IJAE
Vol. 119, n. 3: 286-303, 2014
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

Invited Review in Histology and Cell Biology

Features of intestinal lesions in the clinical course of Inflammatory Bowel Diseases

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Submitted May 31, 2014; accepted revised August 20, 2014

Abstract

Inflammatory bowel diseases (IBD), including ulcerative colitis (UC) and Crohn's disease (CD), are chronic, progressive and relapsing inflammatory disorders of unknown etiology. UC is characterized by inflammation of the large bowel mucosa and submucosa, whereas in CD inflammation is trans-mural and may involve various sites of the gastrointestinal tract. Superficial mucosal lesions are most prone to heal, whereas deep ulcers or transmural fissures may heal with more difficulty and may be followed by the development of fibrosis and strictures requiring surgery. Inflammation appears to be necessary to trigger the onset of the fibrotic process, but subsequently plays a minor role in its progression. In IBD, anti-inflammatory treatment does not prevent evolution of fibrosis once the process has started. Therefore, the mechanisms that regulate fibrosis appear to be distinct from those regulating inflammation. Intestinal fibrosis is due to an abnormal accumulation of extracellular matrix proteins producted by activated intestinal myofibroblasts. Increased evidence indicate that a number of molecules are involved in the development of the disease and a crosstalk between TGFβ/Smads pathway and αvβ6 integrin, mTOR and PPARγ could play a crucial role in the development of intestinal fibrosis. Animal models represent a useful tool to investigate the molecular and cellular mechanisms of intestinal inflammation and fibrosis and to test the effectiveness of novel therapeutic strategies for the prevention and treatment of intestinal fibrosis that still remain the major cause of surgical intervention.

Key words

Ulcerative colitis, Crohn's disease, inflammation, immunohistochemistry, treatment.

Key to abbreviations

αSMA	=	alpha smooth muscle actin
ACE	=	angiotensin converting enzyme
ANG-II	=	angiotensin II
BMP	=	bone morphogenetic protein
CD	=	Crohn's disease
CTGF	=	connective tissue growth factor
DAMP	=	damage-associated molecular patterns

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http://www.fupress.com/ijae
DOI: 10.13128/IJAE-15562

ECM = extracellular matrix
EGF = epidermal growth factor
ET = endothelin
HGF = hepatic growth factor
IBD = inflammatory bowel disease(s)
IFN = interferon
IGF = insulin like growth factor
IL = interleuchin
LAP = latency associated peptide
MMPs = matrix metalloproteinases
mTOR = mammalian target of rapamycin
mTORC = mammalian target of rapamycin complex
mTORis = mammalian target of rapamycin inhibitors
NO = nitric oxide
PAMP = pathogen-associated molecular patterns
PDGF = platelet-derived growth factor
PIKK = phosphatIdylinositol 3-kinase-related kinases
PPARγ = peroxisome proliferator-activated receptor gamma
RAS = renin angiotensin system
ROS = reactive oxygen species
RXR = retinoid X receptors
Smad = small mother against decapentaplegic
TGFβ = transforming growth factor beta
TIMPs = tissue inhibitor of metalloproteinases
TNBS = trinitrobenzene sulfonic acid
TNFα = tumor necrosis factor alpha
UC = ulcerative colitis
VEGF = vascular endothelial growth factor
WT = wild type

Introduction

Inflammatory bowel disease (IBD), including ulcerative colitis (UC) and Crohn's disease (CD), are chronic, progressive and relapsing inflammatory disorders of unknown etiology. Genetic, environmental and intestinal microbial factors play a pivotal role in the etiology and pathogenesis of IBD (Schirbel et al., 2010).

The incidence and prevalence of IBD are increasing with time and in different regions around the world, indicating its emergence as a global disease (Molodecky, et al., 2012). IBD may occur at any time, from early childhood to late adulthood, with the pick of incidence in the second or third decade of life (Jager et al., 2013).

Intestinal lesions and symptoms may range from mild to severe, causing disability and a poor quality of life. (Abraham et al., 2009; Clevers H. 2009).

Ulcerative colitis is characterized by inflammation in the large bowel mucosa and submucosa, whereas in CD inflammation is trans-mural and may involve various sites of the gastrointestinal tract. IBD becomes symptomatic when lesions are extensive or distal, or when associated with local complications.

Approximately 50% of the patients present a slightly evolutive disease with a low prevalence of relapses, hospitalizations, and complications (Cosnes et al., 2011). Other patients have a more severe course and may develop complications that require surgery. In UC, the main complications include toxic megacolon, massive hemorrhage or colon perforation. In CD, intestinal fibrosis and strictures, internal or perianal fistulas and abscesses are frequent, being reported in approximately one-third of patients. Fibrosis and stricture formation is the result of an uncontrolled and excessive process of intestinal healing, while perforation and fistulas of a defective process of the tissue repair (Rieder et al.,; 2012, Speca et al., 2012).

