy may be left alone, or gently dilated to prevent progression. Mild to moderate strictures that receive an ileoscope can be treated with balloon dilation for effective yet controlled expansion of the strictured segment. Severe strictures that do not easily receive an ileoscope or allow only a wire to cross may also be treated this way, but must be performed with great caution as risk for perforation or luminal hemorrhage is great and risk should be balanced with benefit of proceeding. Strictures at the ileostomy closure site may be dealt with in a similar way with cautious and gentle balloon dilation to minimize risk for complication [26].

Most recently, endoscopic needle-knife strictureplasty, has been proposed as a method of endoscopically 'coring' the fibrotic ring of the strictured segment instead of breaking the fibrotic ring with outward pressure, as is the case with balloon dilation [27].

This technique requires specialized skill, comfort with the needle-knife technique, and a readily available and willing surgeon experienced in IPAA repair [28]. For these reasons, it is a technique best performed in high-volume referral centers. Initial reports of the success of this technique are few but promising, and long-term efficacy studies are needed [29, 30].

Often, endoscopic therapies, particularly balloon dilation, result in reformation of scar at the strictured site, and subsequent recurrence of symptoms. If a patient enjoys a

relatively long symptom-free period after the initial dilation, one may consider repeat dilation when symptoms do recur. However, progressively shortened symptom-free periods between dilations may prompt the patient and clinician to consider other options for treatment. In these circumstances, surgical intervention should be considered.

Strictures proximal to the IPAA at the ileostomy closure site or afferent limb are best approached by means of strictureplasty or small bowel resection with primary anastomosis (Fig. 18.7) [31]. If strictureplasty is performed, a Heineke-Mickulitz type is most common, in which a longitudinal enterotomy created on the anti-mesenteric segment of the intestine is closed transversely in a handsewn seromuscular fashion, thus expanding the luminal diameter. These are ideal for short-segment strictures. When a small bowel resection is preferred, the surgeon must divide the mesentery just underneath the bowel lumen to prevent compromise of the blood supply to the IPAA. A primary end-to end anastomosis is best employed to recreate intestinal continuity at these locations (Fig. 18.8); however, a stapled anastomosis is

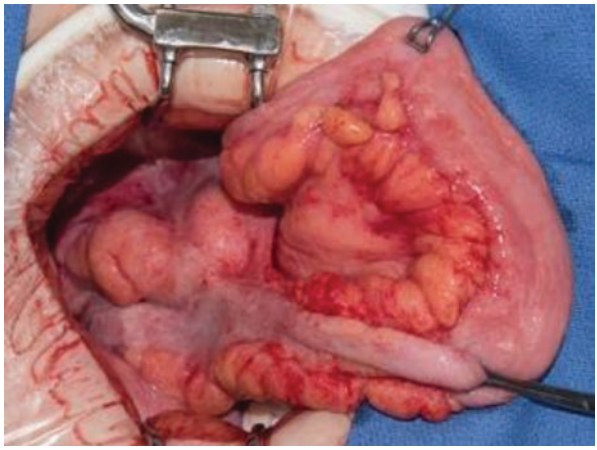

Fig. 18.7 Fibrostenotic Crohn's disease of the afferent limb of IPAA (reuse by permission only JA CCF)

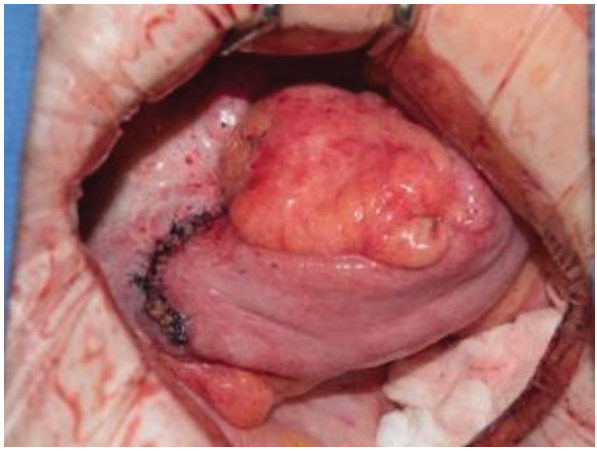

Fig. 18.8 Small bowel hand-sewn anastomosis after resection of afferent limb stricture (reuse by permission only JA CCF)

an effective alternative to this if carefully performed, with transection at the pouch inlet (below the fibrosis) with a linear stapler, careful resection of the affected segment, and joining of the distal ileum to the top of the pouch or tip of J with an EEA circular stapler that is transanally introduced.

Patients who are failing to thrive, immunosuppressed or generally unwell in the perioperative period are best considered for diverting ileostomy at the time of repair to mitigate the potential of anastomotic leak, with ileostomy closure at a later time when the patient has recovered his or her health. Patients exhibiting pre-pouch fibrosis as a result of fibrostenotic Crohn's disease are re-initiated on medical therapy as soon as possible after recovery from surgery.

18.5.2 The Fibrotic IPAA Body

Fibrosis involving the pouch body is a unique and often very morbid situation, and is most commonly a sequelae of chronic ischemia. The IPAA is typically contracted with poor distensibility and is only able to hold low volumes of stool. Patients complain of frequency and urgency of bowel motions, excoriation of the perianum due to frequent soiling and need for frequent cleaning. Those who are severely affected by symptoms should be considered for fecal diversion or pouch excision. In some circumstances, patients may be considered for a redo IPAA if the reason for failure of the first pouch is clearly identified and able to be avoided at the time of a second attempt. One example of this is the situation of a patient who has gained excessive abdominal adiposity since creation of the IPAA. The small bowel mesentery is put on stretch and slowly produces a relative ischemia of the pouch and resultant fibrosis [16, 18]. An acceptable strategy for this patient is for him/her to first pursue adequate weight loss to achieve ideal body weight, followed by redo of the IPAA and strict maintenance of this weight after surgery.

18.5.3 Post-IPAA (Efferent Limb, Anal Canal)

Strictures that are distal to the IPAA are most easily assessed in the outpatient clinic setting, as these are apparent on digital exam. However, although selected patients may be amenable to awake exam or gentle dilation, most will be too uncomfortable for much more than a brief assessment. A thorough exam, typically done in the operating room or sedation suite, is often necessary to determine specifics and etiology of disease, as this is critically important to determining treatment options (Fig. 18.9).

Patients who suffer from fibrostenotic Crohn's disease of the anal canal or pouch-anal anastomosis should first undergo drainage of sepsis with seton or mushroom drains, as soon as possible, to reduce the risk for worsening fibrosis. Those with symptomatic strictures may be offered serial dilations under anesthesia or regular

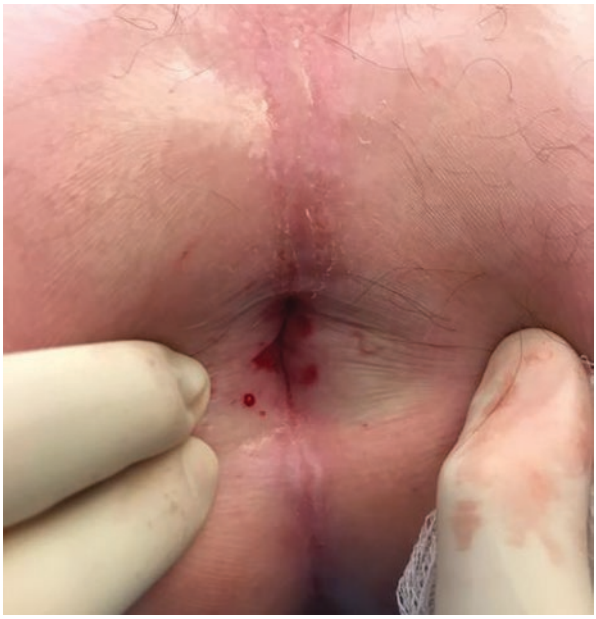

Fig. 18.9 Assessment of a post-IPAA stricture

home dilations performed by patients themselves or willing caregivers [26]. Periodic steroid injection into the fibrotic ring is thought to slow the recurrence of scarring at the time of dilation. Fibrosis will most likely progress, however, and patients deserve a discussion early on regarding the option of fecal diversion to alleviate symptoms if/when they worsen. Severely symptomatic patients are candidates for IPAA excision or proctocolectomy, both with permanent conventional ileostomy.

Outlet strictures that develop for reasons other than Crohn's disease may have treatment options in addition to those described above. Strictures may develop due to chronic ischemia affecting the exit conduit of an S pouch or chronic pelvic sepsis after anastomotic leak in a J pouch. An elongated exit conduit of the S pouch may also develop trauma-related fibrosis over time if transanal intubations are required for emptying. In these cases, surgical correction of the stricture may be performed. Transanal pouch advancement or a combined transabdominal/transanal pouch revision allows for removal of strictured tissues and recreation of a well-vascularized, tension-free anastomosis [3, 7, 32]. Those patients who are appropriate candidates should undergo a full evaluation for redo IPAA if they desire, and should never be dissuaded from pouch excision with permanent conventional ileostomy if they are accepting of this option.

18.6 Conclusion

IPAA-associated strictures present a multi-dimensional challenge that requires a clear understanding of the sequelae of fibrotic disease and is best managed through the combined efforts of physicians and surgeons with appropriate experience and

interest. Medical therapy is not commonly helpful in most cases, and when goals of treatment are not met with endoscopic modalities, early surgical evaluation and intervention is critical to ensure proper treatment and optimal patient outcomes. The ideal surgical approach to IPAA stricture is dependent upon location and severity of disease, as well as the individual goals set forth by this subset of patients with diverse characteristics and desires. The best approach for patients suffering with IPAA-related fibrosis who require intervention involves a multi-disciplinary team approach, early surgeon involvement, and a central focus on goals of curing the disease and avoiding a permanent ileostomy while preserving QOL.

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Chapter 19
Stricturing Crohn's Disease: Strictureplasty

Gabriele Bislenghi and Andre D'Hoore

Abstract The occurrence of strictures as a complication of Crohn's disease is a significant clinical problem which may be present at initial diagnosis or develop many years later. Clinical presentation depends on stricture location and severity of stenosis. Strictures frequently contain a mixture of inflammatory and fibrotic tissue. To date, no antifibrotic agent exists and the effect of anti-inflammatory and immunomodulatory drugs on the fibrotic component of Crohn's strictures remains extremely limited. In this scenario, surgery is frequently unavoidable. Resection of the affected bowel segment has represented for decades the only surgical option. In order to preserve bowel length, non resective techniques such as strictureplasties have gained progressively a role in the treatment of Crohn's strictures and have been proven to be comparable to resections with regard to early and long-term postoperative results and recurrence rate. To better appreciate similarities and differences among the several techniques proposed, strictureplasties can be classified into three main groups including Heineke-Mikulicz like procedures, intermediate procedures (Finney and Jaboulay) and entero-enterostomies (Michelassi like procedures). As ultimate bowel sparing technique, a modified side-to-side isoperistaltic strictureplasty over the ileocecal valve has been recently proposed with encouraging results. Furthermore, mucosal healing has been frequently observed at endoscopic evaluation after surgery. It might be speculated that the alleviation of faecal stasis disrupts the inflammatory process, restores the physiological microbial-mucosa interaction and promote the anatomical and functional recovery of the treated bowel segment. In view of this, the notion of the irreversibility of intestinal fibrosis has to be challenged. Further research should better clarify the mechanism of mucosal healing and delineate therapeutic approaches to trigger the reversal of fibrosis in Crohn's disease.

Keywords Crohn's disease · Fibrosis · Stricture · Surgery · Strictureplasty

G. Bislenghi · A. D'Hoore (✉)
Department of Abdominal Surgery, University Hospital Leuven, Leuven, Belgium
e-mail: gabriele.bislenghi@uzleuven.be; andre.dhoore@uzleuven.be

F. Rieder (ed.), *Fibrostenotic Inflammatory Bowel Disease*,
https://doi.org/10.1007/978-3-319-90578-5_19

19.1 Introduction and Epidemiology

Fibrostenosing Crohn's disease is defined by persistent luminal narrowing. The clinical presentation generally includes the onset of obstructive symptoms [1]. The natural history of Crohn's disease (CD) is highly heterogeneous. Even though the most common initial presentation of CD is purely uncomplicated inflammatory disease, within 10 years of diagnosis more than 70% of CD patients develops a stricturing or perforating complication [2–6]. Stricturing and perforating disease, which may simultaneously coexist, represent the main indication for surgery in CD patients [7, 8]. (Fig. 19.1) Historically, patients with penetrating complications were believed to have more aggressive disease, with an elevated risk of clinical and surgical recurrence. In the Vienna, Montreal and Paris Classifications these patients are scored as having the highest level of disease complications, particularly if the disease is located in the terminal ileum or in the colon. However, this view has been recently reconsidered [9, 10].

Although stricturing CD is relatively uncommon at the time of diagnosis, its occurence increases over time. At the time of diagnosis, strictures may occur in about 5% of patients, whereas up to 30% of the patients develops stricturing complications within 10 years [7, 11, 12]. Distal small bowel and ileocolic anastomosis are the most common sites of involvement while colonic stricturing may occur in up to 17% of patients and proximal small bowel and upper gastrointestinal strictures occur in up to 5% [13]. Furthermore, in this population group, surgery results in high recurrence rates [3]. Up to 30% of patients develop clinical recurrence 1 year after surgery, with a 10% increase after each subsequent year. The clinical recurrence rate can be as high as 20–60%, and the rate of recurrence requiring surgery between 15–50% after only 5 years [14, 15].

Predictors of fibrostenosing CD are clinical (age of diagnosis <40 years, perianal disease at diagnosis, need for steroids during the first flare, and small bowel disease location), environmental (smoking), endoscopic (deep mucosal ulcerations),

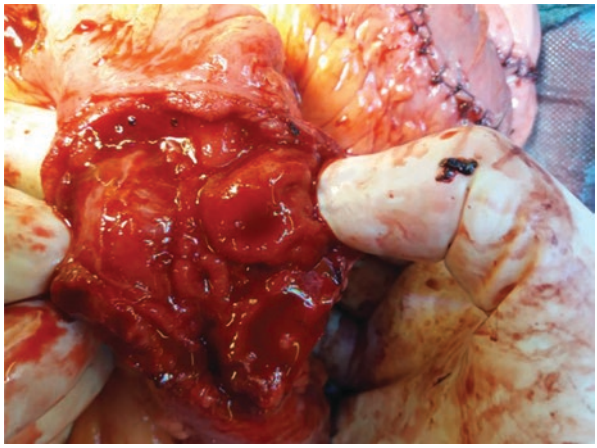


Fig. 19.1 Intraoperative image: longitudinal opening of the terminal ileum reveals severe mucosal inflammation

genetic (NOD2/CARD 15, 5T5T in the MMP3 gene, rs 1,363,670), and serological (antimicrobial antibodies) [7]. Diagnosis during childhood, jejuno-ileal location, and stricturing behaviour are independent risk factors for elevated risk of surgery and high surgical recurrence rates for small bowel CD [16]. In patients with multiple locations not all the diseased segments present with the same level of inflammation or the same type of complication (stricture and fibrosis, perforation, or both). The Montreal and Paris classifications are useful tools and should be used in the surgical reports, since they impact on the post-operative treatment and the long-term prognosis [2–4, 9, 10].

19.2 Pathophysiology

Extracellular matrix accumulation and mesenchymal cell expansion play a major role in the mechanism of stricture formation and result in transmural thickening of the bowel [7]. Inflammation and fibrosis are closely intertwined injury pathways and coexist in intestinal stenosis at varying degrees [7]. The fibrotic process, once initiated by inflammation, can progress independently from inflammation. Currently used anti-inflammatory medications do not delay this development and are only effective in limiting the inflammatory component of the disease process [17]. For that reason, the presence of on-going active inflammation within a strictured segment increases the likelihood of response to anti-inflammatory and immunomodulatory medications. However, even after the introduction of anti-TNF therapy since 1998, the global rates of surgery for CD remain essentially unchanged. All available therapies are essentially unable to reverse the progression of fibrosis in the susceptible patient [18, 19].

19.3 Diagnosis

Radiologic and endoscopic evaluation play a major role in the work-up of patients with fibrostenosing CD, providing information about disease activity and anatomical characteristics that will determine the actual therapy. Up to now, cross-sectional computerized axial tomographic or magnetic resonance imaging (MRI) were basically unable to differentiate between inflammatory and fibrotic stenosis [20]. However, MRI has recently gained an increasing role in the differential diagnosis of CD stenosis [21]. Late images obtained with MRI enterography after intravenous administration of a gadolinium chelate help distinguish between active (inflammatory) and inactive (fibrotic) stenotic lesions. Predominant fibrostenotic lesions show delayed enhancement, whereas active lesions show an early enhancement pattern [22]. Patients who have delayed, homogeneous and full-thickness wall enhancement may be more likely to have a higher fibrotic component of their stricture. This assumption has been recently verified in 41 patients with 44 small bowel strictures

who were scheduled for elective surgery. As result of the correlation between radiological images and histopathologic scores of the resected bowel segments, it was possible to identify patients with severe degree of fibrosis [20].

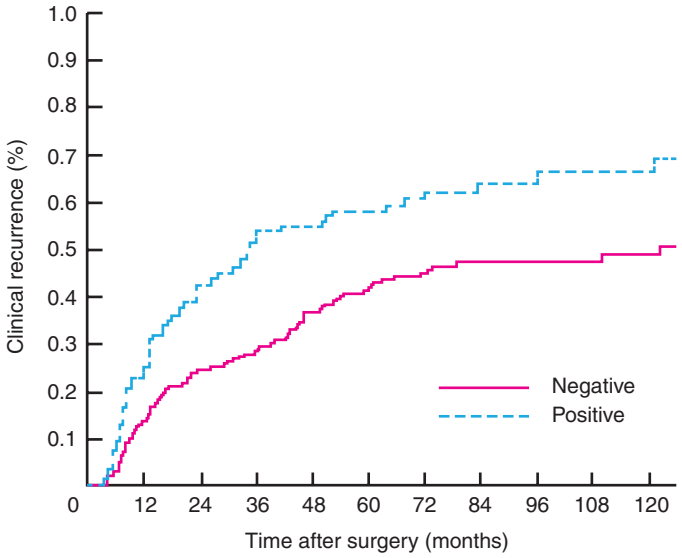
19.4 Surgical Approach

After failure of medical therapy and endoscopic dilatation, or inability to perform endoscopic dilatation, surgical resection of the affected segment is currently the most commonly used treatment strategy [7]. Dilatation is indicated for short accessible strictures in quiescent disease. Balloon enteroscopy facilitates dilatation anywhere in the gastrointestinal tract but most procedure have been described in stenotic ileocolic anastomoses. Multiple small series have demonstrated the safety and short-term efficacy of endoscopic dilatation for stenosis of 15–25 mm length. Long strictures and dilatation of nipple valve anastomosis (telescoped) are associated with technical failure. Balloon dilatation is impractical if multiple strictures are present [23].

19.4.1 Resections

Radical resections did not show a clear advantage compared with limited resections in terms of recurrence. The microscopic presence of inflammation at the resection margin influences the occurrence of postoperative recurrence [24–26]. The only randomized controlled trial from the group of Fazio published in 1994 [24], analyzing data of 152 patients undergoing ileocolic resection for CD, found that extended resection margins (i.e., 12 cm vs. 2 cm for the limited resection) confer no advantage in terms of cumulative recurrence rates (25.3% in the limited resection group and 17.9% in the extended resection group with a median follow-up of 55.7 months, $p = 0.31$). The presence of residual microscopic disease at the resection margins does not appear to give significantly increased recurrence rates compared with free margins (21.4% for CD microscopic involvement and 17.8% for histologically normal, $p = 0.91$). These findings were confirmed in a retrospective study of 77 patients with CD undergoing resection (medium follow-up 5.6 ± 2.8 years), where the recurrence rate of the resections with involved margins was 36% and 38% for free margins resections [25].

Hence, resections for CD should be as limited as possible. Considering the relapsing behaviour of CD and the risk of short bowel syndrome caused by multiple resections, it is common practice to limit the resection to the macroscopically involved bowel, even accepting microscopic positive resection margins. However, we recently published data showing that microscopic involvement of the resection margin have a significant impact on recurrence rates [27]. Further prospective research is needed to further elucidate the importance of positive resection margins for both clinical and surgical recurrence before a more aggressive approach is adopted (Fig. 19.2).



No. at risk											
Negative	316	216	168	143	117	96	74	60	48	39	32
Positive	121	78	56	43	40	34	24	19	15	12	12

Fig. 19.2 Kaplan-Meier curves for surgical recurrence according to microscopic resection margin involvement. P = 0.010 (log rank test) from de Buck van Overstraeten A., BJS 2017

The type of anastomosis does not seem to play a role in disease recurrence rate. In a multicenter, randomized, controlled trial with 139 patients who underwent an ileocolic resection [26] the recurrence rate was similar whether end-to-end anastomosis or side-to-side anastomosis was performed (42.5% in the end-to-end anastomosis group vs 37.9% in the side-to-side anastomosis group, p = 0.55 and 21.9% in the end-to-end group vs 22.7% in the side-to-side group, p = 0.92 for endoscopic and clinical recurrence, respectively). A wide lumen stapled side-to-side anastomosis is found to be associated with decreased anastomotic leak rates and overall postoperative complications compared to end-to-end anastomosis. A meta-analysis including 661 patients with ileocolic resection for CD reported an anastomotic leak rate of 6.7% for end-to-end anastomosis versus 1.2% for side-to-side anastomosis (p = 0.02) and overall postoperative complication rate of 21.2% for end-to-end anastomosis versus 11.2% for side-to-side anastomosis, (p = 0.2) [28]. No significant difference with regard to peri-anastomotic recurrence rates was found [28, 29]. The same was found in a prospective cohort study showing no differences in safety and recurrence rate between hand-sewn side-to-side and stapled side-to-side anastomosis. This may imply that a wide anastomotic luminal diameter is the discriminating factor affecting recurrence, rather than the suturing technique used [30]. A laparoscopic approach is preferable for ileocolonic resections in CD where appropriate expertise is available [31–34].

19.4.2 Strictureplasties

19.4.2.1 History

Although the main goal of surgical interventions is to alleviate symptoms, limiting bowel resection is critical to maintaining the absorbtive function of the small and large intestines. Removing excessive lengths of intestine with traditional segmental resection can result in short bowel syndrome (SBS), in which patients suffer from malabsorptive symptoms and, in some cases, can lead to serious consequences, including a profound decrease in life expectancy [35]. A retrospective analysis of postoperative CD patients found that 8.5% had suffered intestinal failure within 20 years after their initial operation [36]. In attempting to preserve bowel length and to reduce the risk of leak, patients with fibrostenosing jejuno-ileal involvement can be managed by means of strictureplasty. Strictureplasty techniques were originally developed for the upper gastrointestinal tract, where strictures arose from ulcer disease. Nonresective operative techniques were preferred in this region given the anatomical limitations of the pancreaticobilliary system, which often led to unacceptably high rates of morbidity with more traditional resection methods. The three most common procedures performed for stricturing peptic ulcer disease are the eponymous Heineke-Mikulicz, Finney, and Jaboulay strictureplasties. Rather than undergoing resection or bypass, these procedures allowed for increasing the luminal diameter of the bowel, while avoiding segmental resection. The concept of using strictureplasty for multiple small intestinal strictures was first described by Katariya et al. in 1977. In an effort to avoid segmental resection in treating multiple tandem tubercular strictures of the intestinal tract, they demonstrated that the use of strictureplasty not only preserved the intestinal absorptive capacity, but was also a safe alternative to segmental resection or bypass [37]. This work was followed by Lee and Papaioannou from Oxford in 1982, who published their use of strictureplasty for the treatment of Crohn strictures in nine patients, eight of whom were successfully treated with either Heineke-Mikulicz or Finney techniques with follow-up ranging from 8 to 42 months [38].

19.4.2.2 Indications

The main indication for strictureplasty is the presence of multiple small bowel strictures within a long segment of bowel. Although the initial view was that strictureplasty should only be carried out for recurrent disease and in patients who have had previous multiple resections [38], the consensus now is that any patient with a nonphlegmonous fibrotic lesion is suitable, depending on the length of the stricture [39]. The most obvious advantage of strictureplasty over resection is that the risk of short bowel syndrome is lowered.

General contraindications to performing strictureplasty are preoperative malnutrition, the presence of phlegmon/fistula/perforation at the planned strictureplasty site, a stricture next to an already planned resection site, multiple strictures within a very short segment, and any suspicion of small bowel malignancy. Given the limits

of preoperative imaging of the small intestine, specific planning for strictureplasty is often made intraoperatively, and is typically based on the location of active disease. For instance, strictures located in the jejunoileal and ileocolonic anastomotic regions have been shown to respond well to strictureplasty techniques, as opposed to duodenal or colonic locations [40]. The presence of an enteric fistula surrounded by chronic inflammation is not a contraindication in most cases.

19.4.2.3 General Technique

As with all Crohn's disease patients, the operative approach should always begin with examination of the entire small bowel from the ligament of Treitz to the ileocecal valve. This can be achieved either laparoscopically or via laparotomy. This allow the surgeon to create a sort of "roadmap". This is an essential step to design a surgical strategy based on the number, length, and relative location of CD lesions.

During the abdominal exploration at least one stricture site should be identified and opened. Further strictures can be identified either by introducing the index finger into the lumen and passing the gut over the finger in a concertina fashion, or by the use of the balloon 'pull-through' technique. This involves passing an 18-Fr Foley catheter on an introducer into the original enterotomy (Fig. 19.3). Most of small bowel can be pulled over one introducer but sometimes a second enterotomy is needed. If another stricture is detected and incised, this becomes a new entry point for the catheter. Once the catheter is passed up to the duodenum or down through the ileocaecal junction, the balloon is inflated with 8 ml water to give a balloon diameter of 25 mm. The balloon is then withdrawn and is held up wherever the lumen is less than 25 mm. If necessary the lumen can be sized by serial deflation (8 ml = 25 mm, 6 ml = 20 mm, 4 ml = 10 mm). Most 25 and all 20 mm or less strictures are incised longitudinally with diathermy [41].

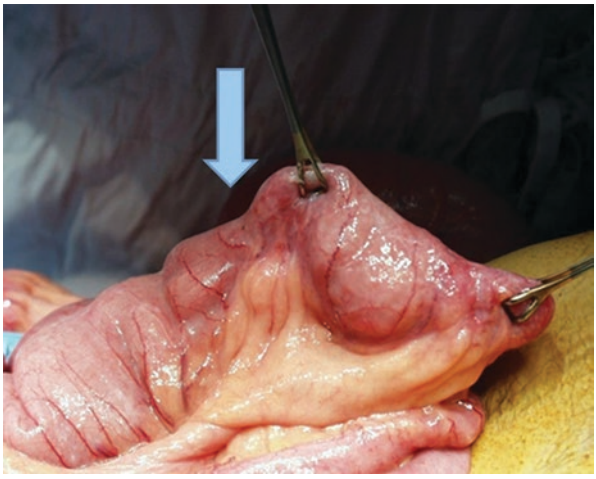


Fig. 19.3 A typical peroperative finding of a localized stricture (short) with creeping fat, luminal narrowing and prestenotic dilation

Table 19.1 Stricturoplasty classification

Conventional strictureplasty
Heinecke-Mikulicz
Modified: Judd
Moskel-Walske-Neumayer
Finney
Modified: Jaboulay
Non-conventional strictureplasty
Side-to side isoperistaltic (Michelassi)
Modified: Poggioli
Sasaki

Adapted from Ambe R, J Gastrointestinal Surgery 2012

At the end of the procedure, small metal clips are used to mark the strictureplasty site extraluminally for future identification in case of recurrent obstructive symptoms. Metal clips can be visualized radiographically on subsequent investigations or intraoperatively at successive operations.

As mentioned above the use of the Heineke-Mikulicz and the Finney strictureplasty in Crohn's stricture was first described more than 30 years ago [38, 42]. Since then modifications of the Heineke-Mikulicz strictureplasties and of the Finney strictureplasty as well as advanced strictureplasty techniques have been proposed [42]. All these techniques can be grouped into various categories: conventional versus non-conventional, short versus long or based on technical difficulty. Yet, the choice of the technique ultimately rests on length, number, and location of strictures.