The crucial steps in the clinical course of IBD include the occurrence of lesions, the manifestation and severity of symptoms, the development of complications, the need for surgery, disability and mortality (Cosnes et al., 2011, Latella et al., 2012). In order to achieve a favorable modification in the disease course, an effective intervention must be carried out at the right time and with a specific therapeutic endpoint. Early treatment is advisable, before the development of severe bowel damage and impaired function.

Features of intestinal lesions

In IBD, progression of intestinal lesions may range from weeks to decades, however, it can be slowed down or reversed by means of medical therapy (Peyrin-Biroulet et al., 2010; Cosnes et al., 2011). Superficial mucosal lesions are most prone to heal, whereas deep ulcers or transmural fissures may heal with more difficulty and may be followed by the development of fibrosis and strictures. IBD becomes symptomatic when lesions are extensive or distal, associated with a systemic inflammatory response, or when associated with local complications such as dilatation (toxic megacolon), massive hemorrhage, strictures, perforation (abscesses and fistulas) and cancer. Colorectal lesions usually present early symptoms, whereas small bowel lesions may remain latent for several years. The disease course is generally characterized by a sequence of flares and remission of varying duration, while approximately one fifth of these patients undergo a chronic, active and continuous disease course. Abdominal pain, abnormal bowel functions and rectal bleeding are the main clinical signs that significantly impact the patient's quality of life. Abdominal pain and diarrhea may also occur as the consequence of smooth muscle or enteric nervous system disfunctions induced by structural damage (Tomita et al., 2000; Vrees et al., 2002; Neunlist et al., 2003; Lomax et al., 2005).

Features and evolution of Intestinal lesions are different between UC and CD.

In UC, the inflammatory lesions are limited to the mucosa and submucosa. Upon presentation, lesions are limited to the rectum (proctitis) in 30-35% of patients, extend to the splenic flexure (left-sided colitis) in 30%-45% and reach the cecum (pancolitis) in 20-25% (Cosnes et al., 2011, Latella et al., 2012). During the course of the disease, after 20 years, the rate of pancolitis may increase reaching 50% of cases. Mucosal lesions are usually diffuse and superficial, deep ulcerations are present only in patients with severe disease. UC appears to be particularly severe in younger patients (especially in children), with a higher frequency of flares that do not respond to medical treatment. Severity of flares and their response to therapy vary and are difficult

to predict. Clinical remission is usually associated with mucosal healing (Pineton de Chambrun et al., 2010).

In CD, the inflammatory lesions are transmural and involve any segment of the digestive tract, but mainly affect the distal ileum and the colon. At diagnosis, approximately 40% of patients show an ileo-colonic disease, about 30% an isolated ileal disease, and another 30% a pure colonic disease (Cosnes et al., 2011, Latella et al., 2012). Approximately 5-15% of patients have associated upper gastrointestinal lesions and 20-30% present perianal lesions (Silverberg et al., 2005; Cosnes et al., 2011; Latella et al., 2012). In CD, the localization of the lesions changes only minimally over time, while the clinical behavior shows an evolution over the course of the disease (Louis et al., 2001; Cosnes et al., 2002; Papi et al., 2005). During the first few years of CD, the inflammatory (non-penetrating/non-stricturing) form predominates, whereas most patients develop a penetrating or a stricturing disease later on. These two forms may co-exist in the same patient, since internal fistulae may complicate long-standing intestinal stenosis. Disease evolution is related to lesion localization, the development of complications (abscess, fistula, stricture) being more frequent and rapid when the small bowel is involved, whereas when the disease is localized to the colon it may remain uncomplicated for many years. There is no relationship between symptoms and progression of the intestinal lesions, since strictures and internal fistulae may develop for several years with only mild symptoms (Cosnes et al., 2011, Latella et al., 2012). Approximately 50% of the CD patients have only a slight evolutive disease, whereas the remaining patients present a more aggressive and evolutive disease with high rates of relapse, complications, hospitalization and surgery.

Taken together, these data indicate the need of strategies aimed at interrupting or delaying the natural evolution of this pathological condition. Current treatment options (antibiotics, steroids, immunosuppressive drugs, biological therapies) may relieve the inflammatory symptoms, but do not improve fibrostenotic obstruction (Van Assche et al., 2004; Dignass et al., 2010; Latella et al., 2011). The results of medical treatment of stricturing CD are still poor (Samini et al., 2010).