Campbell et al. classified these into conventional [Heineke-Mikulicz and Finney] and non-conventional strictureplasties [43]. Up to now 15 different procedures have been proposed over the years in an attempt to provide more options to patients and facilitate the possibility of preserving bowel length. As remarked by Ambe et al. in their review there is much overlap and similarity between these techniques; disparities can be better appreciated when they are classified into three main groups including Heineke-Mikulicz like procedures (Heineke-Mikulicz, Judd, Moskel-Walske-Neumayer, double Heineke-Mikulicz, ileocolic Heineke-Mikulicz,), intermediate procedures (Finney, Jaboulay, combined Heineke-Mikulicz and Finney) and entero-enterostomies (Michelassi: side-to-side isoperistaltic and the modifications of Poggioli and Sasaki) [42]. (Table 19.1).

19.4.2.4 Conventional Strictureplasties

Heineke-Mikulicz Strictureplasty (Fig. 19.4)

The Heineke-Mikulicz is the most commonly performed strictureplasty and best used for short (≤7 cm) strictures [42, 43]. A single longitudinal incision is made on the antimesenteric side of the stricture extending approximately 2 cm beyond the thickened segment of bowel, both proximally and distally. The enterotomy is then closed transversely with a single or double layer closure with single stitches. To facilitate the closure the index finger can be inserted through the incised stricture from an adjacent

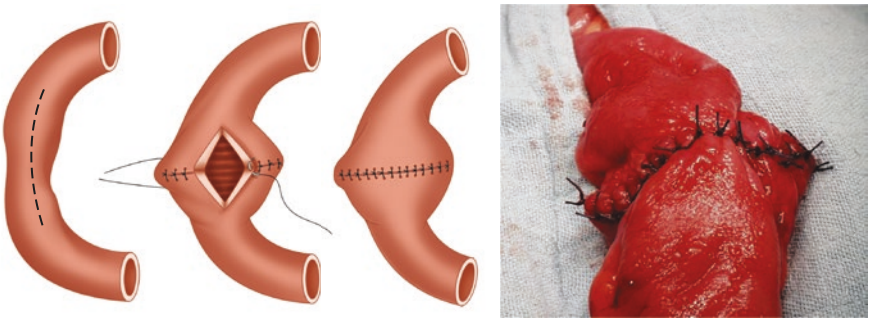


Fig. 19.4 Heineke-Mikulicz strictureplasty on a short segment stricture

opened stricture. The finger stents the anastomosis and helps the cut edges to be everted. Seromuscular sutures are used, sparing the mucosa. The distance between the stitches depends on the thickness of the gut wall.; it is always thinnest in the center of the suture line where there is also the most tension. If the gut is thickened or there is some tension at the center of the suture line, a central mattress stay suture is used. When this is tied, it indicates how much tension there is at the center of the suture line and helps to hold the edges together while performing the remnant suture.

This strictureplasty enlarges the lumen of the diseased intestine and maintains intestinal transit without creating a blind loop or intestinal stasis.

Finney Strictureplasty (Fig. 19.5)

This is one of the conventional techniques used to manage medium-sized strictures usually >10 and <25 cm [44, 45]. Multiple adjacent or confluent strictures are all opened longitudinally until normal gut is reached proximally and distally. The incised gut is bent over in a loop, folding the strictured segment in a "U"-shape manner. The posterior wall is sutured with a continuous seromuscular stitch. A stay suture helps to approximate the apices of the long incision. When the posterior suture meets the apical stay suture, they are knotted together and the suture is continued up the anterior wall.

Finney strictureplasty results in the creation of a lateral diverticulum and subsequent functional bypass while relieving obstruction. The lateral diverticulum can result in luminal stasis and bacterial overgrowth and blind loop syndrome.

19.4.2.5 Modified: Conventional Strictureplasties

Judd Strictureplasty

In this technique, the strictured segment has an associated fistulous opening at its center. In this technique, the fistulous site is excised, and the remainder of the short segment (<10 cm) stricture is then opened in a longitudinal manner, encompassing the opening of the excised fistula. The defect is then closed as in the HM technique.

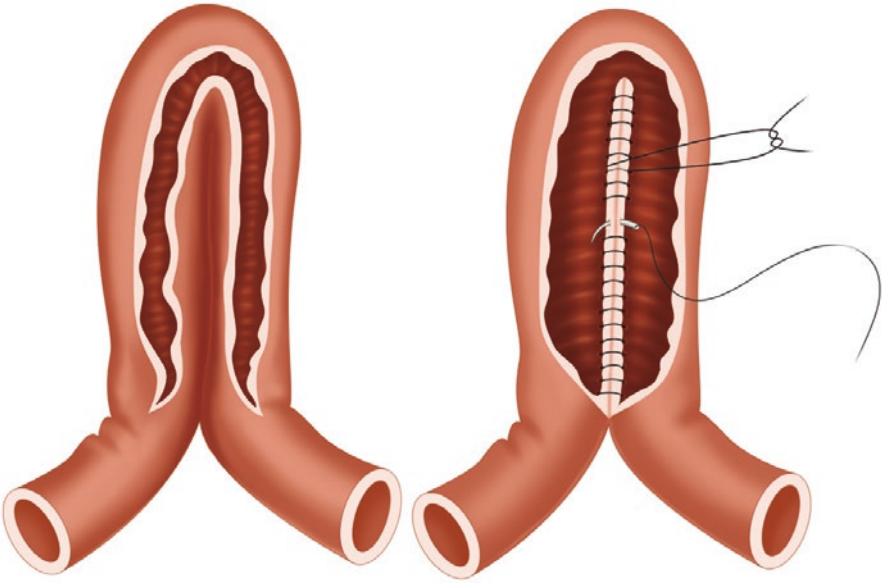


Fig. 19.5 Finney strictureplasty

This technique confers the benefits of HM strictureplasty, as it is technically easy to perform with no significant tension on the suture line [45].

Moskel-Walske-Neumayer Strictureplasty

This type is suited for short segment strictures (<10 cm) in which there is dilatation of the proximal portion of the bowel. A Y-shaped longitudinal enterotomy is made across the stricture with the fork of the "Y" pointing toward the dilated portion. The defect is then closed in the HM fashion (Fig. 19.3). This technique finds favor due to the fact that it is technically easy to perform and reduces proximal dilatation of the bowel while conferring a gentle transition from dilated to nondilated bowel.

Jaboulay Strictureplasty

This technique was initially described back in the late nineteenth century by Mathieu Jaboulay. It is very similar to the Finney procedure and consists in a side-to-side enteroenterostomy. The Jaboulay strictureplasty is suitable for medium-sized (>10 and <25 cm) strictures and can also be performed with the stapler technique. With this technique, bowel length is spared; however, there is the creation of a lateral diverticulum with resulting blind loop and stasis in the strictured segment.

Limitations of the conventional strictureplasties is the length of stricture that can be treated. Taking into account implications of geometry, sequential use of HM stricutreplasty is limited to lesions at a distance of about 10 cm from each other to allow a 5 cm distance between strictureplasties. Furthermore, long Finney type strictureplasties create a "diverticulum" on the small bowel and result in possible stasis of content with bacterial overgrowth. This certainly is the case if the Jaboulay strictureplasty bypasses longer segments of diseased bowel.

These shortcomings are addressed by the new non-conventional isoperistaltic strictureplasties.

Side-to-Side Isoperistaltic Stricutreplasty (Referred to as Michelassi Stricutreplasty) (Fig. 19.6)

The Michelassi side-to-side isoperistaltic strictureplasty is indicated for significantly long strictured segments (>20 cm) or a long portion of bowel containing multiple short strictures in tandem, making the creation of multiple HM strictureplasties unsafe [46]. The mesentery of the small bowel loop to undergo the strictureplasty is first divided at its center. The proximal small bowel loop is then moved over the distal one in a side-to-side fashion. The stenotic segments of one loop are placed adjacent to the dilated segments of the other loop. The two loops are then approximated by a layer of interrupted seromuscular Lembert stitches with nonabsorbable 3-0 sutures. A longitudinal enterotomy is performed on both loops, with the intestinal ends tapered to avoid blind ends. Hemostasis is achieved with suture ligatures or electrocautery. The outer suture line is reinforced with an internal row of running full-thickness 3-0 absorbable sutures, continued anteriorly as a running Connell suture; this layer is reinforced by an outer layer of interrupted seromuscular Lembert stitches with nonabsorbable 3-0 sutures. The benefits of this technique include relief of intestinal obstruction created by multiple strictures in sequence, avoidance of resecting a long segment of bowel containing normal absorbing intestine in between strictures, and avoidance of blind and bypassed loops of bowel. This technique may be challenging to perform in the presence of a thickened and shortened mesentery (Figs. 19.7, 19.8, and 19.9).

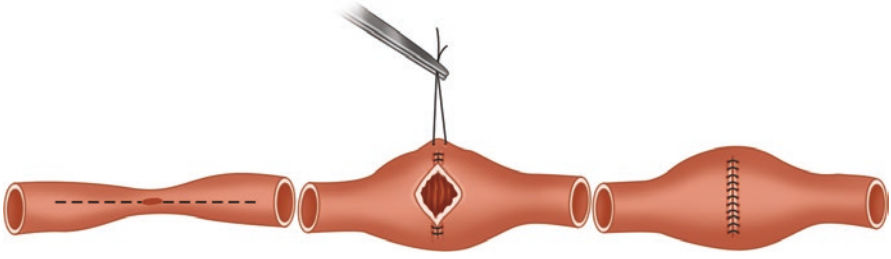


Fig. 19.6 Judd stricutreplasty

Fig. 19.7 M-W-N stricutreplasty

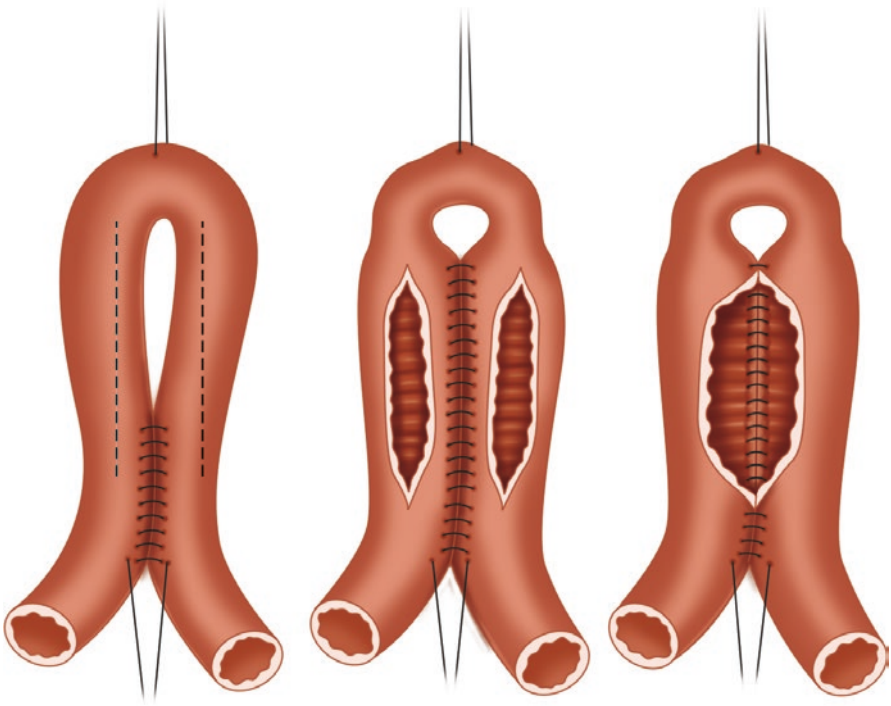


Fig. 19.8 Jaboulay stricutreplasty

Poggioli Strictureplasty

A modified form of Michelassi's side-to-side isoperistaltic enteroenterostomy has been proposed and published by two groups: Poggioli et al. [47] and Di Abriola et al. [48] respectively. These authors describe a technique whereby a long strictured segment (>20 cm) of bowel is plastied using a modification of the side-to-side isoperistaltic strictureplasty technique described by Michelassi. The technique begins by severing the bowel and dividing the mesentery at the proximal junction of the stricture. The non-diseased bowel is then advanced over the strictured segment. A

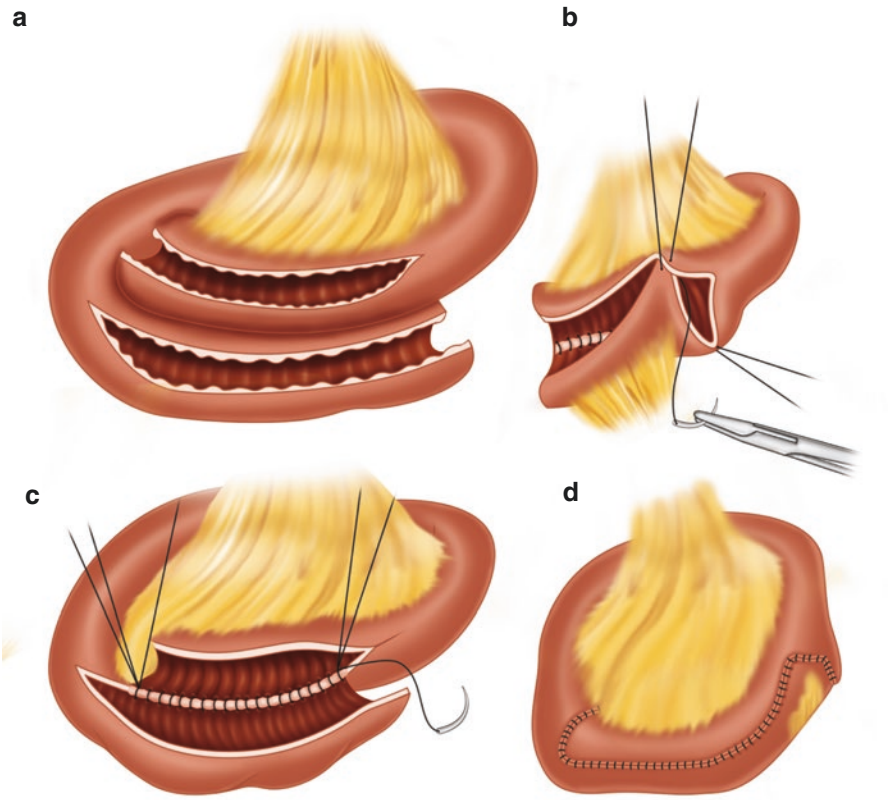


Fig. 19.9 Michelassi strictureplasty

longitudinal enterotomy is made on both overlapping segments, and a side-to-side enteroenterostomy is then performed in the usual manner. The use of proximal, non-diseased bowel offers better laxity of the mesentery and better suture line integrity. However, it should be cautioned that this technique is challenging to perform and carries the inherent risk of a potential two-fold bowel loss should the repair fail or a complication arise (Fig. 19.10).

Allthough the initial diameter of the small bowel is larger in the Poggioli stricutreplasty the main disadvantage is the risk of extensive bowel loss shall the stricutreplasty need to be resected.

Sasaki et al. [49] describe a variant of Michelassi's technique in which Heineke–Mikulicz strictureplasty is added to both ends of the strictureplasty (Figs. 19.11 and 19.12).

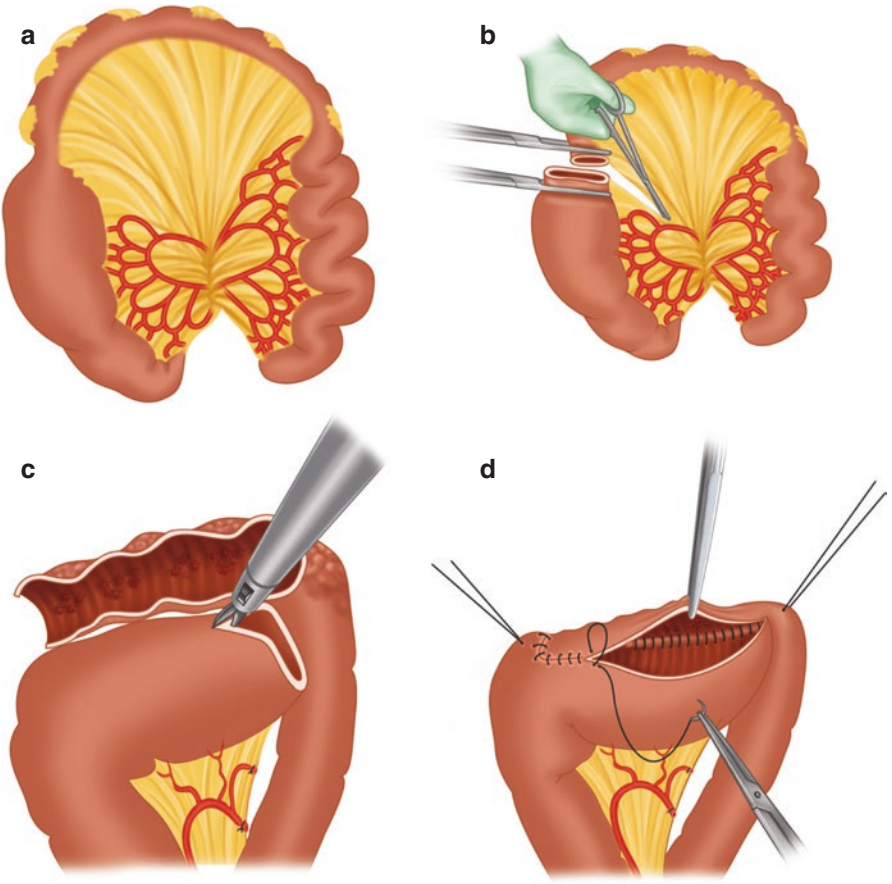


Fig. 19.10 The Poggioli isoperistaltic stricutreplasty

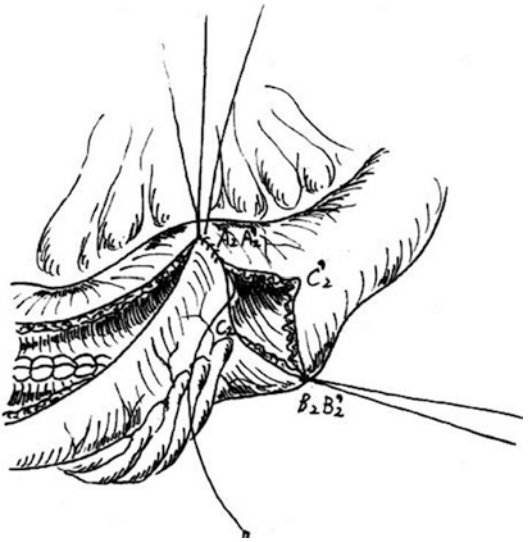


Fig. 19.11 Sasaki modification at the in- and outlet of the isoperistaltic stricutreplasty to avoid any early stenosis at the inlet

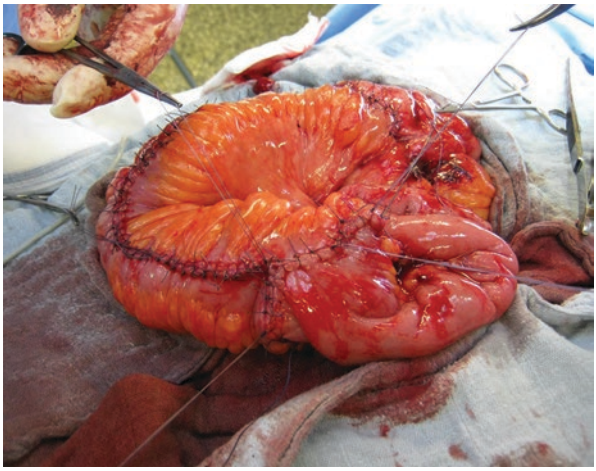


Fig. 19.12 Peroperative view an a long isoperistaltic strictureplasty with a Sasaki modification at the inlet of the strictureplasty

19.4.2.6 Results

Short-Term Results

The safety and feasibility of strictureplasty for Crohn's disease has been well validated. In a meta-analysis by Yamamoto et al. [40], 1112 patients who underwent 3259 strictureplasties were studied. The overall morbidity rate was 13% and the mortality rate was nil. Only 4% of patients developed a septic complication, such as anastomotic leak, abscess formation, and fistula. Less than half of these patients required a laparotomy for sepsis. The strictureplasty site was commonly associated with sepsis (78% of patients). The postoperative hemorrhage rate was 3%. In a more recent systematic review and meta-analysis more than 1600 patients who had over 4500 stricturoplasties were described [43]. The reported overall complication rate was low, ranging from 5% to 20% and mortality rate was nil. Long-term recurrence rates ranged from 25% to 70%. Interestingly, half of the patients included had strictureplasties as the first surgical procedure.

The most common complications after strictureplasty were small bowel obstruction (2.6%), intra-abdominal septic complications and suture leakages (4.2%), and intra-luminal and intra-abdominal bleeding (3.2%), with a cumulative re-operation rate of 2.8%. The main risk factors claimed to influence post-operative complications were malnutrition, hypoalbuminemia, unscheduled surgery, peritonitis, intra-abdominal septic complication with peritoneal contamination, anemia, and older age. In contrast, steroid use, synchronous bowel resection, and number, site, or lengths of strictureplasties were not significant risk factors. Only 5 cases of adenocarcinoma of the small bowel arising at a strictureplasty site have been reported (0.3%.) Therefore, routine biopsy with frozen section before performing a strictureplasty is not advised [40, 42, 43, 50, 51].

Strictureplasty is safe and does not confer increased morbidity when compared with small resections and anastomosis. In a meta-analysis by Reese et al. [52] 662

patients, who underwent strictureplasty or bowel resection were examined. The overall early postoperative complication rate of strictureplasty was 12.7% compared to 19.1% in the resection group, septic complications occurred in 8.1% of strictureplasties, and 11.2% of intestinal resections, and postoperative hemorrhage rates in 3.0% vs. 6.7%, respectively. None of these differences was statistically significant.

A word of caution about the actual role of strictureplasty in small bowel Crohn's disease: the indications of strictureplasty has been expanded over the last years probably because many authors reported the procedure as safe. If strictureplasty is an alternative treatment to resection for fibrotic strictures under all circumstances remains unclear. Several studies have compared the outcomes of the two techniques. However, in those studies strictureplasty was mainly used for short fibrotic strictures and resection was used for phlegmonous disease, long strictures, abscess and fistula. The inclusion of a wide variety of disease presentations obscures their comparison. Therefore, there is no clear direction for surgeons in choosing one or the other procedure. Well-designed prospective studies are necessary to compare the outcomes of strictureplasty and resection.

Data for procedure-specific recurrence rates are available only for a few strictureplasty techniques.

Campbell's meta-analysis has compared the efficacy and safety of conventional strictureplasty techniques (Heineke-Mikulicz, Finney) to nonconventional strictureplasty techniques (modified Finney, combined Heineke-Mikulicz and Finney, modified Heineke-Mikulicz, Michelassi, and others). The Heineke-Mikulicz technique was the most commonly (>90%) used conventional strictureplasty. The Michelassi technique was the most commonly used non-conventional strictureplasty (>80%). Nonconventional strictureplasty had the same, if not lower rates, of complications compared with the more conventional techniques. Specifically, long-term (recurrent stricture, small bowel obstruction, reoperation, carcinoma, and deaths) and short-term (small bowel obstruction, sepsis, postoperative bleed, other infections) complications were analyzed. Early complication rates were 15% for conventional strictureplasty versus 8% for nonconventional strictureplasty, while late complications were 29% for conventional strictureplasty versus 17% for nonconventional strictureplasty. Non-conventional, advanced strictureplasty techniques do not confer a higher postoperative morbidity risk than conventional, simpler strictureplasty techniques [43].

The Michelassi side-to-side isoperistaltic strictureplasty should be discussed separately. This type of strictureplasty has been validated as feasible and safe in several smaller studies. An international multicenter observational study of 184 patients with Crohn's disease who underwent a side-to-side isoperistaltic strictureplasty determined that the overall morbidity rate was low, ranging from 5.7 to 20.8%. In this series, the length of diseased bowel selected for strictureplasty ranged from 20.8 ± 9.9 cm to 64.3 ± 29.3 cm and synchronous strictureplasties were performed in 41.9–83.3% of cases [51].

Long-Term Results

Strictureplasty sites are not immune from disease recurrences. Reese et al. [52] found no difference in terms of recurrence or need of surgical treatment between strictureplasty and resections (37.8% vs 31.0%). Patients undergoing strictureplasty were 8% more likely to experience surgical recurrence than patients undergoing resection ($p = 0.01$). The site of the recurrence was not specified. Similarly, Bellolio et al. reported a reoperation rate after strictureplasty of 45.7% at a median follow-up of 63 months. The surgery free-survival after 5 and 10 years was 70.7% and 26.6% respectively. Again, the site of recurrence was not reported [53]. In the series with the longest mean follow-up (107 months), 54% of patients had developed a symptomatic recurrence and 44% required surgery at 10 years [54]. Dietz et al. [55] in a retrospective review of 314 patients who underwent 1124 strictureplasty procedures reported an operative recurrence rate of 34% within a 7.5 years of follow-up period. Yamamoto analyzing more than 3200 strictureplasties found a 5-year site-specific recurrence rate of only 3% [40]. Fichera et al. published data about 78 patients with 134 sites requiring operative intervention (85 requiring resection and 49 amenable to strictureplasty). Significantly fewer recurrences at strictureplasty sites compared to resection sites (45% vs. 70%; $P < 0.05$) were observed.

Data for procedure-specific recurrence rates are available only for a few strictureplasty techniques. Campbell et al. reported recurrent structuring disease in 32% of patients with conventional strictureplasties and 17.8% of patients with unconventional strictureplasties over a mean follow-up of 50 months. In the series of Tichansky et al. [56] recurrence rates ranged from 23% for the Finney strictureplasty to 32% for the Heineke-Mikulicz strictureplasty. Interestingly, only 8% of recurrences occurred on a previous strictureplasty site, most of the recurrences occurring away from it. Similarly, Yamamoto analyzes data from 3259 strictureplasties and assesses an overall symptomatic recurrence rate of 39% for jejunoileal strictures (161 out of 411 patients) and 36% for ileocolonic strictures (9 out of 25 patients). Strikingly, only 3% or 20%, respectively, of strictures were found at the previous site of strictureplasty [42].

Over the years ambiguous long-term results have been reported when the Finney-like strictureplasties have been compared to the Heineke-Mikulicz. In some papers this technique has been reported to have a higher recurrence rate, probably due to the creation of a large, lateral diverticulum with faecal stasis and bacterial overgrowth [55]. Experience with recurrences and need of surgery is accumulating slowly after performance of a side-to-side isoperistaltic strictureplasty. One observational study [51] reported recurrence rates of 7.6% after a mean follow-up of 35 months, with most recurrences sited at the inlet and outlet of the side-to-side strictureplasty. As consequence of this observation, some authors began to advocate performance of a Heineke-Mikulicz strictureplasty at the inlet and outlet, respectively. Recently, Tonelli et al. published long-term results about 91 patients undergoing side-to-side strictureplasty. This is one of the series with the longest follow-up. Fourty-four percent of patients developed a recurrence at a median follow up of

55 months. The recurrence involved the strictureplasty site in 24 patients (28.9%) after a median follow up of 48 months, being surgical in 15 patients. The surgical recurrence affected the SISS body in 8 patients, the inlet in 4 patients and the outlet in 3 patients. Age at diagnosis, family history and smoking habit were found to be independent factors of relapse. The S-S plasty lead to a resolution of symptoms in more than 90% of cases. Even after long-term follow-up data suggest encouraging results for this type of strictureplasty [57].

19.5 Future Perspectives

There is an ongoing quest to avoid classical ileocaecal resection and to encorporate strictureplasties over the ileocecal valve. This idea is not new but was limited to the length that could be treated with the classical sitructreplasty (Fig. 19.13).