Progression of intestinal lesions

Acute intestinal inflammation is usually followed by physiologic healing of the damaged tissue and restoration of the normal structure and function of the intestine (Rieder et al., 2012). If this does not occur, chronic inflammation can develop, characterized by continuous events of injury and repair that may lead to the development of fibrosis (Speca et al., 2012). In IBD, it is still unclear which factors trigger chronicity and what sets the stage for the later development of intestinal fibrosis.

Inflammation appears to be necessary to trigger the onset of the fibrotic process, but subsequently plays a minor role in its progression (Speca et al., 2012). Anti-inflammatory treatment in IBD and in other chronic inflammation-associated fibrotic conditions in various organs (lung, liver, kidney) does not prevent evolution of fibrosis once the process of excessive extracellular matrix (ECM) deposition has started. The mechanisms that regulate fibrosis, therefore, appear to be distinct from those regulating inflammation.

Fibrosis represent a common complication of IBD and follows the distribution and location of inflammation (Burke et al., 2007; Rieder et al., 2009). In UC, the deposition

of ECM is restricted to the mucosal and submucosal layers of the large bowel and can induce structural changes (haustral loss, colonic shortening), and motility disorders of the colon. In CD, fibrosis can involve the entire bowel wall of the gastrointestinal (GI) tract including the mucosa, muscularis mucosa, submucosa, muscularis propria and serosa layers and can lead to strictures and obstruction that require surgery. The higher prevalence of fibrosis in CD is probably a consequence of trans-mural bowel inflammation which expose all the cells producing ECM to inflammatory mediators released by the activated immune and non-immune cells (Burke et al., 2007). Of note, course and extent of intestinal fibrosis in IBD display significant variability among individual patients, suggesting that the susceptibility to intestinal fibrosis may have a genetic component. Host genetic factors are likely to play key roles in the modulation of intestinal fibrosis and to contribute to the overall variability in disease progression (Gazouli et al., 2010).

Current anti-inflammatory therapies used in IBD do not prevent nor reverse established intestinal fibrosis and strictures, which may present years after remission of active inflammation. Despite the therapeutic advance in the treatment of IBD in the last two decades, the incidence of intestinal strictures in CD has not significantly changed (Cosnes et al., 2005; Spinelli et al., 2010; Angelucci et al., 2011). This implies that control of intestinal inflammation does not necessarily affect the associated fibrotic process. The lack of efficient and well-tolerated anti-fibrotic drugs is partly due to the fact that the main and specific cellular and molecular pathways leading to fibrosis remain to be identified (Fiocchi et al., 2011; Speca et al., 2012).

Mediators of intestinal fibrosis

Intestinal fibrosis is a chronic and progressive process acting through complex cell/matrix/cytokine and growth factor interactions but it may be a reversible event (Speca et al., 2012). It is characterized by abnormal production and deposition of ECM proteins by activated myofibroblasts, which are also called ECM-producing cells. Fibrosis depends by the balance between production and degradation of ECM proteins. These are produced by the activated myofibroblasts, stellate cells, pericytes, and intestinal or bone marrow stem cells; myofibroblasts derive not only from resident mesenchymal cells (fibroblasts, sub-epithelial myofibroblasts and smooth muscle cells) but also from epithelial and endothelial cells, by a process known as epithelial/endothelial-mesenchymal transition (Fiocchi et al., 2011; Speca et al., 2012). ECM degradation is mediated by matrix metalloproteinases (MMPs). The fine balance between MMPs and tissue inhibitor of metalloproteinases (TIMPs) appears to be altered in IBD and related intestinal fibrosis (Ravi et al., 2007). Myofibroblasts are activated by a variety of mechanisms including paracrine signals derived from immune and non-immune cells, autocrine factors secreted by myofibroblasts, pathogen-associated molecular patterns (PAMPs) derived from micro-organisms and the so-called damage-associated molecular patterns (DAMPs) derived from injured cells (Fiocchi et al., 2011).

All intestinal cell types that produce ECM proteins act synergistically and are under the control of various biological mediators, such as growth factors, cytokines, chemokines, proteolytic enzymes, complement components, vasoactive amines and peptides (Wynn T.A. 2008). The most important pro-fibrotic mediators include trans-

forming growth factor-β (TGF-β), activins, integrins, connective tissue growth factor (CTGF), platelet-derived growth factor (PDGF), insulin-like growth factor (IGF-1&2), epidermal growth factor (EGF), endothelins (ET-1, -2, -3), various cytokines, products of oxidative stress, components of the renin-angiotensin system (RAS), angiogenic factors (e.g. vascular endothelial growth factor-VEGF) and the mammalian target of rapamycin (mTOR) Speca et al.,2012). Several molecules with anti-fibrotic properties have also been identified including peroxisome proliferator activated receptors (PPARs), IFN-a, IFN-g, IL-7, IL-10, IL-12, Smad7, adiponectin and nitric oxide (NO).

Although the TGF-β/Smad pathway represents the major driving force of fibrosis, several pro-fibrogenic and anti-fibrogenic molecules seem to interact directly with the TGF-β/Smad pathway.

Blockade of TGFβ signaling, either at the extracellular or intracellular level, offers a strategy to prevent/treat fibrosis (Wynn et al., 2012; Latella et al., 2013a). Since TGFβ, however, is also involved in cellular differentiation, proliferation, transformation and immunoregulation, its blockade is problematic as TGFβ, Smad2 and Smad4 disruptions are lethal. Targeting of individual intracellular pathways, however, could lead to the selective blockade of TGFβ fibrotic responses without involving physiologically vital TGFβ responses. Disrupting Smad3 results in mice that survive to adulthood and also confers resistance to intestinal fibrosis (Zanninelli et al., 2006; Latella et al., 2009).

We reported that Smad3 knockout model is useful to evaluate the role of TGFβ/Smad3 signaling pathway in the development of intestinal inflammation and fibrosis (Zanninelli et al., 2006). 2,4,5-Trinitrobenzene sulphonic acid (TNBS) induced chronic colitis in Smad3 wild-type (WT) and in Smad3 knock out mice showed macroscopic and microscopic features of colonic fibrosis which were significantly more pronounced in WT than in null mice. The colon of Smad3 WT mice appeared shorter and dilated and displayed thickened oedematous walls while that of TNBS-treated Smad3 null mice showed a normal aspect. In Smad3 WT mice, histology and immunohistochemistry showed an abnormal accumulation of collagen in the submucosal and serosal layers altering the architecture of the colonic wall. On the other hand, in Smad3 null mice the colonic structure was preserved, demonstrating that Smad3 null mice are resistant to the development of intestinal fibrosis and that TGFβ1-/Smad3 signaling plays an important role in this process (Latella et al., 2009) (Fig. 1).

There is increasing evidence of an extensive crosstalk between TGFβ/Smad3 pathway and αvβ6 integrin, PPARγ and mTOR that may lead to strong effects on fibrosis development.

Integrins regulate interactions between cells and the ECM and influence cell growth, differentiation, wound healing and fibrosis (Van der Flier et al., 2001; Margadant et al., 2011). In normal conditions αvβ6 integrin is not expressed, but it is up-regulated and colocalizes with TGFβ following tissue injury, during wound healing, in epithelial cancer and fibrosis (Jennkins G., 2008; Wipff et al., 2008; Latella et al., 2013b). The ligands of αvβ6 include fibronectin, tenascin, vitronectin and latency associated peptide (LAP). Interaction with LAP activates latent TGFβ and promotes fibrosis, while various genetic and pharmacologic interventions targeting αvβ6 integrin reduce TGFβ1 activation and fibrosis (Hahm et al., 2007; Horan et al., 2008; Nadler et al., 2009; Sullivan et al., 2010; Katsumoto et al., 2011). Inhibitors of αvβ6 also significantly reduce tissue levels of profibrogenic transcripts, including procol-

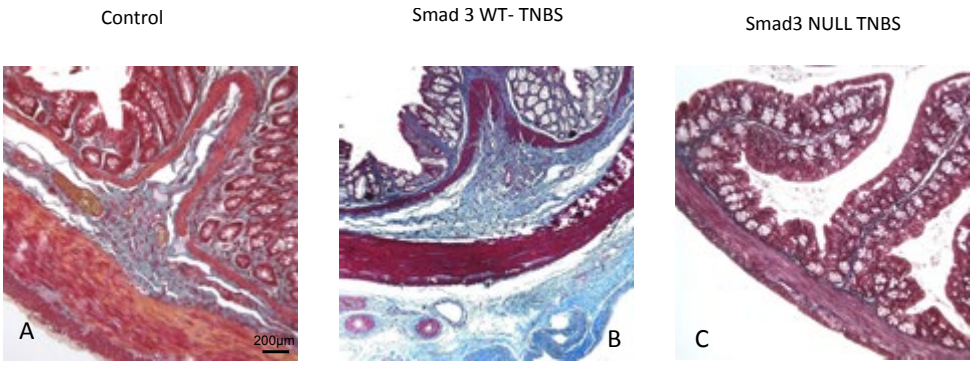

Figure 1 – Following TNBS treatment, colon from Smad3 WT mice showed marked changes due to abnormal deposition of connective tissue in the serosa and to a lesser extend in lamina propria and submucosa (B), whereas the colonic wall of null mice appeared normal (C), similar to control mice (A). Masson trichrome, 4X.

lagen α1(I), αSMA, TGFβ1, CTGF, TIMP-1. Inhibition $\alpha v\beta 6$ integrin could thus be a novel therapeutic strategy against fibrosis, as it may inhibit TGFβ activation at sites of injured organ, where $\alpha v\beta 6$ integrin is up-regulated, without affecting other vital homeostatic TGFβ roles in inflammation and immunity.