However, there was a recent interest to adapt the isoperistaltic stricutreplasties as an alternative for resection. As the ultimate bowel sparing techniques, a modified side-to-side isoperistaltic strictureplasty over the ileocaecal valve for the treatment of terminal ileal Crohn's disease has recently been proposed [58, 59]. Several surgeons have used a side-to-side isoperistaltic strictureplasty to treat terminal ileal

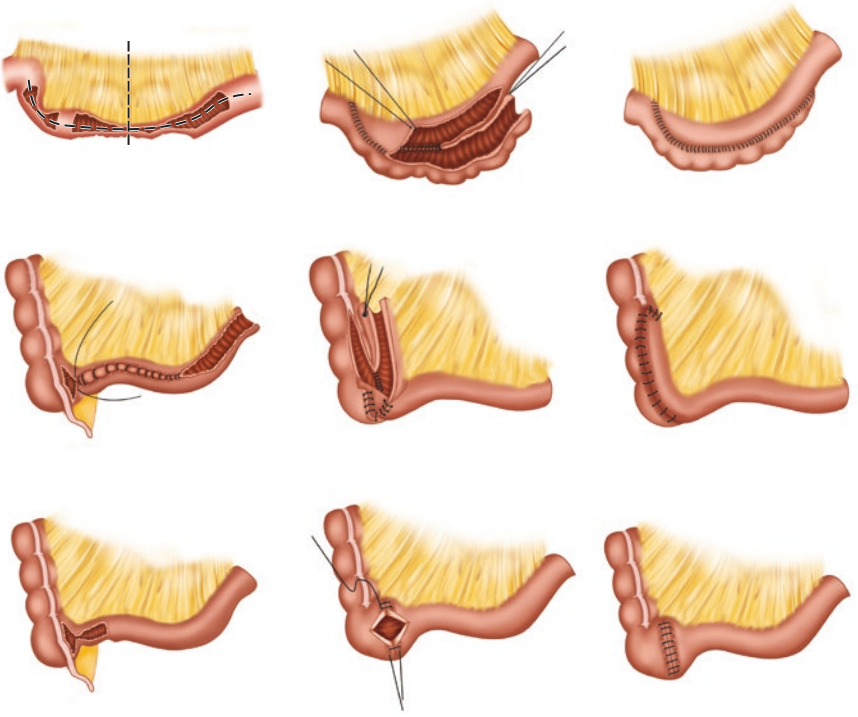


Fig. 19.13 Finney and HM over the valve for short terminal and valve strictures. These techniques are merely reserved for short anastomotic strictures

Crohn's disease (CD) and some have performed a side-to-side isoperistaltic ileocolic strictureplasty (SSIS), incorporating the ascending colon into the strictureplasty. Others have combined a segmental resection with an SSIS. Our preference is not to incorporate the terminal ileum into the strictureplasty unless it is diseased. This avoids the loss of healthy bowel when a resection of the strictureplasty might be necessary because of leakage or surgical recurrence. When performed the strictureplasty is extended through the ileocaecal valve, which is often affected by disease. After laparoscopic mobilization of the right colon, the terminal ileum and proximal part of the ascending colon are exteriorized through an umbilical incision. The length of the diseased segment is measured. A suitable point to divide the bowel and mesentery is selected in the middle of the loop. A short healthy area of bowel avoiding excessive fibrosis or inflammation is preferred to avoid too much traction on the strictureplasty. The bowel and part of the mesentery are divided to enable mobilization of the proximal part through the ileocaecal valve. The most proximal loop is opened longitudinally by monopolar cautery and the strictureplasty is started at the outlet, using interrupted Vicryl 20 sutures (Johnson & Johnson (New Brunswick, New Jersey, USA); Vicryl suture 2-0, V323H) for the posterior suture line. The more distal loop is then opened longitudinally to include the ileocaecal valve and the anterior suture line is inserted. Additional separate stitches are used as required for haemostasis. An appendectomy is routinely performed in all cases. The strictureplasty ends at the inlet, giving the opportunity to perform the Sasaki modification, which incorporates enlargement at the inlet by an HM strictureplasty (Fig. 19.14).

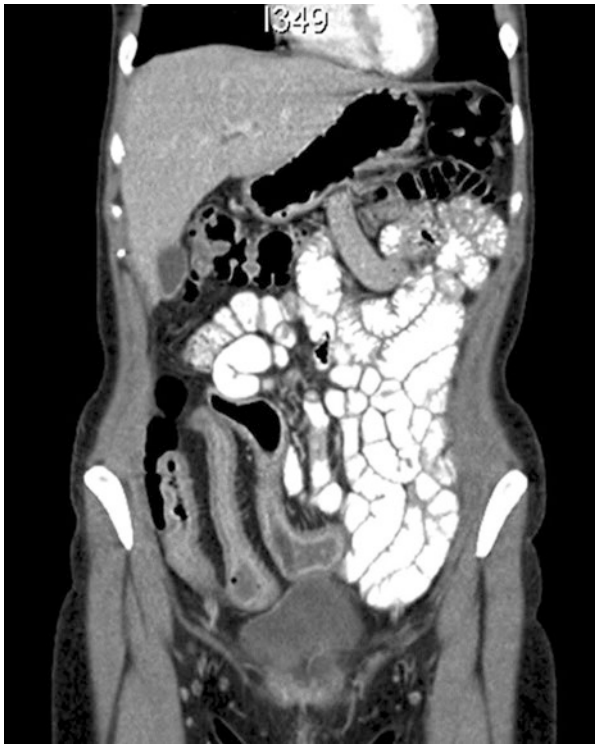


Fig. 19.14 MR enterography demonstrating a long diseased terminal and preterminal ileum. Classical surgery would indicate ileocaecal resection

To allow bowel sparing in this case a long stricutreplasty isoperistaltic can be performed (Fig. 19.15).

The same technique is very useful if multiple sequential strictures are present in the ileal region adjacent to the valve. Performing sequential HM or Finny will lead to stasis and recurrence (Fig. 19.16).

Results of over 40 patients have been published with an acceptable low rate of early postoperative complications (2 anastomotic leakage). One patient had to be reoperated on owing to the formation of stenosis at the inlet and a new short strictureplasty (HM) was performed. Another patient underwent adhesiolysis for adhesion obstruction. To date, no resection of the strictureplasty has been required. After a median follow-up of 33 months 27 patients (68%) are free of symptoms. Of them 9 patients (22.5%) remained in remission without any adjuvant medical treatment. Thirty-one patients (77.5%) needed postoperative medical treatment. Thirteen (32%) fail to reach clinical remission (Figs. 19.17 and 19.18).

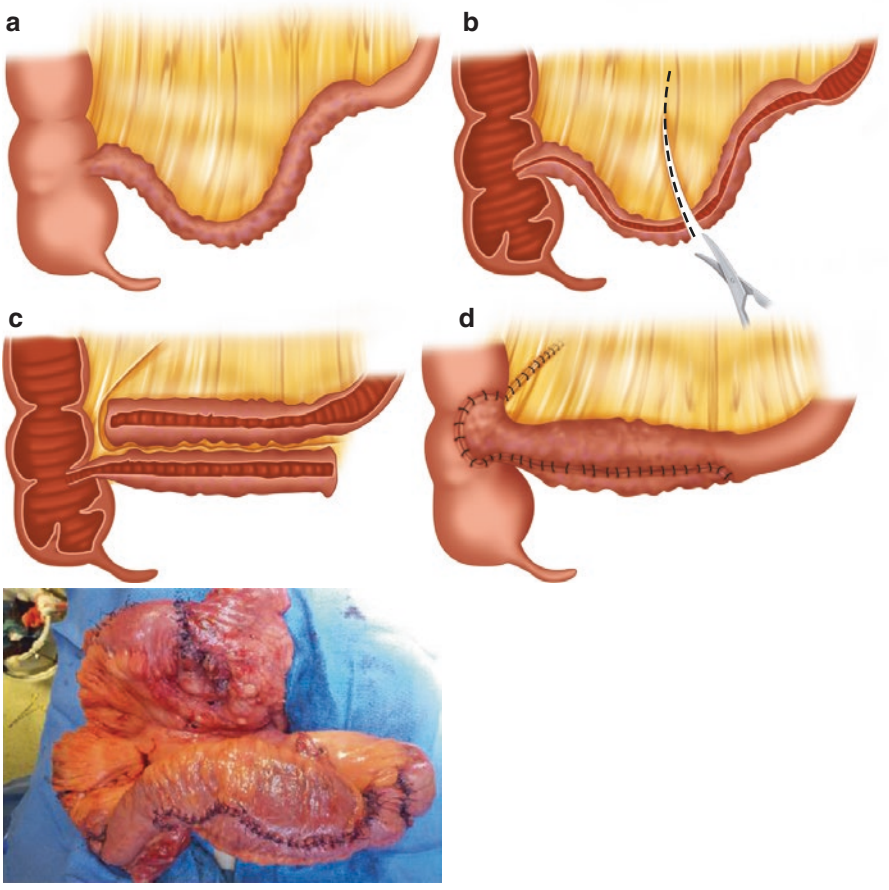


Fig. 19.15 Modified over the valve side-to-side isoperistaltic strictureplasty as further developed in our department to avoid resection of the terminal and perterminal ileum

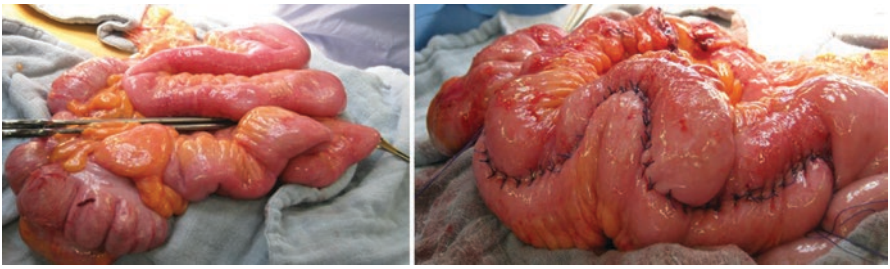


Fig. 19.16 Multiple strictures are treated using one long side-to side stricutreplasty over the valve

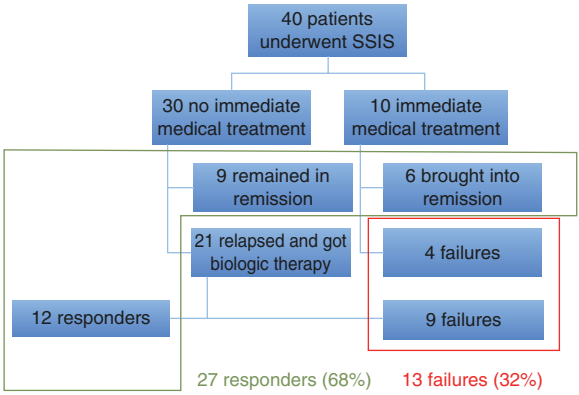


Fig. 19.17 Long-term results of the modified side-to-side isoperistaltic strictureplasty over the ileocaecal valve

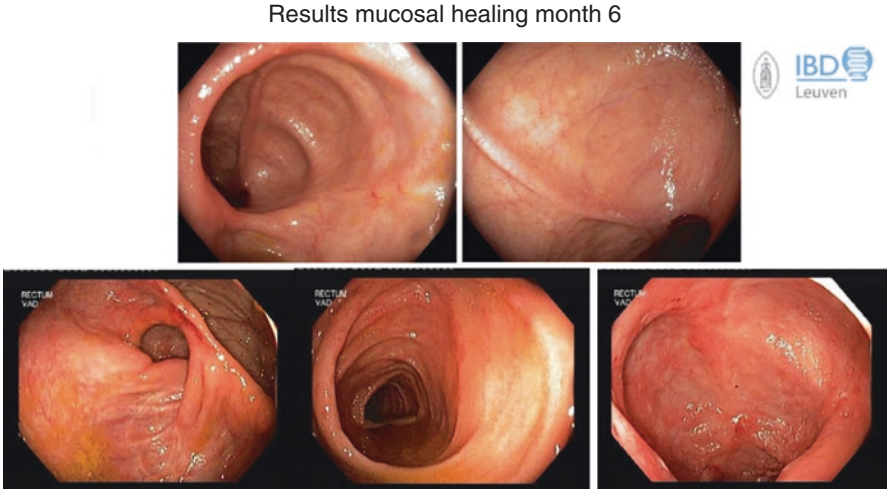


Fig. 19.18 Endoscopic results 6 months after stricutreplasty over the valve. Not always a complete healing has been observed but there is a remarkable reduced inflammation

The most intriguing observation of stricturoplasty is the normalization of the bowel wall and a low site-specific recurrence rate, which has been reported between 2% and 5% at 10 years. The surgical intervention seems to have the potential to stop progressing or even to reverse intestinal fibrosis. Based on Cleveland Clinic data, following strictureplasty operations, patients have been encouraged to undergo follow-up small bowel series after an interval of at least 6 months. Of the 44 asymptomatic patients who complied, recurrence at the strictureplasty sites with narrowing of the caliber of the bowel was noted in only 11% of patients after a median interval of 2 years [60]. Maconi et al. performed serial ultrasound examinations in patients after strictureplasty and found a reduced thickness of the intestinal wall, suggesting a possible mechanism and further fueling the promise of reversibility of intestinal strictures [61].

Repetitive surgery for recurrences typically shows macroscopically normal bowel segments previously treated by stricturoplasty from 6 months after surgery [58, 59, 62–64]. Although healing was not complete in every patient, important improvement was however clearly visible. Endoscopic improvement appeared to be the consequence of surgery, since most patients did not receive any Crohn's medication postoperatively. It could be speculated that the alleviation of faecal stasis may play a key role in postoperative mucosal healing, modifying the microbial-mucosal interaction. Functional recovery of strictureplasty has never been investigated because of the difficult accessibility of the treated segment for investigational purposes. Performing a strictureplasty over the ileocaecal valve or ileocolic anastomosis gave the opportunity for flexible endoscopic monitoring, offering a good clinical model for research on the healing process and functional recovery of the treated segment. It remains however unclear whether the diseased bowel segment returns to normal function after stricturoplasty. Further research should therefore focus on the mechanisms of healing and on the assessment of functional recovery, in terms of motility and absorptive function, of the operated segment. Indeed, should clear functional recovery be observed, it would make sense to save a short strictured segment, whereas saving a bowel segment without any functional recovery would be unnecessary. Research on the mechanisms of healing and functional recovery will help surgeons to decide in which cases performing strictureplasty in order to conserve bowel length would be most appropriate.

In view of the above the notion of the irreversibility of intestinal fibrosis has to be challenged. Up to now bowel fibrosis has been considered a progressive and irreversible process that leads irremediably to stricture formation. The chronic progressive nature of stricturing CD lead to the common belief that fibrosis is a one-way street from fibrosis to stricture formation with intestinal obstruction followed by the eventual need for surgical resection.

The concept of reversibility of intestinal fibrosis is in concordance with various observations from other organs such as the improvement of skin scarring [61] and reduced skin thickening in systemic sclerosis, [65] decreased proteinuria in patients with renal interstitial fibrosis, [66] the improvement of vital capacity in idiopathic pulmonary fibrosis [60, 67] the successful therapeutic reduction of myocardial collagen content in hypertensive patients [68] and reversibility of myocardial fibrosis

after adrenalectomy [69]. In addition, there is a growing body of evidence indicating that liver fibrosis is a potentially reversible and bidirectional process overcoming the former paradigm of liver cirrhosis being an irreversible process. Repetitive histological evaluation via liver biopsies could prove reduction of the fibrosis grade after removal of the liver injury-causing triggers in patients with hepatitis C, [70] hepatitis B, [71] non-alcoholic steatohepatitis (NASH) [72] or autoimmune hepatitis [73].

Although the gut comprises unique features compared to other organ fibroses, such as severity and chronicity of inflammation in the context of IBD, the quality and quantity of the commensal microbiota or environmental influences on the disease course, intestinal fibrosis shares essentially all core mechanistic features with fibrotic disease of the above-mentioned organs [74–77]. Therefore, it appears to be reasonable to consider these mechanisms and therapeutic approaches and apply them as promising approaches for the reversal of stricturing CD.

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Chapter 20
Challenges of Translation of Anti-Fibrotic Therapies into Clinical Practice in IBD

Gerhard Rogler

Abstract Fibrosis is an important clinical problem and affects a high number of patients with inflammatory bowel diseases (IBD). Anti-inflammatory therapies may not be sufficient to prevent intestinal fibrosis in IBD patients. Several anti-fibrotic treatment approaches have been developed. However, there are significant challenges in translating these anti-fibrotic therapies into clinical practice in IBD.

Anti-fibrotic therapy approaches in IBD are complicated by the fact that an effective and intact wound healing response and effective repair mechanisms are essential in Crohn's disease and ulcerative colitis patients. This implies that the anti-fibrotic therapies must not interfere with repair and tissue regeneration. Strategies interfering with transforming growth factor (TGF)β expression and activation are promising in other fibrotic diseases but may lead to more inflammation in IBD. The specific pathophysiology of IBD makes it difficult to extrapolate clinical data obtained with anti-fibrotic agents in other diseases than the gut. Another challenge is the lack of clear-cut clinical endpoints and readout for clinical trials for intestinal fibrosis. At present, the development of anti-fibrotic therapies takes place in other diseases such as lung and liver fibrosis. It will be important to develop new clinical endpoints for intestinal fibrosis trials to test new anti-fibrotic treatment strategies in IBD to benefit from progress in other fibrotic diseases.

Keywords Inflammatory bowel disease · Imaging · Clinical end points · Fibrosis markers · Translational medicine

G. Rogler
Division of Gastroenterology and Hepatology, University Hospital Zürich, Zürich, Switzerland
e-mail: gerhard.rogler@usz.ch

F. Rieder (ed.), *Fibrostenotic Inflammatory Bowel Disease*,
https://doi.org/10.1007/978-3-319-90578-5_20

Abbreviations

CD Crohn's disease
IBD Inflammatory bowel disease
IL Interleukin
UC Ulcerative colitis

20.1 Introduction

Up to two thirds of patients with CD may develop either a stricturing or penetrating disease course within 10 years after diagnosis [1]. Up to 80% of all CD patients undergo surgery at least once during the course of their disease [2–4]. In half of these patients intestinal obstructions and strictures are the indication for surgery. Recent data by Pittet and coworkers from the Swiss IBD Cohort group indicate that over a period of 40 years still more than 75% of patients have to undergo surgery [5]. The most frequent reason for surgery right after diagnosis of CD is fibrosis [5]. Over the first 25 years thereafter an almost a linear decrease in the proportion of surgery-free patients can be observed.

Whereas we are able to control for inflammation better and better, an effective preventive therapy for fibrosis or a pharmacological approach that could even reduce fibrosis is literally absent. Most gastroenterologists believe that surgery can be avoided by preventing or reducing inflammation. This concept also has been brought forward by Pariente and colleagues [6]. In this concept, surgery is necessary due to a chronic subclinical inflammation and subsequent fibrosis caused by smoldering inflammation [6]. The evidence to support this concept is weak. To some extent, fibrosis might be independent from the inflammatory process. Recent epidemiologic data indicate that early treatment intervention may prevent a B1–B3 development of disease subtypes but not B1–B2 in CD patients [7].

It is obvious, that fibrosis research and development of potential therapeutic avenues is much more advanced in other fibrotic diseases. Therefore, it is important to "think out of the box" and to learn from those areas to improve the situation of patients with CD and UC. Whereas there is some progress in basic research on fibrosis in IBD, clinical research on the prevention and therapy of fibrosis in IBD is still largely absent. Pathophysiological mechanisms leading to fibrosis in IBD have recently been reviewed [8–10].

20.2 Which Therapeutic Targets Have Been Identified in Other Fibrotic Diseases?

Basic research in liver fibrosis not only focuses on anti-inflammatory strategies as is presently the case in CD [11–16]. Several other interesting approaches have been investigated for the treatment of liver fibrosis: Inhibitors of proliferation and

angiogenesis were tested successfully for the prevention of liver fibrosis. An interesting target is the Hedgehog signaling pathway [17, 18]. This pathway transmits information in embryonic cells and is required for proper development. An involvement of Hedgehog signaling has recently been discussed for idiopathic pulmonary fibrosis [19, 20] and liver fibrosis [17, 18]. The Hedgehog pathway was found to be activated in lungs of patients with idiopathic pulmonary fibrosis where it is contributing to progression of fibrosis by increasing the proliferation, migration, extracellular matrix production, and survival of pulmonary fibroblasts [19].

Direct fibrogenesis inhibitors have been tested in animal models of pulmonary of liver fibrosis. Among those direct fibrogenesis inhibitors are TGFβ1 and TGFβ1 receptor antagonists [21–25], hepatocyte growth factor (HGF) agonist [26], angiotensin-receptor antagonists [27, 28], ACE inhibitors [29], connective tissue growth factor (CTGF) antagonists [30, 31], cannabinoid receptor 1 antagonist [32–34] and lysophosphatidic acid receptor type 1 (LPA1) antagonists [35, 36].

Instead of inhibiting fibrosis a successful strategy may be the stimulation of extracellular matrix degradation [37, 38]. In respective fibrosis models inhibitors of tissue inhibitor of metalloproteinases (TIMP) [39], TGFβ antagonists and inhibitors of lysyl oxidase like 2 (LOXL2) [40] were tested. The LOXL2 was targeted also clinically by a specific antibody in clinical trials in idiopathic lung fibrosis and liver fibrosis, however respective trials were negative or stopped [41–43].

20.3 Why Is Translation of Anti-Fibrotic Therapies into Clinical Practice in IBD So Difficult?

The development of anti-fibrotic therapies in IBD is difficult for two major reasons. First, there is a lack of suitable animal models that would allow to test a series of different compounds and identify promising candidates for IBD. There are some models of intestinal fibrosis available; however, they all have specific disadvantages. Animal models of fibrosis have been recently summarized and reviewed by Theresa Pizarro [44, 45]. In these animal models of intestinal fibrosis the initiation of fibrosis usually is either induced by chemicals such as dextrane sodium sulfate (DSS) [46–48] or 2,4,6-trinitrobenzenesulfonic acid (TNBS) [49–56] or by bacterial cell wall products such as peptidoglycan (PG-PS) [57, 58]. Of course, this way of induction of fibrosis is quite artificial. A spontaneous model, the SAMP1/YitFc mouse strain was studied by Pizarro et al. [45, 59]. This mouse model has the great advantage that intestinal fibrosis develops without chemical induction. Unfortunately, this model seems to depend on local factors in the animal facilities and most likely the local microbiota [60].

A recent heterotopic transplant model adapted from a bronchial transplant model [61, 62] has the advantage of a reliable and rapid induction of fibrosis in isolated parts of the small intestine [63]. Small bowel resections are transplanted subcutaneously into the neck of recipient animals [63]. A rapid fibrosis occurs within 7–14 days associated with increased expression of fibrosis-mediators such as $alpha_v beta_6$ integrin, interleukin (IL)-13, and TGFβ [63]. In this model of intestinal

fibrosis pirfenidone and antibodies against MMP-9 proved to be effective and prevented the development of strictures whereas antibodies against LOX-L2 were not successful [64, 65]. This may indicate that indeed targets and compounds can be screened with this model that could be promising for further clinical development.

The second important challenge in the translation of anti-fibrotic therapies into clinical practice in IBD is the lack of clinical scores and objective endpoints for such clinical trials.

20.4 Why Do We Have No Clinical Trials on the Prevention of Intestinal Fibrosis?

At present, there is no reliable biomarker that would fulfill the criteria for a good endpoint in a respective clinical study. There are no serum markers of intestinal fibrosis that accurately correlate with the process of fibrosis or the degree of collagen deposition. YKL-40 has been reported to be a marker for liver fibrosis [66]. Increased levels have also been found in patients with intestinal strictures but the correlation coefficient is only $r = 0.457$ and serum levels are also increased during active inflammation [67] making this marker not a good candidate for clinical trials. All further "marker-candidates" do not show a sufficient correlation with the degree of intestinal fibrosis to be useful for monitoring of an anti-fibrotic therapy. Several new markers for liver fibrosis [68–70] have not been investigated in sufficient detail in intestinal fibrosis. Most likely the volume of the fibrotic area in the intestinal wall is too small to be reliably represented by a serum marker. In general, this important aspect discriminates intestinal fibrosis and stricture formation from liver fibrosis or lung fibrosis. Both are large organs and even in cases where the fibrosis is not completely homogeneous it affects the whole organ. In intestinal fibrosis, the majority of the organ remains unaffected.

Besides the lack of serum markers there is a lack of clinical scores or indices that have been stablished to quantify the clinical complaints and signs caused by fibrosis. No patient reported endpoints have been validated and are available for respective clinical trials.

In addition, to date the current imaging techniques have not been developed to a point to be useful as clinical endpoints. In CT scans or MRI as well as in ultrasound the evaluation of fibrosis mostly relies on subjective parameters. Contrast enhancement usually is seen as a sign of inflammation. However, active fibrosis could also lead to a contrast enhancement because it is a biologically and metabolically highly active process [71]. Only when fibrosis is already established and a full scar or sclerosis has developed there is no contrast enhancement. A recently developed technique developed for the detection of intestinal fibrosis in MRI is "magnetization transfer" (MT) [57, 58]. MT generates contrast that is primarily determined by the fraction of large macromolecules or immobilized phospholipids in cell membranes in tissue [58]. Connective tissue such as bone, cartilage and muscle show an intense

Table 20.1 Current trials on fibrosis, strictures and Crohn's disease

Trial nr	Title	Target	Sponsor
NCT01986127	A randomized, double-blinded, placebo-controlled study on the effects of adalimumab intralesional intestinal strictures of Crohn's disease patients	Administration of intralesional adalimumab (directly injected in the stricture) associated to endoscopic dilatation. Success rate at week 8 compared with placebo in patients with Crohn's disease with confirmed intestinal stenosis (3 stenosis as maximum)	Investigator initiated; Hospital Clinic of Barcelona
NCT02675153	Efficacy and safety of sirolimus in the treatment of Crohn's disease with stenosis	Efficacy and safety of sirolimus in the treatment of stricturing Crohn's disease	Investigator initiated; the second Hospital of Nanjing Medical University
NCT02395354	Comparative prospective multicenter randomized study of endoscopic treatment of stenosis in Crohn's disease: metal self-expanding prosthesis balloon dilatation	To evaluate the efficacy of endoscopic treatment (prosthesis vs dilation), determined by the percentage of free patients of a new therapeutic intervention (dilatation, prosthesis or surgery) for symptomatic recurrence at 1 year follow-up	Investigator initiated; Grupo Espanol de Trabajo en Enfermedad de Crohn y Colitis Ulcerosa

signal in MT. Also fibrotic strictures in the mucosal wall show an intense MT signal: In normal, non-fibrotic bowel wall segments, an intermediate MT ratio of 25.4 ± 3.4% was measured, whereas, to the contrary, the MT ratio was significantly increased in bowel wall segments with fibrotic areas (35.3 ± 4.0%, $p < 0.0001$) [72]. MT could become an option to quantify fibrosis in intestinal segments. On the other hand, new ultrasound techniques such as shear wave elastography may be promising [73–77].

The difficulties of translation of anti-fibrotic therapies into clinical practice in IBD are reflected by the fact that only three studies are currently active for patients with Crohn's disease and fibrosis (see Table 20.1). All of them are investigator initiated illustrating that the pharmaceutical industry has not understood the potential of this indication or does not want to face the outlined challenges in defining endpoints and scores.

20.5 Which Endpoints Are Used in Clinical Trials on Fibrosis in Other Diseases?

The lack of an easily determined clinical endpoint is a major disadvantage for trials on intestinal fibrosis. What reliable endpoints are used in other diseases?

For liver fibrosis Fibroscan® is used [78] but liver biopsy is still seen as gold standard. There is a very reliable endpoint for clinical trials in idiopathic pulmonary fibrosis: the "forced vital capacity" [79]. The vital capacity is the maximum amount of air a person can expel from the lungs after maximum inhalation. A patient's vital capacity can easily be quantified by a spirometer without any invasive methods, can be easily repeated and is of no risk for the patient [79]. Subsequently, idiopathic pulmonary fibrosis is the main indication for the development of anti-fibrotic drugs for the pharmaceutical industry despite that the number of patient with idiopathic pulmonary fibrosis is low.