The mammalian target of rapamycin (mTOR), a phosphatidylinositol 3-kinase-related kinase (PIKK), forms at least two distinct complexes (Tsang et al., 2007). The mTOR complex 1 (mTORC1) controls protein synthesis, cell growth and proliferation, as well as autophagy, angiogenesis and fibrosis. The mTOR complex 2 (mTORC2) is involved in cell proliferation and survival, metabolic regulation and actin cytoskeleton organization. mTOR signaling is activated by hormones, growth factors, amino acids, stress and alterations in cellular energy status (Tsang et al., 2007). mTOR inhibitors (mTORis) exert direct antifibrotic activities by reducing fibroblast and myofibroblast numbers and also by down-regulating the production of profibrogenic cytokine (IL-4, -6, -13, -17), TGFβ1 and collagen (Poulalhon et al., 2006; Wang et al., 2010; Osman et al., 2011).

Antifibrotic effects of mTOR inhibitors have been reported in many fibrotic diseases (Humar et al., 2002; Ong et al., 2007; Korfhagen et al., 2009; Geissler et al., 2010; Patsenker et al., 2011). The combined immunosuppressive and antifibrotic action of rapamycin, and its analogues sirolimus and everolimus as well as tacrolimus, may thus be a promising treatment of intestinal fibrosis in CD (Dumortier et al., 2008; Massey et al., 2008; Reinisch et al., 2008).

PPARs are nuclear receptors, which regulate gene transcription by binding to retinoid X receptors (RXR) (Xu et al., 2001). PPAR-γ is present in colorectal mucosa, liver, vascular tissue and in adipocytes, monocytes, macrophages, dendritic cells, B and T cells. It is involved in the modulation of adipocyte differentiation, glucose homeostasis, lipid metabolism, inflammatory and immune processes, as well as fibrosis (Rousseaux et al., 2006). PPAR-γ activation strongly correlates with the TGFβ/Smad pathway as it markedly reduces Smad3 phosphorylation and downregulates CTGF expression which is a downstream effector of of TGFβ/Smad pathway (Zhao et al., 2006; Zang et al., 2009). Overexpression of PPAR-γ prevents tissue fibrosis, whereas

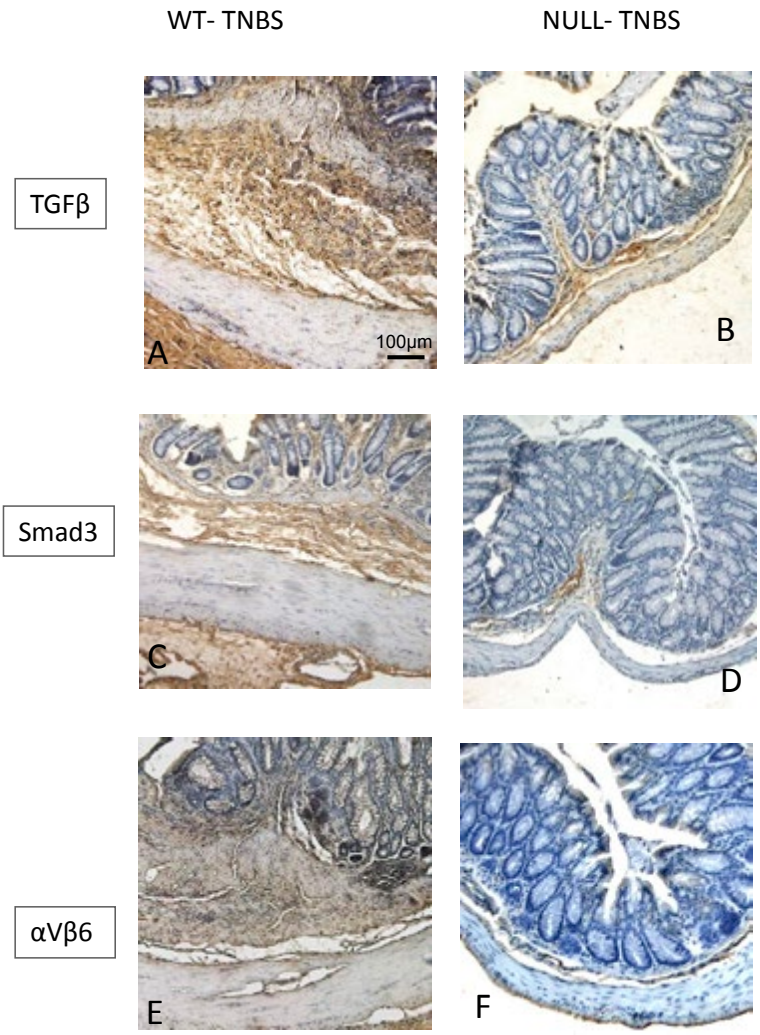

Figure 2 – TGFβ, Smad3 and αVβ6 immunostaining (A,C,E respectively) were increased in colonic submucosa and serosa of Smad3 WT TNBS-treated mice compared to that of Smad3 null mice (B, D, F respectively). Immunohistochemistry, 10X.

its loss increases fibrosis (Latella et al., 2013a; Nan et al., 2011; Kapoor et al., 2009; Wei et al., 2010). PPAR-γ agonists attenuate fibrosis in several organs including the intestine and these antifibrotic effects are abolished by PPAR-γ selective antagonists (Wu et al., 2009; Kawai et al., 2009; Aoki et al., 2009; Yang et al., 2010). PPAR-γ is an innate protector against excessive fibrogenesis and a potential anti-fibrotic target.

In a recent study we have reported that TNBS-induced colorectal fibrosis is at a same time dependent on by TGFβ/Smads, αvβ6 integrin, mTOR and PPARγ expression. In fact, TGFβ, Smad3, αvβ6 and mTOR were markedly increased in submucosa

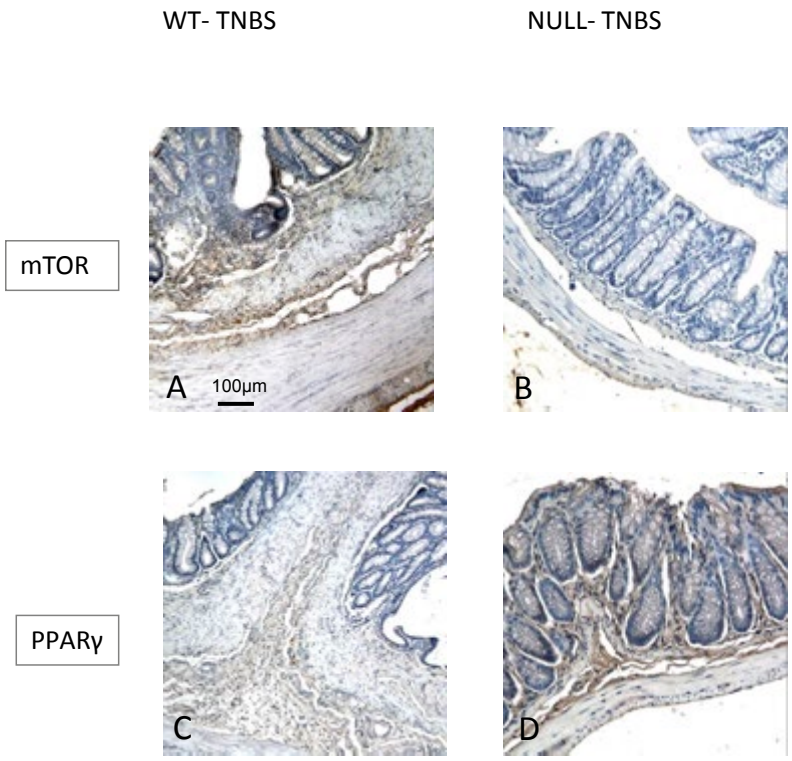

Figure 3 – mTOR immunostaining was increased in colonic submucosa of Smad3 WT TNBS-treated mice (A) compared to that of Smad3 null mice (B), whereas PPARγ was decreased in WT-TNBS (C) and increased in Null TNBS mice (D). Immunohistochemistry, 10X.

and serosa layers of the large bowel from Smad3 WT with TNBS-induced chronic colitis and fibrosis, while PPARγ was decreased. On the other hand, Smad3 null mice receiving TNBS did not develop intestinal fibrosis and showed a reduced expression of PPARγ and an increased expression of that of TGFβ/Smads, αvβ6 integrin and mTOR (Latella et al., 2013b) (Fig. 2-3). Increased expression of αvβ6 integrin, TGFβ, Smad3 and mTOR results to be associated with intestinal fibrosis development, whereas upregulation of PPRAγ appears to be protective towards fibrosis.