The factors that have been identified to play a role in the pathogenesis of idiopathic pulmonary fibrosis are similar to those identified to be relevant during intestinal fibrosis. Data show that platelet derived growth factor (PDGF), endothelial growth factor (EGF), transforming growth factor (TGF)α and β or endothelin 1 also play an important role in promoting intestinal fibrosis and deposition of collagen [80–83]. This indicates that results derived from animal studies and clinical trials in idiopathic pulmonary fibrosis may be transferable to intestinal fibrosis requesting for more scientific interaction between pulmonologists and gastroenterologists.

20.6 Summary

As obvious from the above mentioned clinical trials anti-fibrotic treatments are mainly studied in idiopathic pulmonary fibrosis and to a lesser extend in hepatic fibrosis whereas there are almost no trials going on in intestinal fibrosis. This is mainly due to the facts that we do not have reliable animal models of intestinal fibrosis and that there are no reliable endpoints for clinical trials. Magnetization transfer and new ultrasound techniques may provide us with some objective endpoints in the future.

Competing Interests Gerhard Rogler has consulted to Abbot, Abbvie, Augurix, Boehringer, Calypso, FALK, Ferring, Fisher, Genentech, Essex/MSD, Novartis, Pfizer, Phadia, Roche, UCB, Takeda, Tillots, Vifor, Vital Solutions and Zeller; Gerhard Rogler has received speaker's honoraria from Astra Zeneca, Abbott, Abbvie, FALK, MSD, Phadia, Tillots, UCB, and Vifor; Gerhard Rogler has received educational grants and research grants from Abbot, Abbvie, Ardeypharm, Augurix, Calypso, Essex/MSD, FALK, Flamentera, Novartis, Roche, Takeda, Tillots, UCB and Zeller.

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Chapter 21
What Distinguishes Mechanisms of Fistula and Stricture Formation

Michael Scharl

Abstract Wound healing is a common process in the intestinal tract, in particular during chronic intestinal inflammation. Recent studies suggested a so-called regenerative inflammation that plays a crucial role for the regeneration of injured tissue. While this self-limiting acute inflammation protects the tissue, an overwhelming and chronic ongoing inflammatory process might lead to development of fibrosis or even cancer. Intestinal fibrosis and the resulting strictures represent, in addition to fistulas, frequent complications in IBD patients. To date, treatment options for fistulas and strictures are limited and no preventive treatment for intestinal fibrosis and stricture formation has been approved. As a result, irreparable organ damage and surgery is a frequent event in IBD patients. The onset of fibrosis often precedes fistula formation in the intestinal tract suggesting a pathophysiological connection between both of the processes. Nevertheless, our understanding of the pathogenetic mechanisms underlying intestinal fibrosis and fistula development is limited. An involvement of epithelial-to-mesenchymal transition (EMT) has been demonstrated for both, intestinal fibrosis as well as fistula development. It is anticipated that fistulas and fibrosis may result from chronic and severe intestinal inflammation and deregulated wound healing mechanisms. However, current knowledge also demonstrates fundamental differences between fibrosis and fistula development. Taken together, further research efforts are clearly required to gain a better understanding of the complex pathophysiology of fistula and intestinal fibrosis development. This would finally help to foster the development of novel treatment options for those intestinal complications.

Keywords Fistula · Fibrosis · Wound healing · Epithelial-to-mesenchymal-transition · TGF · IL-13 · MMPs

M. Scharl
Department of Gastroenterology and Hepatology, University Hospital Zurich, University of Zurich, Zurich, Switzerland
e-mail: michael.scharl@usz.ch

F. Rieder (ed.), *Fibrostenotic Inflammatory Bowel Disease*,
https://doi.org/10.1007/978-3-319-90578-5_21

21.1 Introduction

Tissue remodeling and wound healing are crucial mechanisms how the body reacts to cell and tissue damage. This damage occurs continuously during a lifetime and is caused by infectious, toxic, neoplastic or immune-mediated events. Depending on the affected organ system, the reacting cell types, the duration, type and intensity of the damaging event the ensuing response of the body can be very different. Nevertheless, some degree of tissue inflammation is commonly involved [1]. Inflammation itself can exert protective as well as detrimental effects. If adequately controlled, inflammation protects the body from pathogens and is involved in tissue repair and regeneration. In contrast, uncontrolled, chronic or overwhelming inflammation results in cell death, tissue damage, fibrosis, autoimmunity, and tumor development [2]. Crohn's disease as well as ulcerative colitis are both associated with chronic, possible life-long, inflammation, often resulting in severe and permanent organ dysfunction as well as tissue remodeling that is finally associated with the development of intestinal fibrosis and neoplasia [3] (Table 21.1).

21.2 Wound Healing

During IBD disease course, chronic and severe mucosal damage requires efficient wound healing mechanisms in the intestinal tract. Regeneration of the intestinal epithelium is dependent on transmembrane receptor Lgr5 expressing intestinal stem cells (ISC). Those ISC are located at the base of the intestinal crypts between the Paneth cells which produce factors being essential for ISC survival and proliferation [4, 5]. Upon tissue injury the ISC expand, repair the mucosa and restore epithelial barrier function.

Intestinal tissue damage is the result of inflammation what causes infiltration of immune cells into the mucosa. Severe inflammation results in local tissue destruction

Table 21.1 Common and distinct mechanisms in the pathogenesis of CD-associated fistula and fibrosis development

	Fistula		Fibrosis
Similarities	Triggered by chronic inflammation		
	Induction of TNF expression		
	Onset of EMT		
	Imbalance of MMPs and TIMPs		
	Invasive behaviour of Myo-fibroblasts		
Differences	Signaling cascade after TNF upregulation		Signaling cascade after TNF upregulation
	Strong expression of TGFβ IL-13 MMPs SNAIL1 and β6-integrin	*Regulation:* TGFβ also induces DKK-1, which limits IL-13 expression	Strong expression of IL-13 TGFβ MMPs excessive ECM deposition

as indicated by loss of epithelial cells and degradation of extracellular matrix in the submucosa. Mainly the infiltrating mononuclear cells secrete reactive oxygen radicals and tissue-degrading enzymes, such as matrix metalloproteinases (MMP) and release pro-inflammatory cytokines, chemotactic and cell-activating peptides [6, 7]. This further enhances the extent of ongoing inflammation and tissue damage resulting in the continued infiltration of immune and non-immune cells into the inflamed tissue. In the case of severe tissue damage, finally so-called myofibroblasts migrate into the affected areas. These myofibroblasts are able to contract the wound area and to produce extracellular matrix (ECM) what is supposed to limit the extent of tissue damage [6]. Since MMPs are able to degrade secreted ECM components the balance between MMPs and their inhibitors, tissue inhibitors of MMPs (TIMP), is critical for the extent of tissue damage during inflammation [6]. A damage to the intestinal epithelium also allows the entry of commensal microbes and microbial macromolecules into the mucosa. This process leads to the generation of damage-associated molecular pattern (DAMP), pathogen-associated molecular pattern (PAMP) and reactive oxygen species (ROS), finally activating immune cells that then produce a broad number of cytokines, such as interleukin (IL)-6, IL-10, IL-17, IL-22 or tumor necrosis factor (TNF). Those cytokines not only are involved in the inflammatory response, but also in initiating the regenerative response by modulating ISC proliferation [1]. In the absence of microbiota-associated inflammation, a so-called sterile inflammation is induced by tissue damage and cell death [8], mainly mediated by DAMPs and activating pattern recognition receptors (PRR), such as toll-like receptors (TLR) and NOD-like receptors (NLR) as well as MMPs [9–11].

During acute intestinal inflammation, the activation of the immune system and matrix remodelling enzymes causes limited tissue damage what frequently results in a complete restitution of the damaged tissue due to sufficient wound repair mechanisms. More severe acute or moderate chronic inflammation causes severe or chronic tissue degradation and damage. These events are regularly followed by tissue repair, what might already cause fibrosis and scars. Severe acute and continuously ongoing chronic tissue damage frequently results in severe tissue fibrosis. This finally promotes the development of intestinal strictures and clinically symptomatic intestinal obstruction as the end point of inflammation-induced tissue injury [6].

21.3 Crohn's Disease Fistula

In contrast to the physiologic process of wound healing, intestinal fistulas represent a severe and frequent complication of CD. CD-associated fistulas occur in up to 50% of patients [12, 13]. They still represent an unresolved medical problem in the treatment of CD patients, since permanent fistula healing is hardly achievable and recurrences are frequent. Fistulas in CD patients often impair the quality of life because of the above mentioned limited treatment options. The cumulative incidence in population-based cohorts and meta-analyses of fistula formation varies

widely between 17 and 50% [14–19]. Most of the fistulas are located in the perianal region (54%), 24% are entero-enteric, 9% rectovaginal and 13% involve other locations, such as entero-cutaneous, entero-vesical, and intraabdominal fistulas [16].

Morphologically, a fistula represents a tract between two epithelial-lined surfaces. The prevalence of perianal fistulas increases with disease duration and more distal localization of intestinal disease [12, 20]. Noteworthy, especially perianal fistulas are not specific for CD and also occur during infection, hidradenitis suppurativa and malignant processes [21, 22]. Histologic features of CD fistulas are nonspecific. Fistula tracts may be detectable microscopically and lined by granulation tissue and/or "squamous" epithelium. The tracts are typically filled with debris, erythrocytes and acute inflammatory cells [12]. Chronic inflammation and surrounding fibrosis are regularly visible. It is hypothesized that fistulas arise as a consequence of an acute inflammatory process with infection and suppuration [23]. A deep penetrating ulceration located in the rectum or the anus might fill with fecal material. The luminal pressure then forces this material into underlying tissue layers. Additionally, also anal gland or duct abscesses might serve as a point of origin. The process of tissue destruction may then be maintained by ongoing inflammation as well as luminal antigens and bacteria. Interestingly, CD fistulas are commonly surrounded by fibrotic tissue [12, 24].

To date, only a small number of studies have investigated the pathogenesis of CD-associated fistulas. About 30% of intestinal and perianal fistulas from CD, but also from non-CD patients feature flattened intestinal or narrow squamous epithelium and are surrounded by granulation tissue. "Non-epithelialized" fistula areas exhibit a lining of myofibroblast-like cells (so-called "transitional cells") forming a new basement membrane (BM). Only fistulas from CD patients, but not from non-CD patients, show regions with disordered myofibroblasts and fragmented BM suggesting different mechanisms of fistula formation in CD vs. non-CD patients [12]. Additionally, a characteristic composition of inflammatory cells has been described in and around fistulas. CD fistulas typically feature a central infiltrate consisting of $CD45R0^+$ T cells, an underlying band of $CD68^+$ macrophages as well as a dense infiltrate of $CD20^+$ B cells in the outer fistula wall. Fistulas from non-CD patients, in contrast, commonly present a dense macrophage infiltrate and only few $CD20^+$ B cells and $CD45R0^+$ T cells [12]. Recent data also suggest accumulation of CD4+ $CD161^+$ T cells with a Th17, Th17/Th1 and Th1 phenotype in CD perianal fistulas [25].

21.4 Epithelial to Mesenchymal Transition (EMT) and CD Fistulas

Current knowledge suggests that the driving force for the development of CD-associated fistulas is EMT. While EMT is as a physiological process involved in embryogenesis, organ development, wound healing and tissue remodelling, it also plays an important role for pathological processes such as tissue fibrosis and

cancer progression [26, 27]. During EMT, a differentiated, resident epithelial cell loses its epithelial cell shape, down-regulates epithelial-cell specific proteins as E-cadherin or claudin-4. And acquires a mesenchymal cell shape accompanied by the upregulation of mesenchymal proteins, such as vimentin [26].

The process of EMT has been clearly demonstrated in pathogenesis of CD-associated fistulas [12, 24]. In particular, tracts of CD fistulas are covered by intestinal epithelial cells (IEC) as well as by "transitional cells" (TC). The transitional cells develop from intestinal epithelial cells via EMT and express typical mesenchymal cell markers, such as vimentin and alpha smooth-muscle-actin (α-SMA), in addition to their epithelial markers, such as cytokeratins (CK)-8, CK-20 or E-cadherin [24]. Additionally, in cells undergoing EMT, nuclear localization of β-catenin and of the EMT-associated transcription factor SLUG, can be detected. A further hint for an involvement of EMT in fistula development is the strong expression of transforming growth factor (TGF) β, the most powerful driving force for EMT, in cells along as well as surrounding the CD fistula tracts [24, 28]. Immunohistochemical studies have also detected Snail family transcription factors in cells lining the fistula tract as well as around CD-associated fistulas. On the one hand, SNAIL1 is detected in nuclei of transitional cells lining the fistula tracts. On the other hand, SLUG (SNAIL2) is expressed in cells of fistula surrounding tissue, but almost absent in transitional cells [29].

21.5 Molecules Involved in CD Fistula Formation

IL-13 is strongly expressed in cells lining the fistula tract and also, to some lesser extent, in fistula surrounding fibrotic tissue layers. On a molecular level, TGFβ is able to induce IL-13 secretion from colonic lamina propria fibroblasts (CLPF) derived from CD patients with fistulizing disease, but not from non-IBD control patients or CD patients without fistulas. This suggests a specific amplification loop in CD fistula tissue [13, 24]. Recent data have also shown that IL-13 induces expression of the EMT transcription factor SLUG as well as of β6-Integrin, a protein that is associated with cell invasiveness, in an in vitro model of EMT using HT29 IEC spheroids [13]. Further support for the "amplification loop" theory is given by the fact that TNF and TNF-receptor 1 are also strongly detectable in cells lining the CD-associated fistula tracts [29]. We and others have demonstrated that TNF induces EMT and the expression of EMT-associated genes in IEC spheroids [30, 31]. TNF and TGFβ also induce the expression of the Wnt-antagonist, Dickkopf-homolog 1 (DKK-1) in CLPF derived from CD patients with fistulas. DKK-1 is expressed along fistula tracts in CD patients and limits TGFβ-induced IL-13 expression (32). Support for the hypothesis that the intestinal microbiota is somehow involved in fistula formation in CD patients is provided by the observation that the bacterial wall component, muramyl-dipeptide (MDP), induces not only EMT in IEC, but also the expression of fistula-associated molecules in IEC and fistula CLPF [30].

With respect to matrix remodelling enzymes, a strong expression of MMP-3 and MMP-9 can be observed in CD fistulae, while levels TIMP-1, TIMP-2 and TIMP-3 are lower compared to their expression level in colon tissue from non-IBD patients. This observation supports the assumption that fistula formation is associated with a dysbalance of matrix remodelling enzymes, in particular of MMPs and TIMPs, what contributes to the development of fistulas through enhanced ECM degradation [32]. All of those observations strongly suggest EMT-like processes in the pathogenesis of CD-associated fistulae [12, 13, 24, 29, 30, 33]. Additionally, fistula-associated molecules seem to be associated with the development of so-called fistula-carcinomas in CD patients, a very severe and important complication of CD and CD-associated fistulas [34–36].

21.6 Pathogenetic Differences Between Stricture and Fistula Formation

Inflammation is a crucial trigger for tissue regeneration, but nowadays knowledge suggest that uncontrolled or chronic inflammation might also be an important trigger for both fibrosis and fistula formation [1, 37]. Unfortunately, to date, this has not been demonstrated formally in mammalian animal models. Mouse and rat models only rarely and late develop fibrotic alterations in the intestine and the onset of clinically relevant strictures or fistulas is very rare. Nevertheless, the general concept of an inflammatory trigger for development of fibrosis and fistulas is generally accepted.

From a clinical point of view, there is no medication available that directly targets fibrosis and anti-inflammatory treatment in IBD patients at the same time and is also sufficient to treat fibrosis once excessive ECM deposition has occurred [38, 39]. Subsequently pathophysiological mechanisms perpetuating fistula and/or fibrosis formation may be distinct from the ones regulating the onset of fibrosis and fistula formation. In particular, inflammation seems to play an important role in the beginning of fibrotic tissue alterations and fistula development. However, the impact of inflammation in later stages of the disease is unclear. This aspect becomes very interesting when considering novel therapeutic strategies, such as SMAD7 antisense oligonucleotides that affect TGFβ function.

While treatment options for intestinal inflammation in IBD patients become more and more sophisticated, options for treatment and prevention of intestinal fibrosis or fistula formation are still very limited. This is also due to the fact that our understanding of the pathophysiological mechanisms of fistula and fibrosis development is scarce. Of course, fistulas and fibrosis share some common pathogenetic features, such as EMT, but have also clearly distinct pathways and triggers. Currently, the most promising approach to prevent fibrosis and likely also fistula development might be to control inflammation before the complication has occured. Therapeutic interventions to control inflammation once stenosis/fibrosis or fistulas have been formed are in general not successful [38].

From a molecular perspective, the development of fibrosis is defined as the excessive accumulation of ECM what finally causes organ dysfunction or even organ failure [26]. Current knowledge suggests that the key factors for fibrosis are chronic tissue damage due to chronic inflammation, overwhelming or defective wound healing mechanisms and expanding mesenchymal cells, mostly fibroblasts, myofibroblasts and smooth muscle cells [40]. Fibroblasts are continuously producing ECM as part of continuously ongoing tissue regeneration mechanisms. Following injury or inflammation, mesenchymal cells are able to rapidly proliferate and to invade the affected sites of injury or inflammation from within and without the intestinal tract. Hereby they follow a chemical gradient which is produced by growth factors. Finally, the fibroblasts become activated by a cocktail of cytokines that is produced by and secreted from immune as well as non-immune cells [40]. As a consequence, the mesenchymal cells produce excessive amounts of collagen and other components of the ECM [41]. Nevertheless, expression and activity of MMPs and their inhibitors TIMPs are elevated in the intestine of CD and UC patients. This suggests that the development of intestinal fibrosis in IBD patients is not only due to excessive ECM production, but rather dependent on an imbalance in regular tissue-remodeling processes [6]. Here, a clear correlation to the development of CD-associated fistulas is seen. Aberrant matrix remodeling, production of ECM components and a deregulated ECM turn-over are characteristic features of the development of both, fistulas and fibrosis. Noteworthy, CD fistulas are commonly surrounded by fibrotic tissue: A possible explanation for this observation might be the fact that the body aims at initiating wound healing around the fistula tracts. Since the fistula itself can be already result from defective wound healing mechanisms, the onset of fibrosis and the development of fibrotic tissue around the fistula tract might serve to limit ongoing tissue damage as well as further fistula growth. In this regard, the onset of fibrosis around fistula tracts would represent a rescue mechanism of the intestinal tissue. A further hint to his theory is the fact that fibroblasts which are isolated from dense fibrosis tissue reveal a clearly higher migratory potential as colonic lamina propria fibroblasts (CLPF) isolated from fistulas. These observations suggest that fibroblasts in fistula areas might exert a lower capacity to repair tissue defects. As a compensatory mechanism, intestinal epithelial cells might be reprogrammed via EMT into mesenchymal cells. This allows them to migrate to the affected tissue regions what finally promotes fistula formation [42].

While the involvement of cytokines and growth factors, such as IL-13, TNF or TGFβ in the pathogenesis of intestinal fibrosis has been well documented [6, 40], recent studies also suggest an involvement of TNF and IL-13 as well as of their receptors in fistula formation. Those molecules are highly expressed in TC lining fistula tracts. These observations support the hypothesis that comparable mechanisms might contribute to the onset fistulas and fibrosis in the intestine. This assumption is even more underlined by the observation that EMT is crucial for fistula development and that hallmarks of EMT are be detected in areas of intestinal fibrosis in CD patients [28, 43]. TGFβ, the most powerful inducer of EMT, is highly detectable in fistula as well as fibrotic regions of IBD patients [24, 44]. Additionally, the EMT-associated molecule, β-catenin, is less expressed in the membrane, but strongly

expressed in the nucleus, in fibrotic areas and fistulas hinting at its enhanced transcriptional activity. While the EMT-related transcription factor SNAIL1 is strongly expressed in both, fistula tissue and fibrotic tissue, expression of SLUG transcription factor can only be observed in the nuclei of mesenchymal cells in fibrotic areas. In transitional cells along CD fistulas SLUG expression is only poorly detectable [28, 29]. Interestingly, in a case report of a patient with a fistula-associated anal adenocarcinoma, a remarkable staining of SLUG transcription factor was shown not only in TC lining the fistula tract, but also in the carcinoma tissue originating from those cells [34]. As a limitation, however, one must mention that all of the current literature studying the pathogenic role for EMT in Crohn's disease intestinal fistulas and fibrosis are based on descriptive results obtained by haematoxylin-eosin staining, immunohistochemistry and electron microscopy only. Due to this lack of functional studies and in particular in vivo studies on this topic, the true relevance for EMT in fistula and fibrosis development in CD patients warrants further confirmation.

Increased levels of IL-13 in the fibrotic intestine of CD patients are produced by a population of cells expressing high levels of IL-13Rα_1 [45]. According to their phenotype, those cells (KIR$^+$ CD45$^+$ CD56$^\pm$ CD3$^-$ IL-13Rα_1^+) might be innate lymphoid cells (ILC). Fibroblasts down-regulate levels of MMP-2 as well as of TNF-induced MMP-1 and MMP-9 protein in response to IL-13 [45]. A very interesting observation in fibrosis and fistula development are the effects of IL-13 and TGFβ. In the pathogenesis of fibrosis, IL-13 induces TGFβ secretion. In contrast, in fistula development fistula-derived myofibroblasts secrete IL-13 following stimulation with TGFβ [13, 44]. Nevertheless, conflicting results have been demonstrated with respect to the impact of IL-13 in the development of intestinal fibrosis in stricturing CD [46] suggesting that the possible pro-fibrogenic role of IL-13 in CD needs to be critically reassessed. A further hint to the complexity of fistula and fibrosis development is the observation that IFNγ is able to induce fibroblast apoptosis following co-treatment with TNF in an in vitro model of fibrosis [47, 48]. However, TNF is able to induce intestinal fibrosis by inducing collagen accumulation and inflammation [48].

Fistula formation in CD patients and development of intestinal fibrosis exhibit several similarities, but they also features remarkable differences. In the pathogenesis of CD fistulas, less migratory potential of myofibroblasts and an aberrant ECM production occurs, while as a compensatory mechanism, intestinal epithelial cells invade the wounded area to close the wound area. In contrast, increased proliferation as well as migration of myofibroblasts followed by enhanced matrix synthesis might be the critical mechanism in the development of intestinal fibrosis [6].

21.7 Summary

A well-balanced wound healing response represents a critical repair mechanism of the intestinal tract during acute and chronic intestinal inflammation. Defective wound healing promotes the onset of fistulas or fibrosis, the latter possibly resulting in clinically relevant stenosis or strictures. On a molecular level, EMT might play a

crucial role for the development of both, fistulas and fibrosis. However, the exact mechanisms for their development are not yet determined. This clearly suggests that further studies and if possible *in vivo* studies, are needed to gain a better understanding of both pathologies, which would be essential for the development of novel therapeutic strategies aiming at preventing and healing fistulas and stenosis. It is necessary to consider fistula formation as a pathological process which is distinct from inflammation. This has also been discussed for the development of intestinal fibrosis. It will certainly be a great achievement to better understand the pathways of fistula formation and to compare those mechanisms to those of frequently coincident fibrosis formation. Since treatment options for fistula and fibrosis therapy are limited to date, this represents one of the big unmet goals in IBD therapy and further research is clearly needed [37].

21.8 Conflict of Interest Statement

No conflicts of interest exist.

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Chapter 22
The Pathogenesis of Intraabdominal Adhesions: Similarities and Differences to Luminal Fibrosis

Edward Macarak and Joel Rosenbloom

Abstract Essentially every organ in the human body, including the intestine, can be affected by fibrotic reactions. Under normal homeostatic conditions these reactions are self-limited and constitute an important reparative process aimed at the restoration of the functional integrity of injured tissues. However, under pathologic circumstances the homeostatic regulatory mechanisms evolve into an uncontrolled fibrotic process characterized by the accumulation of large amounts of fibrotic tissue, which disrupts normal organ architecture and ultimately leads to organ failure. Even though their etiology varies greatly, all fibrotic reactions share common features. It is universally accepted that myofibroblasts are the cells ultimately responsible for the pathologic fibrotic process. Myofibroblasts, expressing α-smooth muscle actin (α-SMA), comprise a distinctive population of mesenchymal cells. When activated, they markedly increase the production of fibrillar collagens (types I, III, V, and VI) and other extracellular matrix (ECM) macromolecules coupled with an increased inhibition of ECM-degradative enzymes which may result in the production of injurious scar tissue in the intestine. While abdominal adhesions may be caused by infection, inflammation or ischemia, surgical procedures are the primary cause. Unfortunately, adequate therapeutic solutions have proven elusive. The peritoneal surfaces, both visceral and parietal, are covered by a monolayer of mesothelial cells bound to a basement membrane. Because the mesothelial cells are weakly connected, the peritoneal surface is delicate and easily injured, resulting in a series of events, which can be broken down into coagulation cascade and inflammatory stages leading to a fibrous adhesion stage. TGF-β, IL-6 and likely other cytokines and growth factors play critical roles in adhesion formation by mediating the formation of myofibroblasts and stimulating the production of ECM. In the pathogenesis of fibrosis in inflammatory bowel disease (IBD), many factors need to be considered, including a much more sustained inflammatory response, a clear if still poorly understood genetic predisposition, the potential involvement of multiple mesenchy-

E. Macarak (✉) · J. Rosenbloom
The Joan and Joel Rosenbloom Center for Fibrotic Diseases, Philadelphia, PA, USA

Department of Dermatology and Cutaneous Biology, Sydney Kimmel Medical College, Thomas Jefferson University, Philadelphia, PA, USA
e-mail: Edward.Macarak@jefferson.edu; Joel.Rosenbloom@jefferson.edu

F. Rieder (ed.), *Fibrostenotic Inflammatory Bowel Disease*,
https://doi.org/10.1007/978-3-319-90578-5_22

mal cells, exposure of the mucosa to intestinal bacteria and the involvement of the immune system. In IBD, the normal wound healing process triggered by injury and inflammation fails and, instead of resolution, there is continued ECM production by myofibroblasts. Because of a protracted inflammatory response, one could imagine that anti-inflammatory therapy might be an effective approach. Unfortunately, this has not been the case, and it appears that once the damaging fibrotic reaction has been initiated in fibrosis-prone individuals, it is self-propagating. Thus, as in other fibrotic situations, the aberrant myofibroblast becomes the ultimate target. However, unlike adhesions in which the potential instigators can be anticipated and candidate drugs given over a fairly short time, in IBD the pathogenesis is much more protracted. There are a number of FDA—approved drugs capable of intercepting pathways potentially critical in the fibrotic reaction. TGF-β signaling is, of course, the primary target. However, because of the manifold activities of TGF-β, one or more downstream events in the signaling pathways must be judiciously selected so as not to elicit toxic responses. The same caution must be applied when dealing with other potential targets. Because of the inherent redundancy in signaling from multiple cytokines/growth factors involved in fibrotic reactions, it is likely that more than one drug must be administered simultaneously to obtain effective beneficial inhibition.

Keywords Fibrosis · Myofibroblasts · TGF-β · Abdominal adhesions · Inflammatory bowel disease · Crohn's disease · Ulcerative colitis

22.1 Introduction

In order to place abdominal adhesions and luminal fibrosis in inflammatory bowel disease (IBD) on a more comprehensive platform to take advantage of what is known in other systems characterized by fibrotic reactions, we first discuss the pathophysiology of fibrosis in a general sense. Essentially every organ in the human body can be affected by fibrotic reactions. Under normal homeostatic conditions, these reactions are self-limited and constitute an important reparative process aimed at the restoration of the functional integrity of injured tissues through a complex sequence of events constituting tissue repair. However, under pathologic circumstances, the homeostatic regulatory mechanisms evolve into an unrestrained fibrotic process characterized by the progressive and uncontrolled accumulation of large amounts of connective tissue which disrupts the normal organ architecture and ultimately leads to organ failure [1–3]. These reactions can cause multi-system diseases such as Systemic Sclerosis (SSc) [4, 5], as well as fibrotic disorders affecting individual organs including those of the gastro-intestinal system. Despite considerable understanding of the pathogenesis of the fibrotic process attained recently [1–3], disease-modifying therapy for the fibrotic diseases is extremely limited. Even though etiologic agents vary greatly, the fibrotic diseases all share common molecular alterations that result in the exaggerated and uncontrolled accumulation of extracellular matrix (ECM) macromolecules in the affected tissues which may result in the replacement of functioning tissue such as alveoli in the lung, myocytes in the

heart, or nephrons in the kidney either with non-functional fibrotic tissue or injurious scar tissue in the gut [6–9]. At the cellular level, it is universally accepted that myofibroblasts are the cells ultimately responsible for pathologic ECM synthesis in fibrotic disorders [10–13]. Myofibroblasts, expressing alpha smooth muscle actin (α-SMA), comprise a distinctive population of mesenchymal cells, which markedly increase the production of fibrillar collagens (types I, III, V, and VI) and other ECM macromolecules coupled with an increased inhibition of ECM-degradative enzymes [14–16]. Furthermore, such alterations in the ECM produce changes in the biomechanical properties of the affected tissues causing a progressive increase in tissue stiffness, a potentially potent pro-fibrotic stimulus [17–20].

The origin of myofibroblasts, still a contentious issue, may vary depending upon the organ affected and the particular fibrotic reaction [11, 13, 21–23]. There are several potential sources including: [1] recruitment of fibroblast precursor cells (fibrocytes) from bone marrow, [2] trans-differentiation of various cell types including pericytes, adipocytes, and epithelial, mesothelial, and endothelial cells into a mesenchymal phenotype, [3] proliferation and activation of quiescent tissue- resident fibroblasts into a myofibroblast phenotype (see Fig. 22.1) and [4] sub-epithelial myofibroblasts. Although epithelial to mesenchymal transition (EMT), endothelial to mesenchymal transition (EndoMT), or pericyte to myofibroblast transition may play a role under specific circumstances [13, 21–23], the current preponderance of opinion is that the activation of tissue-resident fibroblasts is the major source of activated myofibroblasts. However, even though the *trans-differentiation* of various cell types may not be a predominant source of myofibroblasts during fibrotic disorders, alterations in the phenotype of the *trans-differentiated* cells may result in the production and secretion of pro-fibrotic factors, including TGF-β, which play an important role in the fibrotic process. Furthermore, these phenotypically-modified cells may produce numerous macromolecules which may enhance the fibrotic response such as the EDA form of fibronectin (FnEDA) and other ECM components including proteoglycans and several matricellular molecules [21–26].

22.2 Targeting Myofibroblasts

There are multiple potential levels that could be targeted for inhibition of fibrotic responses, such as elimination of the primary cause as in treatment of viral hepatitis for liver fibrosis, diminution of the immunologic and inflammatory responses in SSc and Idiopathic Pulmonary Fibrosis (IPF), and elimination of the untoward pro-fibrotic activities of myofibroblasts. Regrettably, owing to the lack of a comprehensive understanding of the etiologic mechanisms in the majority of the fibrotic disorders, opportunities for elimination of the originating cause of the fibrotic reaction are rare, and reduction of the immunologic and inflammatory responses has proven to be generally ineffective in abrogating pathologic fibrotic processes. Thus, modulation of the deleterious pro-fibrotic activity of myofibroblasts remains the

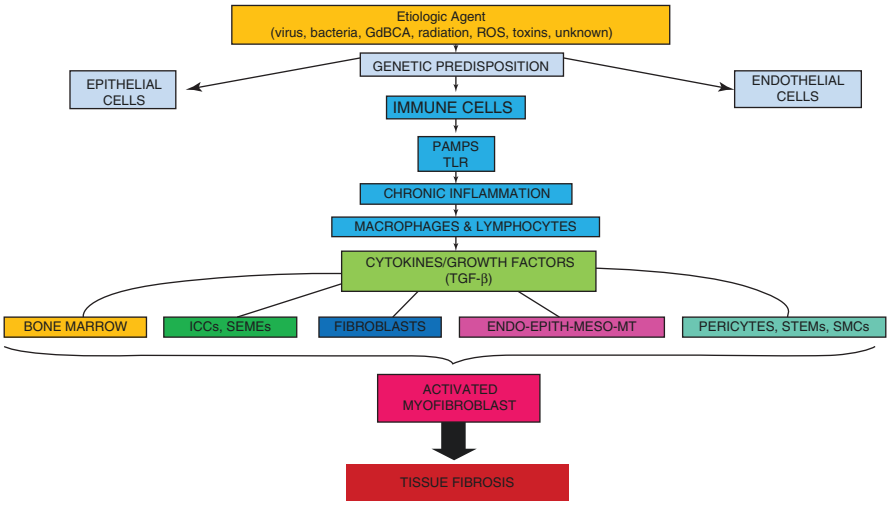

Fig. 22.1 Cellular origins and pathways leading to the formation of an activated myofibroblast. Injuries caused by a variety of putative causative agents such as bacteria, viruses, toxins, ROS, gadolinium-based contrast agents (GdBSAs) or radiation in genetically predisposed hosts result in inflammation. Activated inflammatory cells secrete cytokines and growth factors such as TGF-β and interleukin-6 causing trans-differentiation of resident and non-resident fibroblast cells as well as endothelial, epithelial and mesothelial cells into myofibroblasts. These cells produce excess amounts of structural macromolecules which contribute to fibrosis leading to alterations in tissue architecture causing pathological dysfunction. *PAMPs* Pathogen-Associated Molecular Patterns, *TLR* Toll-Like Receptor, *TGF-β* Transforming Growth Factor beta, *ROS* Reactive Oxygen Species, *GdBSAs* Gadolinium-Based Contrast Agents

most attractive therapeutic approach. This, in turn, requires a precise understanding of the molecular pathways most important in generating the excessive ECM by the myofibroblast as discussed recently [27] and briefly reviewed below.

22.3 The Transforming Growth Factor-β (TGF-β) Pathways

The TGF-β family of growth factors plays wide-ranging roles in numerous physiological processes including embryogenesis, cellular differentiation, immunologic system development, inflammatory response, and wound repair [28–31]. Furthermore, numerous studies have shown that the three TGF-β isoforms are potent inducers of myofibroblasts either through activation of quiescent fibroblasts, or through the phenotypic conversion of various cell types into activated myofibroblasts [32–34]. Owing to their potent pro-fibrotic activities, they have been implicated in the pathogenesis of various fibrotic human diseases [35–39]. The intracellular transduction pathways following TGF-β binding to its cognate receptors are complex and involve both Smad-mediated pathways referred to as canonical and non-Smad pathways referred to as non-canonical [40, 41]. These pathways are diagrammatically illustrated in Fig. 22.2 along with other relevant cytokine/

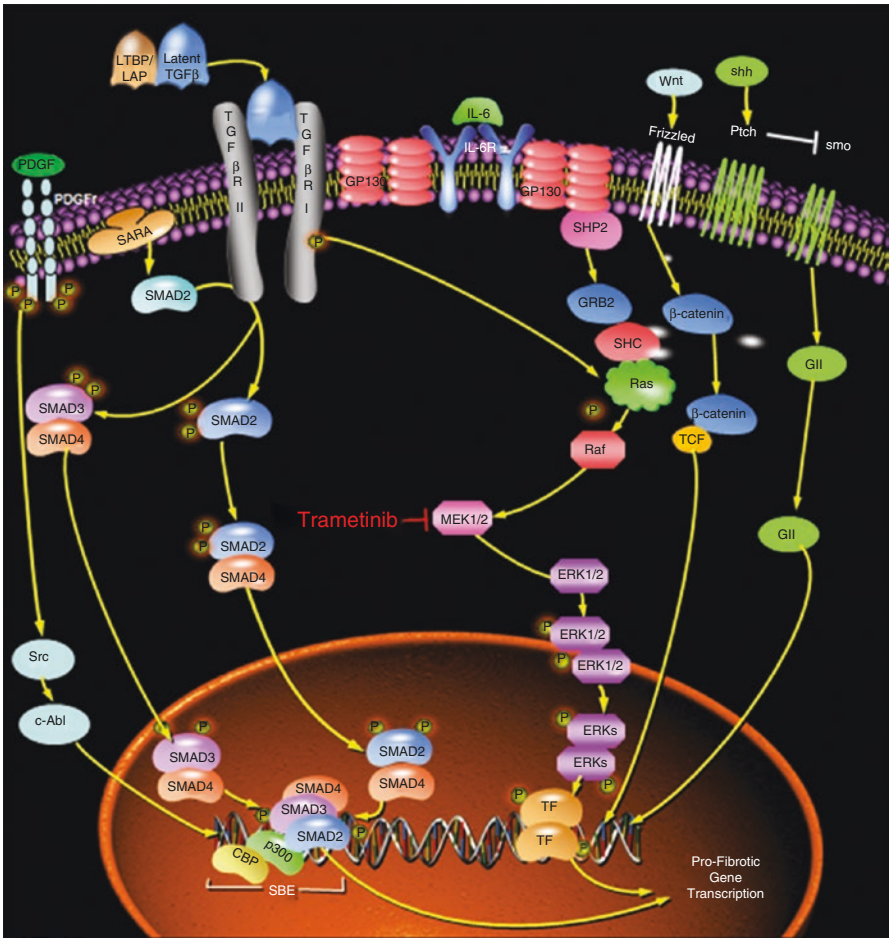

Fig. 22.2 Critical TGF-β, Il-6 and Growth Factor/Cytokine signaling pathways important in fibrogenesis. Illustrated is the canonical pathway originating from a representative dimeric receptor. Following TGF-β binding, the TGFβRll receptor recruits a TGFβRl, either activin-like kinase-1 or activin-like kinase-5 and activates it by phosphorylation. Activin-like kinase 5 (Alk 5) then specifically phosphorylates receptor-regulated Smad2 and Smad3 which then complex with Co-Smad4 resulting in their transport to the nucleus where they interact with various co-activators or co-repressors to regulate transcription of critical pro-fibrotic genes, here represented by connective tissue growth factor (CTGF) fibronectin isoform EDA (Fn^{EDA}) and *C*ol1a1 collagen genes. Also shown is Il-6 binding to its receptor with activation of Ras, Raf and MEK1/2 and ultimately Erk1/2. On the far left and right are illustrated are the signaling pathways for (PDGF), Wnt and Hedgehog. Each of these pathways is activated by ligand-binding to specific receptors, but the subsequent signaling transmission mechanisms between these pathways differ dramatically (see text). For clarity of presentation these pathways have been abbreviated with only the essential features presented. *This figure has been modified from two produced by Protein Lounge. TGF-β* Transforming Growth Factor beta, *TGFβR1 & II* Transforming Growth Factor beta Receptor 1 & II, *LTBP* Latent TGF-binding protein, *Smad* Sma and Mad related family of signal transducers, *ERK* Extracellular signal-regulated Kinase, *TF* Transcription Factor, *Il-6* Interleukin -6, *GP130* Glycoprotein 130, *SARA* Smad Anchor for Receptor Activation, *PDGF* Platelet Derived Growth Factor, *SHP2* Tyrosine phosphatase, *GRB2* Growth factor receptor-bound 2, *SHC* Src homolog 2, *Ras* Fas family of genes, *Raf* Raf kinases, *MEK 1/2* MAPK/ERK pathway, *Wnt* Signal transduction pathway, *Shh* Sonic hedgehog, *Ptch* Protein patched homologue 1, *Smo* G-protein coupled receptor, *Src* Family of protein tyrosine kinases, *c-Abl* Abelson tyrosine kinase

growth factor pathways. In the canonical pathway, the ligand-bound TGF-β receptor II (TβRII) recruits and phosphorylates a TGF-β receptor I (TβRI) which is known as activin-like kinases (ALKs), with ALK-5 being the most important in the context of the fibrotic process. Signaling from the phosphorylated TβRI to the nucleus occurs through the receptor activated RSmads, Smad2 and Smad3, which are phosphorylated by TβR1. The phosphorylated Smad2/Smad3 then bind to the co-Smad, Smad4, forming a complex that translocates across the nuclear membrane. Within the nucleus, the Smad complex, in association with various transcription factors, co-activators and co-repressors, modulates the expression of target genes [39, 40]. Non-canonical TGF-β-initiated signaling cascades are independent of RSmads. These non-canonical pathways can be activated in a cell-specific and context-dependent manner and mediate important TGF-β pro-fibrotic effects [41, 42]. For example, TGF-β stimulation leads to the activation of PI3K, which, in turn, activates two important pro-fibrotic pathways: p21 activated kinase (PAK2)-Abelson kinase (c-Abl) and Akt-mTOR1 [43]. Downstream targets of c-Abl include several mediators involved in the fibrotic response. Activated c-Abl phosphorylates protein kinase C-δ (PKC-δ), a potent pro-fibrotic mediator was recently shown to up-regulate collagen gene transcription in SSc dermal fibroblasts [44]. Phosphorylated PKC-δ has also been shown to in turn phosphorylate the transcription factor Fli-1, reversing its inhibitory effect on collagen gene expression [45]. Another important non-Smad signaling pathway is through activation of Jun-N-terminal kinase (JNK) resulting in the activation of c-Jun, a critical pro-fibrotic transcription factor [41, 42]. Besides serine/threonine phosphorylation, TβRII can also be phosphorylated on tyrosine residues in response to TGF-β [46, 47] leading to activation of Erk1/2 MAPK which play an important role by regulating myofibroblast formation as well as ECM synthesis [48, 49].

One of the important aspects of TGF-β action is the stimulation of other mediators having fibrogenic potential. These include, but are not limited to, connective tissue growth factor (CTGF), platelet-derived growth factor (PDGF) and epidermal growth factor (EGF/Erb-B) ligands. For example, it has been demonstrated that TGF-β-induced ErbB activation was achieved by up-regulation of ErbB ligands through autocrine signaling from the PDGF-receptor (PDGFR) via MEK/ERK [50]. While the molecular changes initiated by TGF-β are complex, and challenging, approaches with therapeutic agents can be carefully designed to take advantage of specific points within critical pathways to abrogate their deleterious pro-fibrotic effects.

22.4 Other Molecular Pathways Involved in the Fibrotic Process

Although the TGF-β family of growth factors plays the most important role in fibrosis, there are numerous other molecular pathways that also participate depending on the specific trigger initiating the fibrotic process and the tissues or organs involved. Although the diverse mechanisms mediated by these pathways result in an extremely intricate picture, the detailed understanding of their components and of their

interactions may provide novel therapeutic targets to modify the devastating effects of fibrotic diseases. Some of these pathways are illustrated in Fig. 22.2 and will be briefly reviewed in the following sections.

Endothelin-1 Endothelin-1 (ET-1), a polypeptide with potent vasoconstrictive activity and a major factor in the pathophysiology of pulmonary arterial hypertension [51, 52], may also have pro-fibrotic activity and play a role in organ fibrosis [53–55]. ET-1 not only can increase the production of ECM macromolecules such as collagen types I and III, but also has been shown to inhibit production of matrix metalloproteinase-1 in normal human fibroblasts [56]. Increased levels of ET-1 have been found in several fibrotic diseases and in experimental pulmonary fibrosis [54, 56, 57]. Additionally, ET-1 may have a potential role in the generation of myofibroblasts through EMT or EndoMT, effects possibly mediated through the endothelin A receptor [58, 59] and through the synergistic stimulation of TGF-β induced EndoMT [60]. These findings, collectively, strongly suggest that ET-1 may play an important role in the pathophysiology of human fibrotic diseases and can be targeted with currently available therapeutics.

22.4.1 Connective Tissue Growth Factor (CTGF/CCN2)

CTGF, also known as CCN2, a growth factor with multiple effects, is now regarded as an important effector in both normal and pathologic fibrotic responses [61–63]. CTGF provokes a potent pro-fibrotic response when added to cultured fibroblasts and elevated CTGF levels have been found in a variety of experimental fibrotic models in mice while suppression of CTGF reduced bleomycin-induced lung fibrosis [61–65]. TGF-β stimulates CTGF synthesis in a variety of cell types, and CTGF can act as a downstream mediator to enhance the pro-fibrotic activity of TGF-β by stimulating the production of ECM components including type I collagen and fibronectin [61, 62]. Importantly, imatinib mesylate, an inhibitor of c-Abl, blocked activation of the Smad1 pathway when normal fibroblasts were stimulated with TGF-β and inhibited stimulation of CTGF expression in SSc fibroblasts [49]. Therefore, at least in some circumstances, c-Abl appears to be required for Smad1 activation. Furthermore, CTGF expression can be stimulated through the RhoA/Rock pathway [66]. Thus, CTGF is a targetable pro-fibrotic mediator.

22.4.2 Platelet Derived Growth Factor

The platelet derived growth factor (PDGF) family consists of four different polypeptides (PDGF-A, -B, -C, -D) which form disulphide-bonded dimers such as PDGF-AA and PDGF-BB as well as PDGF-AB heterodimers. Two structurally related tyrosine kinase receptors, PDGFRα and PDGFRβ bind PDGF ligands which leads to receptor homo- or hetero-dimerization and autophosphorylation of specific tyrosine residues within the receptor cytoplasmic domain [67–69]. This receptor

activation initiates multiple signal translocation pathways including PI3K, Ras-MAPK, Src family kinases and phospholipase Cγ(PLCγ) resulting in important cellular responses including proliferation, chemotaxis and actin reorganization. It is now clear that PDGF can be involved in fibrotic reactions affecting multiple organs, including pulmonary, renal and hepatic fibrosis as well as SSc [70]. Fibroblasts can be regarded as both a major source and target since they secrete PDGF-A as well as express PDGFRα on their cell surface [71–73]. Thus, a PDGF-A/PDGFRα signaling loop can stimulate fibroblasts to synthesize ECM and release pro-fibrotic mediators. Since PDGF-B is released primarily by macrophages and hepatic stellate cells, this suggests a major role for PDGF-B/PDGFRβ signaling in liver fibrosis [74, 75]. PDGF signaling becomes activated upon tissue injury to promote wound closure and is turned off when the repair processes are completed [76]. However, excessive scar formation and tissue fibrosis can result, if PDGF signaling is not terminated.

22.4.3 Wnt-Signaling

While the Wnt proteins, consisting of a multi-gene family of secreted glycoproteins, play crucial roles in embryogenesis, numerous studies now have shown that the Wnt/β catenin pathway is involved in several pro-fibrotic processes including myofibroblast activation via Smad-dependent autocrine TGF-β signaling [77–81]. Besides its structural role, β-catenin plays a critical role in canonical Wnt signaling. In the absence of Wnt signals, β-catenin is phosphorylated by a complex consisting of adenomatosis polyposis coli (APC), axin, glycogen synthase kinase-3β (GSK-3β) and casein kinase which promotes subsequent ubiquitin-mediated β-catenin degradation. When secreted, Wnt proteins bind to cell surface Frizzled receptors (FZD) and lower density lipoprotein receptor-related protein co-receptor (LRP516) and the degradation complex is disrupted resulting in the stabilization of β-catenin which translocates to the nucleus where it binds to T-cell factor/lymphoid enhancer-binding factor (Tcf/Lef) to induce target gene transcription [82, 83]. Aberrant canonical Wnt signaling has been implicated in SSc [80] as well as in pulmonary, renal, dermal and liver fibrosis, in addition to scarring following myocardial infarction and fibrosis accompanying muscular dystrophy [84–86]. Under homeostatic conditions Wnt signaling must be tightly controlled. Indeed, an array of potent negative regulators has been identified, among which the Dickkopf proteins (DHK 1–4) play key roles. Dhk-1 is the best studied of them and it functions as a natural antagonist of Wnt signaling [87, 88].

22.4.4 Hedgehog Signaling

Three different mammalian orthologs of the *Drosophila melanogaster* hedgehog (Hh) morphogen have been identified in humans. They are highly hydrophobic secreted peptides called sonic hedgehog (SHh), Indian hedgehog, and Desert

Hedgehog with SHh being the most important in the present context [89]. Patched (Ptc) a twelve-pass transmembrane protein binds Hh ligands, but in the absence of ligand Ptc interacts with and inhibits Smoothened (Smo), a seven-pass transmembrane protein [90]. However, binding of SHh to Ptc induces conformational changes that prevent Ptc from inhibiting Smo, which, initiates signaling events resulting in stabilization of Gli family zinc finger transcription factors and in enhanced expression of Hh target genes [91]. While Hh signaling is critical during embryonic development, inappropriate activation in adults has been implicated in the pathogenesis of various diseases, including malignancies [92, 93]. In SSc cultured fibroblasts, overexpression of SHh causes accumulation of Gli-2 and increased expression of Hh target genes [94]. An extensive immunofluorescence analysis of affected SSc skin demonstrated intense staining in dermal fibroblasts and endothelial cells. Other results from this study found that TGF-β increased expression of SHh and that SHh induced strong stimulation of fibroblast to myofibroblast transition in normal dermal fibroblasts comparable to that caused by TGF-β [94]. Overexpression of SHh in the skin of mice induced fibrosis and mice lacking one allele of the inhibitor receptor Ptc 1 gene were more sensitive to experimentally-induced fibrosis [95].

22.4.5 Notch Signaling

Also first discovered in Drosophila, Notch signaling is initiated by binding of members of two ligand families, Jagged and Delta-like to Notch receptors, which results in cleavage of these receptors by the secretase complex and release of the active Notch intracellular domain (NICD) [95, 96]. Translocation of the NCID into the nucleus activates transcription of multiple target genes such as Hairy/Enhancer of Split (Hes) [97]. As with Hedgehog, Notch signaling is crucial during embryonic development, and is highly regulated in the adult. There is accumulating evidence for the participation of Notch signaling in fibrotic diseases, although the molecular mechanisms involved in fibroblast activation and enhanced ECM production need further clarification [98–100].

22.4.6 Matrix Stiffness and Rho-Associated Kinases

While activation of myofibroblasts and stimulation of ECM production by TGF-β and other cytokines are complex and incompletely understood, recent studies have focused on the role of actin cytoskeleton reorganization. There is increasing interest in the mechanical properties of the ECM, particularly of the effect of tissue stiffness on the biosynthetic activities of resident fibroblasts/myofibroblasts [18–20, 101, 102]. Although the exact mechanisms whereby increased matrix stiffness stimulates production of polymerized α-SMA remains to be elucidated, this effect can result in nuclear translocation of MKL-1 (MRTF-A), a transcription factor that plays a critical role in the stimulation of expression of fibrotic genes [101]. Furthermore, matrix

stiffening can promote RhoA production and activation, resulting in increased ROCK activity and enhanced fibroblast contractility. In addition, there may be cross-talk between the MAP kinase ERK 1/2, a potential downstream target of ROCK, and TGF-β [103]. All these findings suggest that as the fibrotic process proceeds and tissues become increasingly stiff, a vicious cycle gets established in which the matrix stiffness itself promotes further ECM production.

22.5 Pathophysiology of Fibrotic Abdominal Adhesions

While peritoneal adhesions may be caused by infection, inflammation or ischemia, surgical procedures are primarily responsible and can cause pelvic pain, bowel obstruction and infertility [104–107]. Although modern advances in surgical technique, including laparoscopy, have led to a decrease in their incidence, abdominal adhesions still pose a very significant medical as well as economic problem. Unfortunately, similar to other fibrotic reactions, adequate therapeutic solutions have proven elusive. This section briefly reviews the present state of affairs, including consideration of existing therapies and then places abdominal adhesions in the context of fibrotic reactions in general. Within this framework, an argument is developed suggesting that drugs which target signaling pathways known to be instrumental in the pathogenesis of many other fibrotic reactions be tested for their efficacy either in preventing or ameliorating intestinal adhesions.

The visceral peritoneum covering the gut and other viscera accounts for about 80% of the total peritoneal surface, while the parietal peritoneum lining the walls of the abdominal cavity accounts for the remaining 20%. These surfaces are covered by a monolayer of mesothelial cells bound to a basement membrane which, in turn, rests on a bed of connective tissue containing fibroblasts, macrophages and other cell types [108]. This layer also contains a rich capillary network and lymphatics. Because the mesothelial cells are weakly connected, the peritoneal surface is delicate and easily injured. Injury to the peritoneum exposing the basement membrane causes a local inflammatory response resulting in deposition of a fibrin-rich exudate as part of the haemostatic process. While a fibrinous exudate is essential for normal repair, if it is not resolved in a timely fashion, it can provide a matrix for invading fibroblasts and blood vessels leading ultimately to adhesion formation.

Activation of the coagulation cascade results in the formation of thrombin from prothrombin, which converts fibrinogen into fibrin monomers and which polymerize forming a fibrin clot, a reaction which can be inhibited by anti-thrombin. An essential feature of the restoration of normal tissue architecture is the degradation of fibrin by the proteolytic enzyme, plasmin, which is formed from the inactive precursor, plasminogen, by tissue plasminogen activator (tPA) or urokinase plasminogen activator (uPA) with tPA being the most important in the present context. A very significant inhibitor of tPA and uPA is plasminogen activator inhibitor (PAI-1) which is produced by a variety of cells and whose production is enhanced by a number of factors including surgery, inflammation, IL-1 and tumor necrosis factor (TNF). The control of these competing reactions is not well-understood at the present time.

22.6 Stages in Abdominal Adhesion Formation

Successful treatment of abdominal adhesions is bedeviled by several confounding factors, not the least of which is the complex pathogenesis. A variety of causes can be responsible for initiation of adhesion formation which then likely proceeds through a common pathway. This process has been broken down into several stages, each one of which has been considered as a therapeutic target.

22.6.1 Coagulation Stage

As discussed above, the coagulation cascade is a critical factor in adhesion pathogenesis [109, 110]. This sequence of reactions involves a number of protein factors which facilitate or inhibit the ultimate formation of a fibrin clot. While, in many cases, the formation of a clot is essential to limit injury, resolution of the clot, in a timely manner, is necessary to prevent adhesion formation. Thus, the balance between fibrin clot formation and its lysis is critical. Much attention has been devoted to the measurement of factors such as thrombin that promote the clot formation as well as those like plasminogen activator that resolve it. These studies provide a rational basis for enhancing clot lysis as a therapeutic strategy. However, in practice, this has proven difficult.

22.6.2 Inflammatory Stage

Although this stage can overlap with the coagulation stage, it is clear that a major inflammatory response is initiated during abdominal adhesion formation. This stage is characterized by an influx of multiple cell types and production of a variety of cytokines and growth factors is and is elicited by a number of inciting events. This has led to inhibition of inflammation as a therapeutic approach to adhesion prevention which, by and large, has proven to be unsuccessful, although there are some reports of a positive response [111].

22.6.3 Fibrous Adhesion Stage

The final stage in the adhesion process is formation of a connective tissue scar, which is of critical importance since it is this fibrous tissue that causes the most severe complications. The exact inciting events in this final stage have received insufficient attention. However, based upon many studies in multiple organ systems, abdominal adhesion formation shares many attributes with fibrotic reactions found elsewhere in the body. Unfortunately, in none of these fibrotic reactions have any

biomarkers been identified which can be used to detect the early stages of profibrotic pathology. Much more work is needed in the molecular characterization of early adhesion formation and the composition of the scar itself in order to formulate novel therapeutic approaches.

22.7 Focus on Fibrous Adhesion Formation

22.7.1 The Myofibroblast

It is now well-known and accepted that although the causative mechanisms of fibrotic disorders are diverse and vary widely, they all share important cellular and molecular features which provide a fundamental framework for therapeutic approaches. At the cellular level, as discussed above, it is universally appreciated that a particular cell with unique characteristics, the myofibroblast, is responsible in all instances for the formation of the connective tissue scar that disrupts normal architecture and function [112]. The accumulation of myofibroblasts and the uncontrolled persistence of their elevated biosynthetic functions are crucial determinants of the extent and rate of progression of fibrotic reactions and of their clinical course, prognosis and response to therapy. While the origins of myofibroblasts in other organs may differ, in the case of abdominal adhesions, there is a unique cell type, namely the mesothelial surface cells, which likely mediate adhesion formation. These cells can undergo mesothelial/mesenchymal transition and *trans-differentiate* into myofibroblasts which can be facilitated by TGF-β [113, 114]. As previously discussed, both epithelial and endothelial cells can be induced by TGF-β and other cytokines to *trans-differentiate* into myofibroblasts [23, 115–118]. In this transition, the cells loose expression of cadherins, rearrange their cytoskeleton, change morphology, gain expression of α-SMA and produce substantial amounts of ECM [119, 120] .