New therapeutical approaches

The advancements in knowledge about IBD over the past two decades have modified the treatment goals. While in the past the aim of medical treatment was an improvement in IBD symptoms, the current objective is to achieve not only clinical remission but also healing of the intestinal lesions.

Mucosal healing can be considered appropriate for UC which is a disease of the mucosa, whereas the term intestinal healing would be more correct for CD which is

a transmural disease (Caprilli et al., 2012). Reduction or reversal of intestinal fibrosis is an important goal to achieve, but represents a big challenge. At present, there are no approved or effective medical therapies for intestinal fibrosis in IBD (Latella et al., 2013a). Therefore, intestinal fibrosis and associated complications still remain the major causes of surgical intervention (Spinelli et al., 2010; Latella et al., 2011). Surgical correction, by means of intestinal resection or stricturoplasty, is necessary in up to 75% of CD patients during the course of their disease (Cosnes et al., 2011, Latella et al., 2012). However, surgical resection is associated with a high rate of recurrent stricture and the need for repeated surgery is high, therefore exploration of new therapeutic approaches has now become mandatory.

The agents currently used for the treatment of IBD (salicylates, antibiotics, steroids, immunosuppressive drugs, biological therapies) may relieve the inflammatory symptoms, but do not significantly improve fibrosis related strictures and obstruction (Dignass et al., 2010; Caprilli et al., 2012; Latella et al., 2011). There is no doubt that these agents work best when introduced early in the course of the disease, when inflammation predominates and fibrosis is still reversible. Nevertheless, the results of medical treatment for stricturing or penetrating CD are still poor (Samini et al., 2010). Although there are suggestions that biological medications may reduce the need for surgery in the short term, the real impact of biologics on the lifetime risk of surgery remains to be established (Hanauer et al., 2002; Lichtenstein et al., 2005; Schnitzler et al., 2009). Data from population-based cohorts have shown that in the pre-biologic era, the rate of surgery ranged between 27% and 61% within 5 years after diagnosis, and, in the era of anti-TFNα, it ranged between 25% and 33%, thus suggesting that the need for surgery remains high also in the era of biologics (Bouguen et al., 2011).

Increasing lines of evidence suggest that controlling intestinal inflammation alone is not sufficient to prevent or eliminate the associated fibrotic response (Speca et al., 2012, Latella et al., 2013a).

Since in IBD there is a strong relationship between intestinal inflammation and fibrosis, a new therapeutical approach could be the combination of drugs with anti-inflammatory actions and drugs with anti-fibrotic action.

Several antifibrotic drugs (chemical and biological) have been tested in experimental models of tissue fibrosis being able to inhibit, mitigate or even reverse the fibrotic process (Rieder et al., 2008; Latella et al., 2013a). Their antifibrotic activity seems to be related to different mechanisms of action, by reducing the proliferation and increasing the apoptosis of the ECM-producing cells, by reducing their profibrogenic effects at various levels (differentiation, activation, motility, contraction, ECM deposition), or by promoting ECM degradation. Besides efficacy, safety of antifibrotic drugs is important as they have to be administered chronically.

Inhibitors of several pro-inflammatory and profibrotic molecules like TGF-β, Smad3, $\alpha v\beta 6$ integrin, IL-13, CTGF, mTOR, PDGF, ACE and ANG-II and ROS have been developed and are currently being investigated for the treatment of several fibroproliferative disorders of various organs including the intestine (Latella et al., 2013b). Anti-inflammatory and anti-fibrotic molecules like hepatic growth factor (HGF), bone morphogenetic protein 7 (BMP-7) and PPARγ agonists attenuate fibrosis in various organs including intestine (Latella et al., 2013a)

Among numerous drugs that have been investigated for their antifibrotic potential, botanicals represent a significant component (Owens at al., 2004; Rieder et al., 2008).

Botanical extracts with antifibrotic properties have been evaluated both in experimental and clinical studies showing efficacy and safety in liver, lung, kidney and joint fibrosis , (Rieder et al., 2008; Owens at al., 2004; Van der Flier , 2001; Margadant et al., 2010).

Combined oral administration of *Boswellia serrata* extracts with anti-inflammatory activity and *Scutellaria baicalensis* extracts with anti-fibrotic activity significantly improved the course and macroscopic finding of TNBS-induced chronic colitis in rats (disease activity index, colon weight, length, adhesions, strictures, dilatation, oedema, ulceration, and extension of damage), as well as the histological degree of the colorectal fibrosis, compared to TNBS rats treated with either *Boswellia* or *Scutellaria* extracts alone (Fig. 4).