Prior to becoming a myofibroblast, the precursor cell undergoes a transition to an intermediate stage (proto-myofibroblast) characterized by increased actin-myosin stress fibers and prominent focal adhesion structures [121, 122], which is characterized by the expression of an isoform of the protein fibronectin containing the EDA domain (Fn^{EDA}). It is expressed at the cell surface during embryonic development, but is not normally found in adults, which distinguished it from the plasma form which is secreted by the liver into the blood stream. Expression of this cellular form of fibronectin (Fn^{EDA}) characterizes the intermediate stage of myofibroblast formation and results from the splicing of a unique exon into the primary mRNA transcript [123]. This exon encodes for an extra 91 amino acids in the final Fn^{EDA} molecule and is only synthesized in adults as a consequence of tissue damage, inflammation and wound healing [124]. Thus, the proto-myofibroblast synthesizes Fn^{EDA} but not α-SMA [125]. Clearly, Fn^{EDA} represents a potential diagnostic and therapeutic target.

22.7.2 Critical Cytokines, Signaling Pathways and Therapeutic Strategies

While the underlying etiology of a particular fibrotic reaction is frequently unknown, certain signaling pathways activated by several cytokines and growth factors undoubtedly play key roles in the pathogenesis with the TGF-β family playing a critical and predominant role by mediating the formation of myofibroblasts and stimulating the productions of ECM [126]. TGF-β is secreted into the ECM as a large latent complex which can undergo several alternative proteolytic or conformational activating events prior to binding to its cognate receptor. While the intracellular transduction pathways following TGF-β receptor binding can directly stimulate molecular effectors involved in the pro-fibrotic response, another important aspect of TGF-β action is the stimulation of other mediators having pro-fibrotic potential. In addition, a number of other cytokines and growth factors have been shown to be involved in adhesion formation, each of which may involve signaling via unique pathways and which can be targeted therapeutically.

Because of the multiplicity of signaling pathways elicited by TGF-β, multiple potential therapeutic targets exist. However, judicious choices must be made since TGF-β has numerous, and diverse critical functions. Thus, inhibiting all of its manifold activities by blocking its cell surface receptor, for example, is not desirable since toxic effects may ensue. Importantly, the use of this approach has been largely unsuccessful in past clinical trials [127–129]. Therapeutic strategies must be developed which limit the effect, as much as possible, to inhibiting signaling reactions crucial to, and only to, the fibrotic response. This implies that the most desirable targets are those that are essential for the response, but are as far "down- stream" in the relevant signaling pathways as possible. Furthermore, recent studies have shown that the c-Able-PKCδ pathway participates in the process of endothelial-mesenchymal transition. Additionally, the Akt-mTOR pathway plays an important role in various cell processes including regulation of cell proliferation and metabolism as well as being involved in some epithelial/mesenchymal transitions. These pathways are potential therapeutic targets.

It is likely that there are several cytokines in addition to TGF-β that may participate in adhesion formation. Of these, IL-6 is of considerable importance. The diverse functions of IL-6 are mediated by several protein components which include a receptor that is specific for IL-6 (IL-6R) and gp130 which together form a heterodimer complex and activate two pathways: the JAK/Stat-pathway and the Ras-MAPK (mitogen-activated protein kinase) pathway [130]. Most importantly, IL-6, in addition to TGF-β, was also found to be elevated in peritoneal fluid during abdominal surgeries. In a model of repetitive peritoneal inflammation, IL-6 was found to be capable of mediating a peritoneal fibrotic process [131]. Studies have shown that IL-6 can promote EMT in colorectal cell lines [132]. These data, as well as recent evidence from our laboratory [114], demonstrated that MMT can be induced by IL-6 and thus may represent an important cellular mechanism which can mediate the formation of abdominal adhesions.

22.7.3 Role of Hypoxia

It has been shown that hypoxia may play a role in abdominal adhesion formation by several mechanisms based largely on experimental model systems. Hypoxia can decrease tPA and increase PAI expression thereby inhibiting fibrin lysis. The PAI-1 gene promoter contains hypoxia response elements HRE-1 and HRE-2 which bind the oxygen-regulated transcription factor HIF-1α resulting in increased PAI-1 expression. HIF-1α also increases the production of vascular endothelial growth factor (VEGF), which plays a critical role in angiogenesis and formation of blood vessels in adhesions. In addition, hypoxia can increase production of TGF-β1 by human mesothelial cells and peritoneal fibroblasts while TGF-β1 can stimulate VEGF and CTGF expression. Hypoxia also increased expression of TIMP-1, thereby potentially causing a decrease in matrix degradation.

22.7.4 Material Barriers

Currently, the most frequent approach in the prevention of adhesion formation is use of material barriers [111, 133]. While a number of such materials are available, including both liquid and solid based ones, no completely acceptable one has been developed for several reasons. These include toxicity, difficulty in handling membrane films, and potentially limited efficacy. However, several of these are currently in use including Seprafilm® which we describe as a typical example. Seprafilm® is a transparent, resorbable membrane composed of sodium hyaluronic acid and carboxymethylcellulose. It degrades in 7 days under physiologic conditions, is safe, and provides some effectiveness in preventing postoperative adhesions after abdominal surgery. However, while Seprafilm® covers the treated tissue, it does not protect remote areas and thus allows adhesion formation at distant sites, and, in addition, it is highly fragile. A sprayable form of Seprafilm® is also currently being tested.

22.7.5 Pharmaceutical Approaches

Attempts have been made to modify adhesion formation by use of pharmaceuticals mainly through use of anti-inflammatory and anti-coagulant agents. For example, these have included systemic and local application of steroids, cyclooxygenase inhibitors, heparin and tissue plasminogen activator (t-PA). In some instances, barrier membranes have been used in combination with an agent e.g., heparin. However, the results have been mixed and no clear-cut beneficial result has been obtained. On a more promising note, efforts have been made to inhibit the effect of the pro-inflammatory peptide substance P by blocking its major receptor, neurokinin receptor (NK1R) which is believed to play a role in adhesion formation, possibly by

lowering expression of metalloproteinases. In animal adhesion models, administration of NK1R antagonists increased metalloproteinase activity, increased fibrinolysis and significantly lowered adhesion formation [111, 133].

22.8 Preventing Adhesions By Blocking Connective Tissue Formation

While abdominal adhesions present a significant and recurring medical problem, unfortunately, there are no really effective therapeutic measures available either for prevention or cure. It is noteworthy that TGF-β1 and IL-6 were found to be elevated in the peritoneal fluid of patients during/after abdominal surgery and that the levels of the cytokines appeared to be related to the severity of abdominal adhesion formation [134, 135]. In contrast to crohn's disease (CD) and IBD, abdominal adhesion formation is unusual because of the potential major role of mesothelial cells. Because of their cellular environment, mesothelial cells are bathed in peritoneal fluid containing high concentrations of both TGF-β and IL-6 and have the potential to undergo MMT providing a major source of myofibroblasts responsible for adhesion formation. The control of MMT is achieved primarily by three families of transcription factors: zinc finger Snail (SNAl1, SNAl2), basic helix-loop helix (Twisted 1) and ZEB (ZEB1, ZEB2) whose expression can be increased by both TGF-β and IL-6. Furthermore, both cytokines can elicit the phosphorylation/activation of a critical intracellular effector molecule, Erk1/2, required for the MMT of peritoneal mesothelial cells. MEK 1/2 is responsible for Erk1/2 phosphorylation and specific inhibition of MEK 1/2 prevents Erk1/2 phosphorylation and MMT. This implies that MEK inhibitors may be good therapeutic candidates for adhesion prevention, e.g., MEK inhibitors such as trametinib and selumetinib have been tested in clinical trials for melanoma [136].

22.8.1 Intestinal Fibrosis in Inflammatory Bowel Disease (IBD)

The pathophysiology of fibrotic reactions such as CD and IBD is considerably more complex in comparison to that of abdominal adhesions which occur secondary to surgery. Although much remains to be determined in the pathogenesis of adhesions with respect to genetic predisposition, firm identification of the source of myofibroblasts and detailed understanding of critical signaling pathways in IBD, the pathophysiology is more multifaceted because potential inciting events are more numerous. Additional considerations include a much more sustained inflammatory response, a clear, if still poorly understood, genetic pre-disposition, the potential involvement of multiple mesenchymal cells/myofibroblasts, exposure of the mucosa to intestinal bacteria and the involvement of the immune system.

22.8.2 Genetic Basis in IBD

While there may be familial pre-disposition in the formation of adhesions, the evidence remains anecdotal and there is no firm supporting evidence. In contrast, significant evidence supports the concept that pre-disposition to development of CD is polygenic with 163 loci contributing to either susceptibility or disease severity including fibrostenosis [137–140]. However attempts to link genetic loci to clinical phenotype remain problematic with the strongest association linked to susceptibility being to NOD2 (polymorphisms with disturbed surveillance of bacterial microflora), IL23 receptor (polymorphisms linked to regulation of adaptive immunity) and ATG16L1 and IRGM (deficit in autophagy) [140]. Individuals with NOD2/CARD15 mutations have a tenfold greater risk of aggressive disease and different genetic variations correlate with distinct phenotypes such as fibrostenosis [141, 142]. Interestingly, deep sequencing of loci identified by genome-wide association study (GWAS) have identified matrix metalloproteinases (MMP-1, MMP-2, MMP-3, MMP-9) and their tissue inhibitors (TIMP-1 and TIMP-2) as prognostic indicators for diagnostic and surgical recurrence in CD [143].

22.8.3 Cells Responsible for IBD Fibrostenosis

As in in other respects, the cellular fibrotic reaction in IBD is considerably more complex than that generally occurring in abdominal adhesion formation. Much of this complexity has to do with inciting events and the extent of tissue involvement. As discussed above, the majority of abdominal adhesions are owing to injury at the serosal surface during surgical procedures. However, in IBD, a multiplicity of factors interact in a complex, and in, as yet, poorly understood fashion, to initiate and prolong the fibrotic response. These factors include genetic predisposition, active participation of a diverse number of cell types including those of the immune system, an extended inflammatory response, exposure to bacterial products from the microbiome, and potential involvement of a number of cytokines and growth factors.

The production of ECM, particularly that of collagens I and III, is a fundamental factor in fibrostenosis formation. In IBD, as in other fibrotic diseases, myofibroblasts play a critical role in the overall pathophysiological mechanism and there are numerous potential sources of them. Unique to the intestine are the cells of Cajal in the submucosa and muscularis [144]. Another major source is the subepithelial myofibroblasts (SEMFs). Interestingly, there is substantial evidence of cross-talk between SEMFs and the epithelium [145]. Conditioned medium from cultured epithelial cells treated with pro-inflammatory cytokines enhanced SEMF migration and production of collagen and MMPs [146]. SEMFs exposed to pro-inflammatory cytokines can themselves then express interleukins IL-6 and IL-8 as well as granulocyte and macrophage stimulatory factors and pro-fibrotic cytokines IL-17A and TGF-β [147, 148]. The SEMFs also express Toll-like receptors (TLR-2, TLR-4 and

TLR-5) which enables them to respond to lipopolysaccharide and flagellin and further cytokine production [149, 150]. Another aspect of the involvement of SEMFs in fibrosis pathogenesis is that they may act as non-professional antigen-presenting cells since they constitutively express class II major histocompatibility complex to promote $CD4^+$ T cell differentiation [151].

Other potential sources of myofibroblasts in IBD include stromal fibroblasts and pericytes which, upon activation, can proliferate, express α-SMA and produce ECM [152, 153]. Pericytes are found in capillaries and small blood vessels surrounding endothelial cells and during inflammation they differentiate into cells producing large amounts of ECM [153]. Another source of myofibroblast-like cells is bone marrow-derived fibrocytes which travel to the tissues through the blood stream. Multipotent bone marrow mesenchymal cells differentiate into fibrocytes which express hematopoietic markers such as CD45, CD11, CD13 and CD6, but they also express collagen and α-SMA [153]. Increased amounts of such cells have been found in the blood and tissues of CD patients.

22.8.4 Smooth Muscle Cells

An unusual feature of the fibrotic response in the intestine is the involvement of smooth muscle cells which are found in the muscularis externa and which represent the largest mesenchymal component in the intestine. This is particularly critical in CD because the full thickness of the intestinal wall is frequently involved. There is marked thickening of the muscularis with cellular hyperplasia, hypertrophy and ECM deposition which contributes to stricture formation [154]. The smooth muscle cells in the muscularis externa and the muscularis mucosae may also be involved in both CD and UC. Smooth muscle cells, in addition to producing ECM, also produce significant amounts of cytokines and growth factors including TGF-β, Il-6, IGF-1, PDGF, and CTGF [155].

22.8.5 Pathogenesis of Fibrosis in IBD

In both CD and ulcerative colitis (UC), injury to the mucosal epithelium exposes the underlying tissues to inflammatory mediators derived from the intestinal microbiome. Such exposure can generate a damaging immune response in susceptible individuals. Such events generate a prolonged and recurrent inflammatory reaction which, in turn, can initiate and extend fibrotic reactions characterized by the activation of myofibroblasts resulting in stricture formation. Such fibro-stenosis occurs much more frequently in CD (30–50%) in which the full thickness of the intestine is affected as opposed to much less extent in UC (~5%) where usually only the mucosa and submucosa are primarily affected [156]. While inflammation is clearly necessary for the initiation of the fibrotic reaction; however, once started, it can progress in the absence of continued inflammation.

22.9 ECM Homeostasis in IBD

The net concentration of ECM is governed by its rate of production and by the rate of turnover of its components. Such turnover is regulated largely by the activity of matrix metalloproteinases (MMPs) of which there are 23 human types. MMPs are zinc and calcium proteases produced by a variety of cells including epithelial, myofibroblasts, and macrophages The MMPs include collagenases, gelatinases, stromelysins and matrilysins and thus comprise a broad level of substrate specificity [157–159]. Increased expression of MMPs such a collagenase MMP8 and gelatinase MMP-9 have been found adjacent to intestinal ulcerations in CD. The activity of these proteases is controlled by four tissue inhibitors of proteases (TIMPs). Cytokines and growth factors such as TGF-β and TNF-α can regulate MMP and TIMP expression. TGF-β down regulates MMP-1 and MMP-3 in intestinal myofibroblasts and enhances TIMP-1 expression, while, contrarily, TNF-α decreases MMP-2 activity and increases TIMP-1 expression [158]. The balance between MMP activity and TIMP inhibitory regulation is disturbed in the intestine of patients with fibrostenotic CD [157–160]. The T-cell derived cytokine, IL-21, that is increased in IBD, can increase expression of MMPs by intestinal myofibroblasts thereby contributing to mucosal ulcer formation [161]. In active CD, increased expression of MMP-1, MMP-3 and TIMP-1 has been found in the intestinal muscularis [162]. Thus, it is likely that these substances play a role in the pathophysiology of CD; however, the mechanism(s) which govern their expression is largely unknown.

22.10 Role of Immune Responses in IBD Fibrotic Reactions

A major difference in the pathogenesis of the fibrotic reaction between IBD and abdominal adhesions secondary to surgery is the pronounced role of the immune system in IBD and its comparatively small role in abdominal adhesion generation. Injury to the intestinal epithelium permits entry of bacteria into the mucosa and the generation of activating molecules including DAMPs and PAMPs which stimulate pro-inflammatory cytokines and chemokines by macrophages which recruit innate immune cells [163]. In a complex subsequent series of events, activated (M1) macrophages secrete interleukins (IL-1, IL-2 IL-23), TNF-α and reactive oxygen species which activate myofibroblasts [163]. Activation of TLRs can also stimulate myofibroblast proliferation and collagen production [164]. In chronic inflammation, macrophages shift to a M2 phenotype and release anti-inflammatory and pro-fibrotic mediators including IL-4, IL-13, IL-10 and TGF-β [163]. A correlation between collagen accumulation and elevated IL-13 and TIMP-1 expression by mononuclear cells has been reported in CD tissue samples [165]. Not surprisingly, the adaptive immune responses are complex. In a Th1 immunity response, interferon (INF)γ was found in early stages of CD and is considered as an anti-fibrotic cytokine blocking

TGF-β and CTGF expression, but INF-γ therapy of fibrotic reactions, for the most part, has not proven successful [166–168]. Th17 immune responses associated with fibrosis have also been reported in CD. IL-17A was elevated in stricturing CD tissue with increased collagen, MMP-3, MMP-12 and TIMP-1 [169]. IL-17 may have other effects in fibrosis by regulating expression of TGF-β and CTGF in myofibroblasts and upregulating collagen production [170].

22.11 Potential Anti-Fibrotic Therapy in IBD

Because the pathophysiology of abdominal adhesion formation may be less diverse in comparison to IBD, potential therapeutic targets are more easily selected as discussed above. Table 22.1 illustrates the greater complexity of IBD versus abdominal adhesions. In IBD there are multiple potential levels at which therapy could be applied. For example, there are several endogenous anti-fibrotic mediators with peroxisome proliferator-activated receptor (PPAR)-γ being the most promising. It has been found that this ligand-activated nuclear receptor, that is widely expressed in various cell types, has been found to decrease inflammatory responses in the intestine and other tissues [171]. PPAR-γ inhibits pro-fibrotic signaling by TGF-β and Wnt-β-catenin [172, 173], blocks TGF-β pro-fibrotic effects such as collagen and fibronectin production [174] and suppresses TGF-β-induced EMT [175]. Since ECM accumulation is governed by the comparative rates of production and degradation, this implies that increased degradation by MMPs might be an effective approach to minimize ECM formation. There is considerable *in vitro* information with respect to the control of the activity of multiple MMPs [158, 176]. Regrettably however, because of severe limitations in present knowledge regarding the ability to up-regulate MMP activity in a controlled manner *in vivo*, this approach is not presently feasible.

Table 22.1 Comparison of fibrosis in adhesions and IBD

	Abdominal adhesions	IBD
Area of involvement	Peritoneal surface	Mucosa, submucosa, muscularis externa
		Full thickness in CD
Myofibroblast sources	Resident fibroblasts	Subepithelial myofibroblasts, cells of Cajal, bone marrow, possibly mesothelial cells , pericytes, smooth muscle cells, EMT, EndoMT
Immune system involvement	Limited	Considerable
Inflammatory response	Acute	Chronic & protracted
Stricture formation	Often	30–50% in CD, ~5% in UC
Feasibility of therapy	Likely	Possible, but questionable because of chronic nature of disease
Pathogenesis complexity	Moderate	Very high

In IBD, the normal wound healing process triggered by injury and inflammation fails and instead of resolution, there is continued myofibroblast activity. Because the inflammatory response is protracted, one could imagine that anti-inflammatory therapy might be an effective approach. Unfortunately, this has not been the case, and it appears that once the damaging fibrotic reaction has been initiated in fibrosis-prone individuals, it is self-propagating. Thus, as in other fibrotic situations, the aberrant myofibroblast becomes the ultimate target. However, unlike abdominal adhesions in which the potential effector cells (mesothelial cells) can be anticipated and candidate drugs given over a fairly short time, in IBD, the pathogenesis is much more protracted. There a number of FDA-approved drugs capable of blocking pathways essential to fibrotic reactions such as trametinib [177] (Fig. 22.2). TGF-β is, of course, the primary target; however, total blockade has not worked because of adverse toxic responses because of its manifold activities. The same caution must be exercised when dealing with other potential targets (Fig. 22.2) [6]. Because of the inherent redundancy in signaling from multiple cytokines/growth factors involved in the activation of genes responsible for ECM synthesis, it is likely that more than one drug must be administered simultaneously to obtain effective beneficial inhibition of profibrotic pathways.

22.12 Conclusions

Although abdominal adhesions and IBD have varying etiologies, they share many features of fibrotic reactions found in other organs and tissues of the body. These include the synthesis of excess extracellular matrix composed of collagen and other macromolecules by myofibroblasts. These fibrotic reactions are driven by various cytokines/growth factors with TGF-β predominating. Despite considerable recently obtained understanding of the pathogenesis of the fibrotic process, disease-modifying therapy for these diseases is extremely limited. However, one potentially productive way forward will be to simultaneously use several FDA-approved drugs which can act together to effectively block multiple pathways preventing the activation of pro-fibrotic genes. In addition, biomarkers need to be identified which can signal the early onset of disease which may permit more effective use of currently available therapeutics.

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Chapter 23
Anti-Fibrotic Therapies from Other Organs: What the Gut Can Learn from the Liver, Skin, Lung and Heart

Calen A. Steiner and Peter D. R. Higgins

Abstract Fibrosis and dysregulated healing can affect nearly every organ system in the body. Often fibrosis represents a final common pathway to end organ failure, and there is evidence for substantial conservation of the mechanisms of fibrosis across many or all of these organs. Given the significant and pervasive impact of fibrosis there is a clear need for effective anti-fibrotic therapies. The study of these mechanisms and therapies is a robust area of research and allows for exciting collaboration. The conservation of mechanisms effectively posits any therapy that demonstrates efficacy in one organ or model of fibrosis as being a potentially viable option in other organs as well. In this chapter we review the current state of anti-fibrotic therapies in organs other the intestine. There are exciting pipeline agents under investigation in multiple organs including the liver, lungs, kidney, skin, and heart. This chapter focuses on agents that are currently in clinical trials and have demonstrated promise as potentially reaching mainstream use.

Keywords Fibrosis · Inflammatory bowel disease · Intestinal fibrosis · Hepatic fibrosis · Pulmonary fibrosis · Renal fibrosis · Dermal fibrosis · Anti-fibrotic · Farnesoid X receptor · FXR · Obeticholic acid · Lysyl oxidase · LOX · Simtuzumab · Statin · Caspase · 5HT · CCR2 · CCR5 · GR-MD-02 · Peroxisome proliferator-activated receptor (PPAR) · Pirfenidone · Nintedanib · Tyrosine kinase inhibitor · mTOR · Lysophospholipid · Prostacyclin · αvβ6 · Endothelin · IL-13 · Connective tissue growth factor · Serum amyloid P · NADPH oxidase · NOX · Pyridoxamine · Janus kinase · JAK · TGF-β · Paquinimod · ACE inhibitor

C. A. Steiner · P. D. R. Higgins (✉)
Department of Internal Medicine, Division of Gastroenterology, Michigan Medicine, University of Michigan, Ann Arbor, MI, USA
e-mail: calens@med.umich.edu; phiggins@med.umich.edu

F. Rieder (ed.), *Fibrostenotic Inflammatory Bowel Disease*,
https://doi.org/10.1007/978-3-319-90578-5_23

23.1 Introduction

The study of mechanisms of fibrosis and potential therapies is a rich area of investigation for numerous organs other than the intestine. Fibrosis is a final common pathway to organ failure in the liver, lungs, kidney, skin, and heart. Despite the diversity of tissues and functions, many mechanisms of fibrosis appear to be similar across organs [1–9]. Although the impact of fibrosis on human health is substantial, there is a stark paucity of therapies currently available to directly treat fibrosis, with the lung being the only organ to boast any approved therapies (Fig. 23.1, Table 23.1). However, there are candidate compounds targeting fibrosis across all of these organs that show promise. Given the conservation of pro-fibrotic mechanisms across tissues and organs, any therapy that effectively treats fibrosis in another organ warrants consideration and potentially investigation as a therapeutic for intestinal fibrosis as well [10]. This chapter will review the current state of anti-fibrotic therapy in the liver, lungs, kidney, skin, and heart, focusing on those agents currently in clinical trials and closer to mainstream use.

Many of these pathways and molecules have been studied in multiple organs. For the purposes of this chapter, we have divided the sections by organ. Each molecule

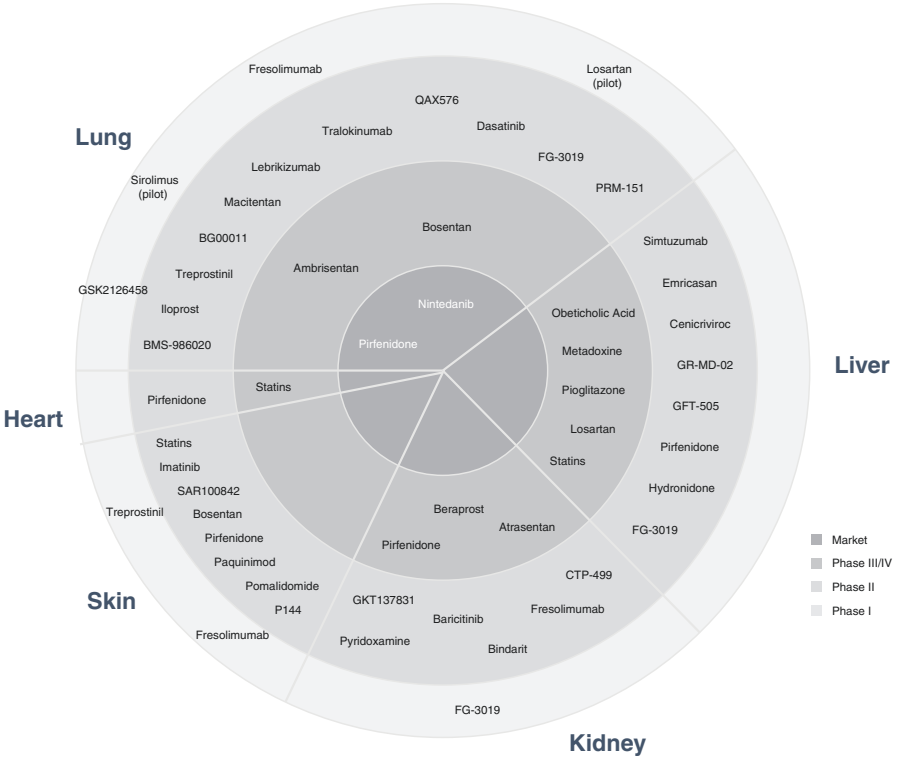

Fig. 23.1 Select anti-fibrotic agents by organ and clinical phase

Table 23.1 Select anti-fibrotic agents by organ and clinical phase

	Lung	Liver	Kidney	Skin	Heart
Market	• Pirfenidone				
	• Nintedanib (tyrosine kinase)				
Phase III/IV	• Ambrisentan (endothelin receptor)	• Obeticholic acid (FXR)	• Beraprost (prostacyclin)		• Statins (HMG-CoA reductase inhibitor)
	• Bosentan (endothelin receptor)	• Metadoxine (5HT)	• Atrasentan (endothelin receptor)		
		• Pioglitazone (PPARγ)	• Pirfenidone		
		• Losartan (ARB/RAAS)			
		• Statins (HMG-CoA reductase inhibitor)			
Phase II	• BMS-986020 (LPA)	• Simtuzumab (LOXL2)	• GKT137831 (NOX)	• Imatinib (tyrosine kinase)	• Pirfenidone
	• Iloprost (prostacyclin)	• Emricasan (caspase inhibitor)	• Pyridoxamine	• SAR100842 (LPA)	
	• Treprostinil (prostacyclin)	• Cenicriviroc (CCR2/CCR5)	• Baricitinib (JAK)	• Bosentan (endothelin receptor)	
	• BG00011 (αvβ6)	• GR-MD-02 (galectin)	• Bindarit (indazolic derivative)	• P144 (TGF-β1)	
	• Macitentan (endothelin receptor)	• GFT-505 (PPARα/δ)	• CTP-499 (PDE)	• Pomalidomide	
	• Lebrikizumab (IL-13)	• Hydronidone	• Fresolimumab (TGF-β1)	• Paquinimod (S100A9)	
	• Tralokinumab (IL-13)	• FG-3019 (CTGF)		• Pirfenidone	
	• QAX576 (IL-13)	• Pirfenidone		• Statins (HMG-CoA reductase inhibitor)	
	• Dasatinib (tyrosine kinase)				
	• FG-3019 (CTGF)				
	• PRM-151 (serum amyloid P)				

(continued)

Table 23.1 (continued)

	Lung	Liver	Kidney	Skin	Heart
Phase I	• GSK2126458 (mTOR)		• FG-3019 (CTGF)	• Treprostinil (prostacyclin)	
	• Sirolimus [pilot] (mTOR)			• Fresolimumab (TGF-β1)	
	• Fresolimumab (TGF-β1)				
	• Losartan [pilot] (ARB/RAAS)				

or pathway is included under the organ in which the most relevant or recent clinical trials are being performed, although many of these molecules will have supporting evidence for use in organs other than the one in whose section they appear.

23.2 Liver

The mechanisms of liver fibrosis are the subjects of intensive investigation, and multiple potential therapies targeting important pro-fibrotic pathways are under study [11, 12].

23.2.1 Farnesoid X Receptor (FXR)

The farnesoid X receptor (FXR) has been implicated as an important player in both inflammatory bowel disease [13] and hepatic inflammation and fibrosis [14–16]. 6-ethylchenodeoxycholic acid (obeticholic acid) is a synthetic bile acid that is an activator of the farnesoid X nuclear receptor [17]. The effect of lipophilic bile acid antagonism of FXR in NASH is thought to be secondary to effects on metabolism, insulin sensitivity, and decreases in circulating triglycerides as well as hepatic gluconeogenesis [17–19].

Recently, a multi-center, double-blind, placebo-controlled trial of (obeticholic acid) for patients with non-alcoholic steatohepatitis demonstrated histological benefit, including improvement in fibrosis [17]. Additionally, a phase 3 trial evaluating the long term benefit of obeticholic acid in patients with NASH fibrosis is currently recruiting (Randomized Global Phase 3 Study to Evaluate the Impact on NASH with Fibrosis of Obeticholic Acid Treatment; REGENERATE Trial. ClinicalTrials.gov NCT02548351).

The mechanism of the anti-fibrotic effect seen in NASH is thought to be primarily metabolic modulation, and may not have translation to intestinal disease. However FXR has been shown to be expressed in the small intestine as well as many other organs [15, 20–22]. Further, FXR is thought to be important in intes-

tinal barrier function as well as immune modulation [23], and FXR activation has demonstrated an anti-inflammatory effect in animal models of inflammatory bowel disease [13].

23.2.2 Lysyl Oxidase (LOXL2)

Lysyl oxidase (LOX) genes represent another potential target for anti-fibrotic therapy. One particular member of this family, lysyl oxidase like-2 (LOXL2), is thought to be a promising target for anti-fibrotic therapy due to its effects in cross-linking the extracellular matrix, and has been linked to fibroblast activation in cancer cells [11, 24]. LOXL2 has been shown to be increased in tissue from fibrotic lung and liver, and inhibition of LOXL2 in mouse cancer models demonstrated a reduction in activation of fibroblasts, decreases in growth factor and cytokine production, and a reduction in transforming growth factor-beta (TGF-β) [25]. LOXL2 has also been implicated as an important pathway in cardiac fibrosis related to heart failure, with increased levels in diseased human cardiac tissue and serum, and a reduction in fibrosis with gene knock-out and anti-LOXL2 antibody treated transaortic constriction mouse models of cardiac disease [26].

Simtuzumab is a humanized IgG4 monoclonal antibody that targets LOXL2. In a phase 2, open label study to assess safety, tolerability, and potential efficacy in liver fibrosis in HIV and/or HCV infected adults, Simtuzumab demonstrated safety and tolerability, but did not demonstrate improvement in fibrosis [27]. Additionally, a phase 2b, randomized, double-blind placebo-controlled trial of simtuzumab in patients with non-alcoholic steatohepatitis (ClinicalTrials.gov NCT01672866) was recently terminated. Simtuzumab has been investigated in the lung as well, but unfortunately failed to demonstrate efficacy in a clinical trial for idiopathic pulmonary fibrosis [28]. Despite this lack of efficacy of Simtuzumab in clinical trials, the promising data from in vitro and animal models combined with safety in human subjects maintains this pathway as worthy of further investigation.

23.2.3 Statins

Statins, or HMG-CoA reductase inhibitors, are actively being investigated as potential antifibrotic agents in liver disease. Traditionally used for their lipid-lowering effects, the potential of statins to impart clinical benefits beyond prevention of coronary and arterial vascular disease are increasingly being recognized and studied [11, 29, 30]. Statins are now thought to have anti-fibrotic, anti-inflammatory, antioxidant, and immunomodulatory effects. The multi-faceted impact of statins is due to their pleiotropic effects. These are a result of a reduction or down-regulation of isoprenoids, which are critical for the function of many GTPases. RhoA is one such GTPase, and a decrease in its activity has been proposed as a potential mechanism for the anti-fibrotic effects.

The anti-fibrotic effect of statins in liver disease has been demonstrated in in vitro and in vivo models of liver fibrosis [31–34]. Further, post hoc analysis of the results of the HALT-C (Hepatitis C Antiviral Long-Term Treatment against cirrhosis) trial have suggested that statin use could potentially reduce progression of fibrosis in chronic hepatitis C patients [35]. In addition to the liver, the anti-fibrotic potential of statins is also being investigated in the lung [36] and heart [37], and there are currently several clinical trials of statins for fibrosis at various stages of completion for fibrosis in several organs including the liver, skin, and heart.

Statins have also been investigated in intestinal fibrosis, and simvastatin has demonstrated anti-fibrotic potential in TNBS-induced colitis mouse model of fibrosis [38]. These encouraging results make statins a potential generic, low-cost, and effective anti-fibrotic therapy.

23.2.4 5-Hydroxytryptamine (5HT)

The serotonin signaling system is thought to be important in exerting both proliferative and anti-proliferative effects on parenchyma in the liver, and antagonism of the 5-hydroxytryptamine (5HT) receptor has been shown to enhance regeneration and reduce fibrosis in hepatocytes [39, 40]. Serotonin derived from platelets has been shown to stimulate extracellular matrix synthesis through 5-HT_{2B} receptors via a TGF-β dependent mechanism [41]. This study utilized dermal fibroblasts, transgenic mice, and a bleomycin-induced dermal fibrosis mouse model to demonstrate the importance of serotonin, primarily from platelets, in the development of experimental skin fibrosis. Further, the use of cyproheptadine and terguride as 5-HT_2 inhibitors in this study established them as potential anti-fibrotic therapies. Terguride has demonstrated anti-fibrotic activity in cardiac fibrosis in a pulmonary artery banding mouse model of right heart failure [42]. The importance of serotonin 5-HT_2 receptors, and the potential therapeutic effect of antagonism of those receptors, has also been demonstrated in a bleomycin-induced mouse model of pulmonary fibrosis [43].

Metadoxine has many pharmacological properties such as restoration of glutathione, NADH, and ATP levels [44]. Some of its effects are thought be related to serotonin 5-HT_{2B} antagonism. Metadoxine is currently approved for alcoholic hepatitis. Metadoxine has shown anti-fibrotic potential in hepatic stellate cells in culture [45], as well as murine models [46]. In humans, a randomized placebo-controlled trial of metadoxine vs. placebo in NASH demonstrated improvement in steatosis, but no difference in ALT, AST, or liver histology [44]. A phase III trial of Metadoxine as therapy for patients with NASH is currently underway (ClinicalTrials.gov NCT02541045). The established safety of Metadoxine and its potential anti-fibrotic effect make the investigation of this drug and others affecting 5HT signaling an intriguing avenue for investigation in liver fibrosis and intestinal fibrosis.

Platelets have been identified as an important source of serotonin in the lung, skin, in wound healing, and in hepatic regeneration and fibrosis [40, 41]. Elevated platelet count and elevated platelet:albumin ratio have been reported to be significant risk factors for surgery in stricturing small bowel Crohn's disease [47]. In addition to direct antagonism of serotonin signaling, anti-platelet therapy represents an intriguing potential anti-fibrotic therapy.

Clopidogrel, which exerts anti-platelet effects via antagonism of the adenosine diphosphate, G-protein coupled receptor $P2Y_{12}$ [48, 49], is currently widely used for the treatment of acute coronary syndrome, myocardial infarction, stroke, and peripheral arterial disease. Inhibition of platelet activation with clopidogrel was shown to be anti-fibrotic in an angiotensin II mouse model of cardiac inflammation and fibrosis [48].

Thus inhibition of the 5HT pathway, and inhibition of platelet activation, represents intriguing potential targets for anti-fibrotic therapies in the intestine.

23.2.5 Caspase Inhibition

Inhibition of caspases represents another potential avenue for anti-fibrotic therapy. Despite apoptosis being well described as less inflammatory compared to necrosis as an inducer. [50, 51] Fas-mediated apoptosis of hepatocytes has been shown to activate fibrogenesis in murine models [52]. Interference with Fas-mediated apoptosis has shown anti-fibrotic potential in bile duct ligation, concanavalin A, and Jo2 monoclonal antibody mouse models of hepatitis and liver fibrosis [52–54]. These findings have led investigators to pursue stellate cell apoptosis as a potential anti-fibrotic therapeutic target. Selecting death receptors specifically as therapeutic targets is complicated by the fact that they exert effects through a variety of intracellular cascades. One such cascade relies on caspases, which are key effectors of apoptosis [55, 56]. Despite the existence of at least 13 mammalian caspases, broad spectrum caspase inhibitors have been synthesized and demonstrated potency as anti-apoptotic agents [53, 57, 58]. One such inhibitor, Emricasan (IDN-6556), has demonstrated efficacy in reducing hepatic fibrosis in murine models [53, 59], and has entered clinical trials [60]. Currently Emricasan is being studied in a phase II clinical trial of patients with liver fibrosis and hepatitis C reinfection after liver transplant (ClinicalTrials.gov NCT02138253). This double-blind, randomized, multicenter trial is evaluating the effects of IDN-6556 versus placebo on liver fibrosis in these patients, and is expected to conclude in early 2018. While this apoptotic pathway is thought to target hepatocellular stellate cells as key mediators/effectors of fibrosis, it is plausible that similar induction of apoptosis in myofibroblasts in the intestine could produce therapeutic benefits in IBD. Furthermore, the ubiquity of caspases as downstream effectors of apoptosis presents a promising potential target that could be effective in multiple organ systems.

23.2.6 Chemokine Receptors CCR2/5

Monocytes and macrophages are recognized as being important mediators of liver fibrosis [61]. Chemokines are critical mediators of cell function and act through G-protein coupled chemokine receptors (CCRs) [62]. Chemokines are recognized as important mediators of inflammation and are also thought to play an important role in orchestrating fibrosis in liver disease [63], and the CCR phenotype profile of monocytes is likely an important determinant of function. The chemokine receptors CCR1, CCR2, and CCR5 are implicated as being pro-fibrotic in murine models of liver fibrosis [64–66]. CCR2 has also been linked to inflammation and fibrosis in the kidney [66–68] and lung. [66, 69, 70] Thus, CCRs represent an intriguing target for anti-fibrotic therapies in the liver and other organs.

Cenicriviroc is a small molecule inhibitor of both CCR2 and CCR5, and has demonstrated safety in early trials of human patients afflicted with HIV-1, [71–73] as well as a phase IIb trial (ClinicalTrials.gov NCT01338883) [74, 75]. Cenicriviroc has also demonstrated therapeutic potential in animal models of liver fibrosis as well as renal fibrosis [76]. A phase II trial of cenicriviroc in patients with NASH and liver fibrosis is planned to complete in late 2017 and (ClinicalTrials.gov NCT02217475). This trial, also called "CENTAUR: Efficacy and Safety Study of Cenicriviroc for the Treatment of Nonalcoholic Steatohepatitis (NASH) in Adult Subjects With Liver Fibrosis," is a double-blind, placebo-controlled, randomized multinational study with the primary endpoint being histologic improvement of NASH activity score without worsening of fibrosis in patients with NASH and liver fibrosis [77]. Results of this study are pending. An open label rollover extension study of participants in CENTAUR began in early 2017 and is ongoing (ClinicalTrials.gov NCT03059446).

There is evidence that CCRs are important in fibrosis in the liver, lung and kidney. At least one molecule that inhibits CCRs has demonstrable safety in human subjects. As such, blockade of CCRs with molecules such as Cenicriviroc represent another potential therapeutic avenue for the treatment of fibrotic inflammatory bowel disease.

23.2.7 GR-MD-02

Galectins are a family of proteins with a carbohydrate binding domain that may be influential in immune and inflammatory processes [78, 79]. One member of this family, galectin 3, is thought to be important in inflammation [80] and has shown to be an important regulator of fibrosis in experimental models of both the liver [81] and lung [82]. Inhibition of galectin-3 with a novel carbohydrate inhibitor, GR-MD-02, has been shown to reduce fibrosis and even reverse cirrhosis in animal models [83]. GR-MD-02 has demonstrated safety and tolerability in a phase 1 trial in patients with NASH [84]. There is a recently completed phase 2 trial of

GR-MD-02 in patients with NASH (NASH-FX) (ClinicalTrials.gov NCT02421094) and an additional phase 2 trial in patients with NASH and portal hypertension (NASH-CX) (ClinicalTrials.gov NCT02462967). Results have not been published for either, but it was announced that GR-MD-02 did not meet the primary endpoint of change in liver fibrosis measured by LiverMultiScan, nor did it meet secondary endpoints of change in liver stiffness measured by MR-elastography or stiffness measured by FibroScan®.

23.2.8 PPAR Gamma

Members of the nuclear receptor peroxisome proliferator-activated receptor (PPAR) nuclear receptor superfamily have an array of effects and are involved in metabolic processes, inflammation, and fibrosis [85, 86]. Thiazolidinediones are ligands for the nuclear receptor peroxisome proliferator-activated receptor gamma (PPAR-γ) that are potent insulin sensitizers but have an array of effects related to modulation of gene expression [85]. Thiazolidinediones are widely used in the treatment of diabetes, and their potential for use as in liver fibrosis is an active area of investigation [11, 12].

Pioglitazone demonstrated an anti-fibrotic effect in a choline-deficient L-amino acid-defined diet rat model of liver fibrosis [87]. It was also shown to be anti-fibrotic in a bleomycin-induced rat model of lung fibrosis [88] The PIVENS trial was a phase III trial evaluating pioglitazone, vitamin E, or placebo in patients with NASH [89]. Pioglitazone failed to achieve significance in the primary outcome (a combination of histological findings, fibrosis and disease activity scores), but did improve steatosis, inflammation, hepatocellular ballooning, and liver enzyme levels [90]. In a proof of concept study in patients with type 2 diabetes or impaired glucose tolerance and NASH, pioglitazone improved liver enzymes, decreased hepatic fat content, and improved some histological findings but failed to significantly reduce fibrosis [91]. Interestingly, in a clinical trial in non-diabetic patients with NASH, pioglitazone did improve fibrosis assessed by histologic features [92].

Pioglitazone also is/has been evaluated in several clinical trials without complete results as of yet. These include a phase IV trial in patients with type 2 diabetes and NAFLD, which is complete but no results have been published (ClinicalTrials.gov NCT01002547); a separate phase IV trial of pioglitazone in patients with NAFLD and type 2 diabetes (UTHSCSA NASH Trial) is completed, (results are available on ClinicalTrials.gov but not analyzed) (ClinicalTrials.gov NCT00994682); and a phase II trial to evaluate long term safety and efficacy in NASH, (results are available on ClinicalTrials.gov but not analyzed) (ClinicalTrials.gov NCT00062764).

Rosiglitazone showed anti-fibrotic activity in a bleomycin-induced mouse model of scleroderma [93], and a bleomycin-induced rat model of pulmonary fibrosis [94]. Rosiglitazone has demonstrated some antisteatogenic efficacy in short but not long term therapy for patients with NASH in the FLIRT/FLIRT-2 trials, and there was no improvement in fibrosis in this trial [95, 96]. In a phase II clinical trial, a different

PPAR-γ agonist, farglitazar, failed to improve fibrosis in patients with chronic hepatitis C [97].

GFT-505 (elifibranor) is a dual agonist of PPAR-α and PPAR-δ being studied for its metabolic effects [98, 99] that demonstrated anti-fibrotic effects in three murine models of liver fibrosis, (western-diet fed human apolipoprotein E2 transgenic mice, methionine-and choline deficient-diet—fed db/db mice, and CCl_4-induced fibrosis rats) [100]. A phase IIb clinical trial of GFT-505 in patients with NASH demonstrated a resolution of NASH without worsening of fibrosis, albeit on a modified definition from the original primary outcome (which was not met) (ClinicalTrials.gov NCT01694849) [101].

PPAR-γ agonists have been identified as potential targets for the treatment of ulcerative colitis given their role in modulation of inflammation and the immune response [102]. In vitro studies of troglitazone and rosiglitazone (PPAR-γ agonists), in human intestinal primary myofibroblasts recently demonstrated anti-fibrotic effects such as reduction in procollagen1A1, fibronectin, and α-smooth muscle actin [103]. If these agents demonstrate consistent anti-fibrotic effects they warrant further investigation for the treatment of intestinal fibrosis, particularly in patients with comorbid metabolic disease.

23.3 Lung

The study of anti-fibrotics for pulmonary disease is a rich and active area of investigation, and pulmonary fibrosis boasts two FDA approved anti-fibrotic medications (pirfenidone and nintedanib).

23.3.1 Pirfenidone

Pirfenidone is a pyridine derivative that has demonstrated anti-fibrotic and anti-inflammatory effects, and is approved for the treatment of idiopathic pulmonary fibrosis [104]. The exact mechanism of action of pirfenidone is yet to be elucidated. In a bleomycin-induced hamster model of lung fibrosis, pirfenidone has been shown to decrease biochemical markers of lung toxicity [105], suppress bleomycin-induced expression of transforming growth factor-beta (TGF-β) and lung procollagen 1 and III [106, 107]. Pirfenidone has also been shown to reduce or attenuate TGF-β mediated profibrotic activity in human lung fibroblasts via suppression or attenuation of α-smooth muscle actin, procollagen, and collagen synthesis and via suppression of human lung fibroblast proliferation [108, 109].

The clinical efficacy of pirfenidone has been studied in four phase III placebo-controlled randomized clinical trials. The first published phase III studied randomized 275 patients and evaluated a primary endpoint of change in vital capacity at week 52 for pirfenidone vs placebo [110]. Pirfenidone demonstrated superiority in

both the primary endpoint ($p = 0.0416$), as well as secondary endpoint of progression free survival ($p = 0.0280$) [110]. The results of the CAPACITY studies were published in 2011 [111]. CAPACITY 004 included 435 patients and demonstrated significance in its primary end point of change in percentage predicted forced vital capacity at week 72 ($p = 0.001$) [111]. CAPACITY 006 included 344 patients and evaluated the same primary endpoint, but failed to show a significant improvement in the pirfenidone group ($p = 0.501$) [111]. A fourth trial, ASCEND, was published in 2014 that included 555 patients and evaluated a primary endpoint of change in percentage of the predicted forced vital capacity at 52 weeks [112]. in the ASCEND trial pirfenidone demonstrated a significant improvement in the primary endpoint ($p < 0.001$), and also had favorable results for several secondary end points such as relative risk of death or disease progression and change from baseline for 6-min walk distance. Further, an analysis of pooled data from the ASCEND and CAPACITY studies included 1247 patients and suggested efficacy of pirfenidone for percent decline in predicted forced vital capacity as well as progression free survival, 6-minute walk distance, and dyspnea at 1 year [113]. Results of an open label extension study of one of the CAPACITY trials, RECAP, have also been published [114]. This study evaluated 178 patients previously randomized to placebo, and reports similar efficacy for forced vital capacity and survival for patients with idiopathic pulmonary fibrosis.

Pirfenidone appears to be generally well tolerated. In a recent prospective, observational, post-marketing surveillance study of 1371 patients, the most common side effects were decreased appetite, photosensitivity, nausea and abdominal discomfort [115]. Safety has also been evaluated in a pooled analysis of the CAPACITY trials, ASCEND trial, and two ongoing open label studies (study 002 and RECAP) [116]. This included analysis of 1299 patients for up to 9.9 years, and concluded that pirfenidone is safe and well tolerated in long term treatment. Most adverse events were mild to moderate, and the most common adverse events were nausea, diarrhea, dyspepsia, vomiting, and rash.

Pirfenidone has also been studied as a potential anti-fibrotic in the liver, kidney, heart, and eye [117]. There are currently multiple clinical trials of pirfenidone at varying stages of completion for the treatment of fibrosis in several organs, including the liver, kidney, skin, and heart.

Pirfenidone has demonstrated anti-fibrotic activity in the CCl_4 and bile duct ligation rat models of liver fibrosis [118]. It has also been shown to have anti-fibrotic effects in patients with chronic hepatitis C in a phase II clinical trial [119]. A randomized, double-blind, placebo-controlled trial of pirfenidone for the treatment of diabetic nephropathy demonstrated improvement in the primary outcome of mean change in eGFR for a dose of 1200 mg/day ($p = 0.026$) but not 2400 mg/day [120]. Despite completion of a clinical trial for pirfenidone in hypertrophic cardiomyopathy (ClinicalTrials.gov NCT00011076), no results have been published.

A second pyridine derivative, hydronidone, has reportedly demonstrated some anti-fibrotic efficacy in rat and mouse models of liver fibrosis, (although these data have not been published), and was well tolerated in a small first-in-human study [121]. A phase II clinical trial of hydronidone in patients with liver fibrosis

secondary to hepatitis B chronic hepatitis is currently recruiting (ClinicalTrials.gov NCT02499562).

These data make pirfenidone and other pyridine derivatives exciting agents for investigation in intestinal fibrosis. However, extrapolating potential efficacy from one organ system to another is made more difficult by the lack of understanding of the mechanism of action. Further complicating the use of pirfenidone for intestinal fibrosis is the incidence of gastrointestinal side effects associated with its use. Despite that, the action on TGF-β signaling, which is also known to be important in fibrosis in many organs including the intestine, highlights pirfendone as a potential anti-fibrotic for a broader array of fibrotic disease.

23.3.2 Nintedanib/Tyrosine Kinase Inhibitors

Tyrosine kinase inhibitors are actively being studied as potential anti-fibrotics in several different organ systems. Nintedanib is an indolinone derivative that inhibits multiple tyrosine kinases including vascular endothelial growth factor receptors, fibroblast growth factor receptors, and platelet-derived growth factor receptors [122, 123]. It is approved for the treatment of idiopathic pulmonary fibrosis, with clinical efficacy established in one phase II trial [124] and two phase III trials (INPULSIS) [125]. The phase II clinical trial was placebo-controlled and randomized 432 patients, and assessed the annual rate of decline of forced vital capacity as the primary endpoint [124]. In this study, the highest dose of nintedanib (150 mg twice daily, reduced the rate of loss of forced vital capacity by 68.4% compared to placebo, but did not reach statistical significance ($P = 0.06$). This study was followed up by the INPULSIS-1 and INPULSIS-2 studies, which were identical randomized, placebo-controlled, double-blind, 52-week phase III trials evaluating the annual rate of decline of forced vital capacity as the primary endpoint [125]. Between the two studies, 1066 patients were randomized. Both studies demonstrated a reduction in rate of decline in forced vital capacity with $p < 0.001$. The most frequent adverse event was diarrhea.

Nintedanib has also shown anti-fibrotic potential in TGF-β activated mouse fibroblasts, LX2 cells, primary human hepatic stellate cells, and the CCl_4 induced mouse model of liver fibrosis [126].

While the efficacy of nintedanib in idiopathic pulmonary fibrosis alone makes this agent worth investigation in intestinal fibrosis, and evidence of anti-fibrotic potential in liver models of fibrosis further supports more investigation, the incidence of gastrointestinal side effects may limit its utility for this purpose, and there has even been one report of colitis associated with its use [127].

At least two other tyrosine kinase inhibitors are under investigation as therapies for fibrotic disease.

Imatinib is another tyrosine kinase inhibitor approved for multiple cancers that has been studied in clinical trials for both idiopathic pulmonary fibrosis [128] as well as diffuse cutaneous systemic sclerosis [129] and scleroderma associated skin

fibrosis [130]. When studied for idiopathic pulmonary fibrosis, a randomized, placebo-controlled clinical trial failed to show improvement in survival or lung function [128]. In a phase IIa open label study for diffuse systemic sclerosis imatinib showed improvement in skin thickness and morphology as well as forced vital capacity [129]. In phase II randomized double-blind study for use in scleroderma-associated skin fibrosis imatinib failed to show efficacy [130]. A phase II study of imatinib for fibrosis in systemic sclerosis has been completed, but no results have yet been published (ClinicalTrials.gov NCT00613171).

Dasatinib is a pan-Src kinase inhibitor [131] approved for Philadelphia chromosome positive leukemia that has shown anti-fibrotic effects in in vitro fibroblasts from systemic sclerosis patients and a bleomycin-induced dermal fibrosis mouse model [132]. Phase I and II trials of dasatinib for scleroderma pulmonary fibrosis have been completed with some results available but not published or analyzed. (ClinicalTrials.gov NCT00764309).

23.3.3 Lysophospholipids

The lysophospholipid lysophosphatidic acid (LPA) is a small lipid that exerts an array of effects via action on G-protein coupled receptors [133]. Recently, several lysophospholipids and their receptors, including LPA, have emerged as potential therapeutic targets for an array of conditions that includes fibrosis, inflammation, and autoimmune disease [134]. LPA signaling has been identified as a potentially important mediator of lung fibrosis [135] and LPA has also been shown to be elevated in systemic sclerosis [136]. LPA antagonism also demonstrated anti-fibrotic potential in a bleomycin induced mouse model of lung fibrosis and a mouse model of scleroderma [137, 138]. The LPA receptor antagonist BMS-986020 has been studied in phase II clinical trials for the treatment of idiopathic pulmonary fibrosis, with results pending (ClinicalTrials.gov NCT01766817). In systemic sclerosis, the LPA inhibitor SAR100842 has also been studied in a phase II clinical trial (ClinicalTrials.gov NCT01651143) and showed a reduction in skin thickness and evidence of target engagement [139]. In the intestine, LPA signaling has been implicated in inflammation and colorectal cancer [140], and the emergence of LPA antagonists as potential anti-fibrotics in other organs certainly represents a potential therapeutic avenue for intestinal fibrosis as well.

23.3.4 mTOR

The mTOR pathway (and an upstream signaling molecule phosphatidylinositol 3-kinase [PI3K]) is thought to be important in immune disease, cancer, and even insulin resistance [141, 142]. The mTOR pathway has also been implicated as having importance in the development of fibrosis in several organs, and inhibition of

this pathway has been described to be anti-fibrotic in the kidney, liver, and lung [143–148]. Two drugs with mechanisms involving mTOR inhibition are currently in clinical trials for the treatment of idiopathic pulmonary fibrosis. GSK2126458 is an orally bioavailable, potent inhibitor of PI3Kα and mTOR that has been shown to inhibit the PI3K pathway in idiopathic pulmonary fibrosis lung tissue, fibroblasts, and bronchoalveolar lavage cells [149, 150]. A phase I proof of mechanism study in idiopathic pulmonary fibrosis has been completed, with results pending (ClinicalTrials.gov NCT01725139). Sirolimus (rapamycin) is an immunosuppressant used in organ transplantation that inhibits the mTOR pathway [151]. Rapamycin has demonstrated anti-fibrotic potential in a unilateral ureteral obstruction rat model of kidney fibrosis [147] and a transgenic TGF-α overexpression mouse model of pulmonary fibrosis [143]. A double-blind, placebo-controlled pilot study of sirolimus in idiopathic pulmonary fibrosis patients is ongoing (ClinicalTrials.gov NCT01462006).

In the intestine, one small retrospective case review found that sirolimus may be an effective rescue therapy in pediatric patients with severe refractory IBD [152].

While the mTOR pathway is an attractive potential anti-fibrotic target for intestinal disease, there are some roadblocks to its utility for this purpose. Rapamycin frequently causes diarrhea that is potentially mediated by reduction in the Na+/H+ exchanger 3 in the intestine, [153] and mTOR gene disruption in mice was shown to cause epithelial cell defects and atrophy following irradiation injury [154]. mTOR inhibition is known to cause well described oral ulceration, also called mTOR inhibitor-associated stomatitis [155, 156]. Perhaps of greatest concern is that mTOR inhibition causes impairment of wound healing, including in the intestine, and impairs healing of anastomoses [157–163]. In case reports, sirolimus has been implicated as a cause of small bowel ulceration [164], and a cause of impaired wound healing after metatarsal resection due to an infected plantar ulcer in a patient with type I diabetes status post kidney and pancreas transplantation [165]. In another case report, sirolimus toxicity has even been implicated as a potential cause of colonic perforation after development of colitis and ulcerations from leukocytoclastic vasculitis [166]. These well described effects of mTOR inhibition raise significant concern when considering their use for fibrosis associated with inflammatory bowel disease.

More investigation may be warranted pending anti-fibrotic efficacy in clinical trials.

23.3.5 Prostacyclin

Prostacyclin is a prostaglandin known to be a vasodilator and inhibitor of platelet aggregation that is increasingly recognized as an important inflammatory mediator in a variety of disease states [167]. Several prostacyclin analogues are currently approved for the treatment of pulmonary arterial hypertension.

Two such agents, iloprost and treprostinil, are currently under investigation in clinical trials as potential anti-fibrotic therapies [12]. Iloprost has been shown to

improve survival and prevent fibrosis in a bleomycin-induced mouse model of pulmonary fibrosis [168]. Iloprost is also used to treat scleroderma and Raynaud's phenomenon, and has been shown to reduce connective tissue growth factor (a pro-fibrotic cytokine) in patients with scleroderma [169].A randomized, double-blind, phase II clinical trial of iloprost in patients with idiopathic pulmonary fibrosis and elevated pulmonary arterial pressure has been completed, but no results are readily available (ClinicalTrials.gov NCT00109681). Treprostinil has been shown to improve digital ulcers in patients with systemic sclerosis [170], and has been studied in a phase I clinical trial in patients with systemic sclerosis (ClinicalTrials.gov NCT00848939). A phase II trial of treprostinil in patients with pulmonary hypertension and idiopathic pulmonary fibrosis was underway but terminated (ClinicalTrials.gov NCT00703339).

A third prostacyclin, beraprost, was shown to reduce renal tubular damage and tubulointerstitial fibrosis in a rat unilateral ureteral obstruction rat model of chronic kidney failure [171]. A phase IIb/III study of beraprost in patients with chronic kidney disease has been completed (ClinicalTrials.gov NCT01090037), but thus far only study design and rationale have been published [172].

The prostaglandin misoprostol has been shown to have effects on gastric mucosal blood flow, cellular permeability and epithelial proliferation, and has demonstrated efficacy for the treatment of NSAID-induced gastric ulcers [173], but is not routinely used for this purpose. Misoprostol has been shown to decrease orocecal transit time in healthy volunteers [174], and in a 3 week randomized double-blind cross over study of nine patients with severe chronic constipation, misoprostol reduced colonic transit time ($P = 0.0005$), increased stool weight ($P = 0.001$), and increased number of stools ($P = 0.01$) [175]. In a small open label trial of misoprostol for chronic refractory constipation, misoprostol enhanced colonic motility (particularly the left colon), but it's use was limited by high rates of withdrawal due to abdominal discomfort [176]. In a larger prospective randomized double-blind trial of misoprostol for improving postoperative intestinal motility, rectal misoprostol did not improve motility and in fact increased nausea and analgesic need [177].

Little work has been done to elucidate the role prostacyclins may play in intestinal fibrosis, but the demonstrable anti-fibrotic potential in the lung, skin, and kidney should position prostacyclins as a viable target for investigation in fibrotic intestinal disease.

23.3.6 Integrin αvβ6

Integrins are cell surface receptors thought to be involved in multiple processes including cell adhesion and migration [178]. αvβ6 is an epithelial-expressed integrin that is thought to be important in tissue repair [179, 180],and may have fibrosis mediating effects through activation of latent TGF-β [181]. αvβ6 has been shown to be up-regulated in a bile duct ligation mouse model of biliary fibrosis [182]. It has additionally been identified as a potential target for the treatment of idiopathic

pulmonary fibrosis [183],and several selective monoclonal antibodies targeting αvβ6 have been generated [184].A humanized monoclonal antibody that targets αvβ6 known as BG00011 (formerly STX-100) has been studied in a phase II clinical trial for idiopathic pulmonary fibrosis, but results are not yet published (ClinicalTrials.gov NCT01371305).

23.3.7 Endothelin Receptor Antagonism

The endothelin receptors ET_A and ET_B are vasoconstrictive, G-protein coupled receptors that bind the peptide endothelin [185]. Endothelin has been shown to be upregulated by TGF-β1 [186], interleukin-1 [187], and TNF-α [188]. Several endothelin receptor antagonists are currently in clinical use for pulmonary arterial hypertension, and there is active investigation into their use as a potential therapy for chronic kidney disease [185, 189, 190]. Endothelin receptor antagonism is also recognized as a potential anti-fibrotic therapy in many organs including the kidney, lung, liver, skin, and heart [12, 185, 191, 192].

Endothelin and ET receptors have long been implicated as important in idiopathic pulmonary fibrosis [193], and have been shown to induce epithelial-mesenchymal transition and reciprocally increase TGF-β1 in alveolar cells [194]. Bosentan is an antagonist of ET_A and ET_B [195, 196]. Bosentan improved fibrosis in a bleomycin-induced rat model of pulmonary fibrosis [197]. Bosentan failed to demonstrate efficacy in clinical trials for the treatment of idiopathic pulmonary fibrosis (BUILD-1, BUILD-3) [198, 199]. Bosentan also failed to demonstrate efficacy in clinical trials for use in interstitial lung disease secondary to systemic sclerosis [200]. Ambrisentan, a selective ET_A antagonist approved for use in pulmonary arterial hypertension, was studied in a phase III randomized, double-blind, placebo-controlled trial (ARTEMIS-IPF) (ClinicalTrials.gov NCT00768300) but was terminated early due to lack of efficacy and risk for respiratory hospitalization and disease progression [201]. Macitentan is another endothelin receptor antagonist used in pulmonary arterial hypertension that showed promise in pre-clinical studies but failed to demonstrate efficacy in a phase II clinical trial for idiopathic pulmonary fibrosis (MUSIC) [202].

Ambrisentan did show anti-fibrotic effect via inhibition of hepatic stellate cell activation and procollagen-1 and TIMP-1 gene expression reduction in a mouse model of NASH [203]. Endothelin receptor antagonism also reduced liver fibrosis and improved portal hypertension in CCl_4 treated mice [204].

Atrasentan is an ET_A receptor antagonist that demonstrated efficacy in reducing residual albuminuria when added to renin-angiotensin system blockade in patients with diabetic nephropathy [205]. A phase III clinical trial of atrasentan in patients with diabetic nephropathy is currently recruiting (SONAR) (ClinicalTrials.gov NCT01858532).

Endothelin receptor antagonists have also been studied for use in dermal fibrosis. Bosentan reduced the number of new ulcers but not the healing of existing ulcers in

patients with systemic sclerosis [206]. A phase II study of bosentan in systemic sclerosis has been completed (ClinicalTrials.gov NCT00318175), but results have not been published.

Despite a general lack of anti-fibrotic efficacy in clinical trials, particularly for pulmonary fibrosis, the pre-clinical data and effects on chronic kidney disease make endothelin antagonism a target worth investigating for inflammatory bowel related fibrosis. A recent study of atrasentan in TNBS-induced mouse model of colitis demonstrated an improvement in the severity of colitis, evidenced by macroscopic and microscopic score reductions and abrogation of levels of IL-1β, keratinocyte chemoattractant, and MIP-2 [207]. These encouraging data indicate a need for further characterization of the role of endothelin in inflammatory bowel disease, related fibrosis, and the potential for endothelin antagonists to be used as anti-fibrotic therapies in the intestine.

23.3.8 Interleukin (IL)-13

IL-13 is a cytokine and inflammatory mediator that is primarily secreted by type 2 helper T cells that increases TGF-β_1 production through action on the IL-13Rα_2 receptor [208, 209]. In animal models, IL-13 has been shown to be an important mediator of bleomycin-induced pulmonary [210] and TNBS-induced intestinal fibrosis [208, 211].

Lebrikizumab is a humanized monoclonal antibody that blocks IL-13 that has been shown to improve lung function in adults with asthma [212] and is being studied in an ongoing clinical trial for the treatment of IPF (ClinicalTrials.gov NCT01872689). A separate human monoclonal antibody against IL-13, tralokinumab, has been studied in two clinical trials for IPF (ClinicalTrials.gov NCT02036580) (completed, results available but not analyzed), and (ClinicalTrials.gov NCT01629667) (terminated due to lack of efficacy). A trial of tralokinumab as add on therapy for the treatment of ulcerative colitis did not improve clinical response (ClinicalTrials.gov NCT01482884) [213], but also did not worsen inflammation in ulcerative colitis. QAX576, another antibody against IL-13, has been studied in two clinical trials for pulmonary fibrosis (either IPF or secondary to systemic sclerosis) that have been terminated (ClinicalTrials.gov NCT01266135, NCT00581997) and one that is complete without results (ClinicalTrials.gov NCT00532233), as well as two trials for fistulizing Crohn's disease (ClinicalTrials.gov NCT01355614) and perianal fistulas in Crohn's disease (ClinicalTrials.gov NCT01316601) that are complete without results.

23.3.9 Connective Tissue Growth Factor

Connective tissue growth factor (CTGF) is recognized as an important mediator of growth factor activity that is involved in TGF-β signaling, has many important contributions to fibrosis including the production of extracellular matrix, and is emerging as a potential anti-fibrotic target [11, 214–216]. CTGF has been shown to be important in liver fibrosis [11, 215, 217], and blockade of CTGF with the anti-CTGF human monoclonal antibody FG-3019 has demonstrated anti-fibrotic effects in animal models of pulmonary [218] and dermal [219] fibrosis. FG-3019 has was found to be well tolerated in early clinical trials for several fibrotic diseases [9], including IPF [220], and diabetes with microalbuminuria [221]. A phase I study of FG-3019 for patients with Type I or II diabetes and diabetic nephropathy has been completed but results are not published (ClinicalTrials.gov NCT00754143) and an additional trial in patients with type II diabetes and kidney disease on ACE inhibitor or ARB therapy was terminated due to suboptimal study design (ClinicalTrials.gov NCT00913393). A phase II trial of FG 3019 for liver fibrosis secondary to chronic hepatitis B infection and beginning therapy with entecavir was unfortunately terminated due to the effects of entecavir alone (ClinicalTrials.gov NCT01217632). FG-3019 is currently being investigated in phase II trials for patients with IPF (ClinicalTrials.gov NCT01890265) (ClinicalTrials.gov NCT01262001).

23.3.10 Serum Amyloid P

Serum amyloid P (SAP) is a pentraxin protein and acute phase reactant similar to C-reactive protein [222]. SAP has been demonstrated to inhibit fibroblast differentiation [223]. This effect has been demonstrated using the serum of patients with scleroderma and mixed connective tissue disease or rheumatoid arthritis [224], a mouse model of ischemia/reperfusion cardiomyopathy [225], murine dermal wounds [226], and in the alveolar fluid in humans with acute respiratory distress syndrome compared to controls [227]. SAP has shown anti-fibrotic potential in transgenic mouse [228] and bleomycin-induced rat and mouse models of pulmonary fibrosis [229], and a radiation-induced hamster model of oral mucositis [230]. The human recombinant form of SAP, PRM-151, was well tolerated in two phase I trials for pulmonary fibrosis, [231, 232] and is currently being studied in a phase II trial for IPF (ClinicalTrials.gov NCT02550873).

23.4 Kidney

23.4.1 Nicotinamide Adenine Dinucleotide Phosphate Oxidases (NOX)

The nicotinamide adenine dinucleotide phosphate (NADPH) oxidases (NOX) are a family of enzyme complexes that are present throughout our body which produce reactive oxygen species (ROS) [233]. The generation of ROS by NOX is thought to play a role in a variety of diseases and NOX is recognized as a potential therapeutic target for the treatment of fibrosis [11, 12, 233]. Activation of NOX and oxidative stress from ROS has been shown to be important in hepatic fibrosis [234–237], pulmonary fibrosis [238–240], and kidney fibrosis [241, 242].

GKT137831 is a small molecule inhibitor of Nox 1 and Nox 4 [243, 244] that has been studied in a recent clinical trial for diabetic nephropathy. Studies of GKT137831 in animal models of fibrosis in the lung, liver, and kidney have suggested that it has potential as an anti-fibrotic therapy. In murine models of liver fibrosis consisting of wild type mice or superoxide dismutase upregulation mutant mice treated with CCl_4 or bile duct ligation, GKT137831 demonstrated anti-fibrotic effects via inhibition of ROS production and decreased fibrogenic gene expression [243]. In a separate study evaluating a bile duct ligated mouse model of liver fibrosis, GKT137831 also demonstrated a reduction in ROS and fibrogenic gene expression [245]. GKT137831 demonstrated partial reversal of age-associated persistent lung fibrosis in mice [238]. The anti-fibrotic potential of GKT137831 has also been demonstrated in a murine model of diabetic nephropathy [242]. A different Nox1/4 inhibitor, GKT136901, also showed anti-fibrotic potential in a murine model of diabetic nephropathy [246].

The NOX pathway has emerged as a clear potential target for anti-fibrotic therapies in a variety of organs, and GKT137831 has been studied in the liver and lung as well as kidney. At this time the results of a phase II clinical trial of GKT137831 in patients with type 2 diabetes mellitus with diabetic nephropathy (ClinicalTrials.gov NCT02010242) are yet to be published.

Along these lines, N-acetylcysteine (NAC) is believed to have a variety of activities including scavenging of ROS [247, 248], and has been studied in a multitude of disease states including pulmonary fibrosis. Open label studies of NAC were initially convincing for use in idiopathic pulmonary fibrosis [249], and a double-blind, randomized, placebo-controlled trial of NAC added to prednisone and azathioprine for the treatment of idiopathic pulmonary fibrosis reported efficacy [250]. A subsequent placebo-controlled trial included the above regimen as well as a NAC monotherapy group, and did not show benefit [251]. Of note, the three drug regimen was discontinued after interim analysis showed increased mortality and adverse events when compared to placebo [249, 251]. Ultimately, the evidence does not support the use of NAC as an anti-fibrotic agent for idiopathic pulmonary fibrosis [249].

The ubiquity of the NOX pathway and universally damaging effects of ROS would suggest good translation of NOX inhibition from other fibrotic diseases to use in the intestine. However, in one study comparing wild type to NOX1 knockout

mice in a dextran sulfate sodium-induced model of colitis, NOX1 appeared to be pivotal in mucosal repair and epithelial restitution [252]. Ultimately, further investigation into the role of NOX and ROS in intestinal fibrosis is needed.

23.4.2 Pyridoxamine

Pyridoxamine is a metabolite of vitamin B_6 that inhibits protein modification from advanced glycation end products and advanced lipoxidation end products [253, 254]. Pyridoxamine produced a decrease in cross-linking of skin collagen as well as inhibition of renal disease progression in a streptozotocin-induced diabetic rat model [255]. In phase II studies in patients with diabetic nephropathy, pyridoxamine reduced urinary TGF-beta 1 and was generally well tolerated [256]. A phase IIb clinical trial of pyridoxamine (Pyridorin) in diabetic nephropathy has been completed, with results yet to be published (ClinicalTrials.gov NCT00734253).

Glycation inhibition represents a clear potential target for chronic kidney disease and subsequent fibrosis given the effect of diabetes on these diseases. The potential benefit of this mechanism for intestinal fibrosis is less clear. However, pyridoxamine did reduce collagen cross-linking in the skin of diabetic animals and showed efficacy in the progression of kidney disease, which argues for a systemic anti-fibrotic effect in animals with diabetes. Should clinical trials of pyridoxamine for kidney disease demonstrate efficacy, this mechanism would warrant a close look for intestinal fibrosis, especially in patients with diabetes and fibrotic intestinal disease.

23.4.3 Janus Kinase (JAK)1/2

Janus Kinases (JAK) are a family of protein tyrosine kinases that are involved in cytokine signaling and serve critical functions in both immunity and inflammation [257, 258]. JAK2 is activated in systemic sclerosis in a TGF-β dependent manner, and in a bleomycin-induced mouse model of dermal fibrosis and a TSK-1 mouse model of systemic sclerosis, inhibition with the JAK 2 inhibitor TG101209 was shown to be anti-fibrotic [259]. Baricitinib (INCB028050) is a potent and selective inhibitor of JAK1 and JAK2 that is orally bioavailable [260], and is currently being developed for use in rheumatoid arthritis. A clinical trial of baricitinib for patients with diabetic kidney disease has been completed, with no results published as yet (ClinicalTrials.gov NCT01683409). A different JAK1/2 inhibitor, ruxolitinib, is approved for use in myelofibrosis and polycythemia vera.

In the intestine, the study of JAK inhibitors for use in inflammatory bowel disease is well under way, with other inhibitors such as filgotinib and tofacitinib actively being studied for the treatment of inflammatory bowel disease [261]. Of note, the authors have tested tofacitinib in three in vitro models of intestinal fibrosis, without any evidence of anti-fibrotic efficacy (personal communication, PDRH). In

addition to evaluating for efficacy in remission and maintenance, the efficacy of JAK inhibition in other fibrotic diseases suggests these drugs should be assessed for anti-fibrotic effects as well.

23.4.4 Bindarit-CCL (MCP) Inhibitor

The importance of chemokines in liver fibrosis has been previously discussed in this chapter. Chemokines are also thought to play pivotal roles in fibrosis in the kidney, lung as well as skin [262]. Bindarit, an indazolic derivative, is thought to exert anti-inflammatory effects via inhibition of monocyte and endothelial cell production of CC chemokine (CCL2) /monocyte chemotactic protein (MCP)-1, CCL7/MCP3, and CCL8/MCP-2 [263]. Bindarit demonstrated anti-inflammatory and anti-fibrotic effects in a porcine model of renal artery stenosis, and this was thought to be primarily due to its effect on MCP-1 [264]. A phase II study of bindarit for the treatment of diabetic nephropathy has been completed but no results have been published (ClinicalTrials.gov NCT01109212).

23.4.5 Phosphodiesterase Inhibition

CTP-499 is a novel phosphodiesterase inhibitor believed to have anti-fibrotic, anti-inflammatory, and anti-oxidative properties [265–267]. The molecule is a deuterium-containing methylxanthine derivative that shares a structure with the primary metabolite of pentoxyfylline with the exception of key hydrogens being replaced by deuterium [265]. Given the similarity in structure to the primary metabolite of pentoxyfylline, CTP-499 may have a similar mechanism to pentoxyfylline, which is also considered a potential therapy for chronic kidney disease [265, 268]. It has demonstrated anti-fibrotic potential in a unilateral ureteral obstruction rat model of renal fibrosis [269]. CTP-499 was well tolerated in a phase I trial [267] and a subsequent phase Ib safety and tolerability trial in chronic kidney disease [265]. A phase II study in type 2 diabetic nephropathy patients has been completed and results are not yet published (ClinicalTrials.gov NCT01487109).

Pentoxyfylline has been studied in experimental colitis, and was shown to inhibit fibrosis in TNBS-induced colitis in rats [270]. Pentoxyfylline-vitamin E was shown to inhibit the TGF-β1 cascade in radiation-induced enteropathy [271]. These data support CTP-499 as a potential therapeutic molecule in the treatment of intestinal fibrosis.

23.5 Skin

23.5.1 TGFβ Targeted Therapies

P144 is a peptide derived from the ligand-binding portion of the type III receptor of TGF-β that binds TGF-β1 and inhibits its activity [272]. Topical P144 treatment of bleomycin-induced skin fibrosis in mice demonstrated decreased dermal fibrosis, suppression of connective tissue growth factor, SMAD2/3 phosphorylation, and alpha-smooth muscle fibroblast development [273]. In cardiac fibroblasts it has been shown to decrease type I collagen synthesis and also inhibit TGF-β1 signaling, as well as a reduce profibrotic gene and protein expression in the myocardium of a spontaneous hypertensive rat model of cardiac fibrosis [274]. In another spontaneous hypertensive rat model, P144 reduced renal fibrosis, and also inhibited NADPH oxidase expression and oxidative stress in the kidney [275]. P144 also demonstrated anti-fibrotic effects in the liver via decreased number of activated hepatic stellate cells in CCL_4 treated rats [272]. Phase II clinical trials of topical P144 for skin fibrosis in systemic sclerosis have been completed but there are no results published (ClinicalTrials.gov NCT00574613) (ClinicalTrials.gov NCT00781053).

Fresolimumab is a human monoclonal antibody targeting TGF-β that was found to be well tolerated in a phase I study in treatment resistant primary focal segmental glomerulosclerosis (FSGS) [276], and is currently being studied in a phase II trial for FSGS (ClinicalTrials.gov NCT01665391). Fresolimumab also inhibited gene expression in TGF-β-mediated genes and showed clinical improvement measured by the modified Rodnan skin score in a phase I trial for patients with systemic sclerosis [277], and a phase I study in patients with IPF has been completed but no results have been published (ClinicalTrials.gov NCT00125385). A separate TGF-β1 specific humanized monoclonal antibody (LY2382770) was recently evaluated in a clinical trial in patients with diabetic nephropathy who were on renin-angiotensin system inhibitor therapy, but unfortunately this failed to improve serum creatinine and was terminated early due to futility [278].

Interestingly, the use of P144 as a topical agent could be potentially translatable to the treatment of intestinal fibrosis. If this molecule has good topical efficacy but poor absorption in the gut, it could constitute a gut-targeted therapy for fibrotic intestinal disease without systemic effects. This line of thinking currently lacks any demonstrated evidence, but should P144 prove efficacious for the treatment of dermal fibrosis, may warrant further investigation.

23.5.2 Thalidomide/Pomalidomide

Thalidomide is a molecule with an array of activities that has been studied in a wide variety of diseases, but its use has been limited due to well-known significant teratogenic side effects [279, 280]. In a bleomycin-induced lung fibrosis mouse model, thalidomide has been shown to inhibit TGF-β1 expression, reduce TGF-β1 and IL-6, and inhibit expression of ERK1/2 and phospho-ERK1/2 [281]. Thalidomide also inhibited lipopolysaccharide induced tumor necrosis factor alpha (TNF-α) [282, 283]. In addition to TNF-α inhibition, thalidomide has been shown to decrease production of TGF-β1, IL-6, VEGF, Ang-1, and collagen synthesis in human lung fibroblasts [284]. Thalidomide reduced the expression of TGF-β1, IL-6, VEGF, Ang-1, Ang-2, and COL1A1 in addition to improving fibrosis on histological examination of bleomycin-induced lung fibrosis in mice [284]. Thalidomide has been studied in clinical trials for idiopathic pulmonary fibrosis, with the only published clinical trial results being for the treatment of cough in idiopathic pulmonary fibrosis in which it did improve cough and respiratory quality of life [285].

The desire to optimize the therapeutic benefit of thalidomide while minimizing side effects gave rise to lenalidomide and pomalidomide, both of which are both significantly more potent inhibitors of TNF-α than thalidomide [279]. Lenalidomide is approved for use in multiple myeloma, myelodysplastic syndrome, and mantle cell lymphoma while pomalidomide is approved for use in multiple myeloma. Pomalidomide has also been studied as an anti-fibrotic in dermal fibrosis. In bleomycin-induced and tight-skin mouse models of dermal fibrosis, pomalidomide decreased expression of PAI-1, CTGF, and COL1A1 [286]. In the same study pomalidomide was also shown to decrease myofibroblast count, hydroxyproline, and dermal thickness. Pomalidomide has been studied in clinical trials for patients with systemic sclerosis and interstitial lung disease (ClinicalTrials.gov NCT01559129), with results not yet published.

Given the universal importance of TGF-β in fibrotic disease, as well as the importance of TNF-α in inflammatory bowel disease, these therapies represent an intriguing potential mechanism for anti-fibrotic therapy in the intestine. Thalidomide and its analogues have been studied for use in inflammatory bowel disease, but there is a paucity of randomized controlled trials or quality support for its use at large, and more studies are needed [287]. Whether or not thalidomide or its analogues could exert specific anti-fibrotic effects in the intestine, or achieve a therapeutic benefit that outweighs their significant side effect profile, remains to be seen.

23.5.3 Paquinimod

Paquinimod (ABR-215757) is a quinolone-3-carboxamide that inhibits the calcium binding protein S100A9 and is thought to have immunomodulatory properties [288, 289]. S100A9 is believed to be important in the modulation of inflammatory and epithelial cells [290]. In the tight skin 1 mouse model, paquinimod reduced skin thickness and TGF-β responsive gene expression [289]. An open label phase II study of paquinimod to evaluate biomarkers and safety in patients with systemic sclerosis has been completed, (ClinicalTrials.gov NCT01487551), but results have not been published.

23.6 Heart

The underlying mechanisms of cardiac fibrosis share many processes already discussed, and the treatment of cardiac fibrosis, particularly related to heart failure and ischemic disease, is an active area of investigation [291, 292]. Many therapies including β-blockers, loop diuretics, endothelin inhibitors, sildenafil, relaxin, ivabradine, and TNF-α antagonists have been considered for their potential anti-fibrotic effect in the heart, but the renin-angiotensin-aldosterone system (RAAS) and TGF-β appear to be the most prominent potential targets [291–293].

23.6.1 Renin Angiotensin Aldosterone System (RAAS)

Inhibition of the RAAS with Angiotensin-converting enzyme (ACE) inhibitors, angiotensin receptor blockers (ARBs), and mineralocorticoid receptor blockers have well known clinical benefits in heart disease but are being increasingly investigated for their anti-fibrotic potential. Therapies modulating the RAAS axis are discussed under the cardiac section given their prominent use in mainstream medicine for cardiovascular disease.

Angiotensin II and its receptor, angiotensin type I receptor (AT1), exert a complicated array of pro-fibrotic effects including activation of macrophages, ROS mediated collagen production, and cardiac fibroblast stimulation [3].

RAAS inhibition also has known renoprotective effects, and angiotensin II is recognized as an important mediator and potential target in renal fibrosis [294]. In the lung, a pilot study of losartan in patients with IPF showed stabilization of pulmonary function [295]. In a CCl_4 induced liver fibrosis model in rats, losartan demonstrated anti-fibrotic effects as well as a reduction in AT1, TGF-β, and alpha smooth muscle expression [296]. A retrospective study including 284 chronic hepatitis C patients showed that patients with hypertension taking angiotensin-blocking therapy had less fibrosis than hypertensive patients who did not [297]. The ARB losartan has been studied in a phase III trial for anti-fibrotic effects in NASH (FELINE) (ClinicalTrials.

gov NCT01051219) and a pilot study for patients with idiopathic pulmonary fibrosis has been completed (ClinicalTrials.gov NCT00879879) with results not yet published.

Both ACE inhibitors and ARBs have demonstrated anti-fibrotic effects on cardiac fibrosis in clinical trials that is separate from their anti-hypertensive effects, and the mineralocorticoid receptor antagonists spironolactone and eplerenone have also demonstrated anti-fibrotic effects in patients with conditions such as heart failure, metabolic syndrome, and LV diastolic dysfunction [292].

The potential for the use of these agents in intestinal fibrosis is supported by studies showing that the ACE inhibitor captopril improved macroscopic and histologic lesions and pro-fibrotic gene expression in a TNBS-induced colitis rat model [298], and losartan similarly decreased macroscopic and microscopic fibrosis scores and TGF-β1 concentration in the TNBS induced colitis rat model [299].

23.6.2 Transforming Growth Factor (TGF)-β

TGF-β is also thought to be critical in the development of cardiac fibrosis [291–293]. Pirfenidone and tranilast have also been proposed as potential anti-fibrotic therapies in cardiac disease due to their ability to inhibit TGF-β [292, 293]. Activin receptor-like kinase 5 (ALK5) is a downstream signaler of the TGF-β pathway [300, 301] Unfortunately, in animal models, inhibition of ALK5 with SM16 and inhibition of TGF-β directly with antibodies demonstrated increased mortality despite anti-fibrotic effects on cardiac fibrosis [292, 301, 302].

23.7 Conclusion

The ubiquity of fibrosis as the pathway leading to chronic organ failure presents challenges, but also opportunities for collaboration across fields. As more breakthroughs occur in a variety of organs and disease states, the potential for these benefits to translate across organs is exciting and attainable (Fig. 23.1, Table 23.1). When approaching the problem of intestinal fibrosis in inflammatory bowel disease, it is important to also consider therapies and mechanisms in other organs, as there is much to be learned and knowledge to be shared.

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F. Rieder (ed.), *Fibrostenotic Inflammatory Bowel Disease*,
https://doi.org/10.1007/978-3-319-90578-5