These observations suggest that a combination of drugs with anti-inflammatory and anti-fibrotic properties could be an effective therapeutic strategy for the prevention and treatment of experimental intestinal fibrosis and their properties seem to be in part related to their effects on the TGFβ/Smads signaling, effects extensively evaluated in a previous study (Latella et al., 2008).

Boswellia and *Scutellaria* extracts have shown several other mechanisms of action.

The gum resin extracts from *Boswellia serrata* have been reported to have anti-inflammatory and immunomodulatory activity (Chevrier et al., 2005; Ammon, 2006). Boswellic acids, ursane type pentacyclic triterpenes, have been identified as the main biologically active components of *Boswellia* extracts (Ammon, 2006). Boswellic acids suppress the synthesis of the leukotrienes by inhibition of 5-lipoxygenase (Ammon, 2006) and decrease, in a dose-dependent manner, the recruitment of adherent leukocytes and platelets into inflamed tissue, mainly by preventing the up-regulation of adhesion molecules (Krieglstein et al., 2001; Anthoni et al., 2006). In addition,

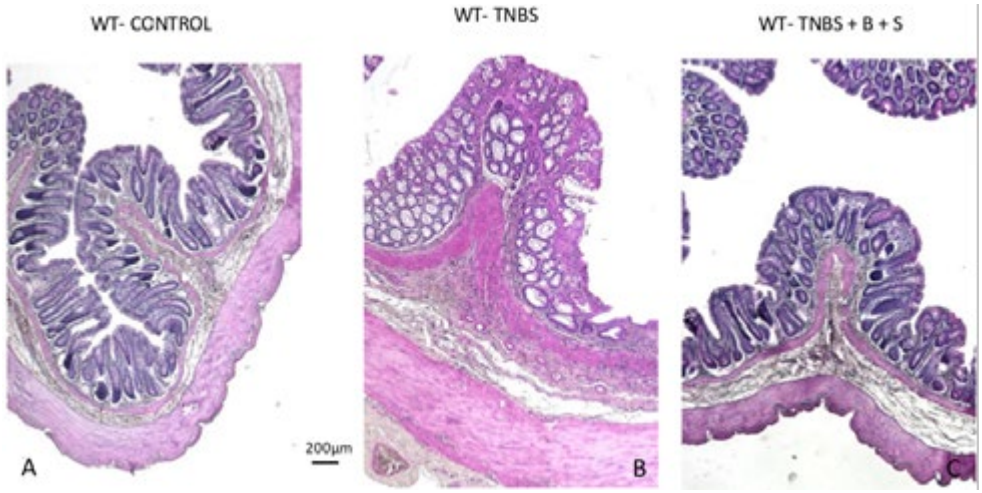

Figure 4 – In TNBS-induced chronic colitis, the colonic wall showed a severe fibrosis both in submucosa and serosa (B) compared to the colon of TNBS-treated rats with both Boswellia and Scutellaria in which there was very mild fibrosis in the submucosa (C), with only minor differences from control (A). Hematoxilin and eosin, 4X.

boswellic acids inhibit leukocyte elastase (Safayhi et al., 1997) and lipopolysaccharide-induced nitric oxide production (Pandey et al., 2005) and suppress nuclear factor (NF)-κB and NF-κB-regulated gene expression (Takada et al., 2006). Boswellic acids also possess anticomplement activity (Kapil and Moza,1992). In clinical trials, promising results have been observed in patients with rheumatoid arthritis, ulcerative colitis, Crohn's disease and bronchial asthma (Gupta et al., 1998, 2001; Gerhardt et al., 2001; Krieglstein et al., 2001; Kiela et al., 2005; Ammon, 2006; Anthoni et al., 2006).

Scutellaria baicalensis and its active flavanoid compounds baicalein, baicalin and wagonin have been reported to possess anti-inflammatory, anti-allergic and anti-infection activities and have been used in the treatment of a variety of inflammatory diseases such as bronchitis, nephritis, hepatitis, asthma and atopic dermatitis (Lin and Shied, 1996). It has been also reported that *Scutellaria* extracts significantly improve the clinical course and the histological, immunological and biochemical parameters in mouse colitis induced by dextran sodium sulphate (Choi et al., 2005). *Scutellaria* extracts exhibit anti-inflammatory activity by binding to chemokine ligands and by their antioxidant ability based on inhibiting the formation of reactive oxygen intermediates (Li et al., 2000; Liu et al., 2002). Furthermore, *Scutellaria* shows potent effects against lipid peroxidation (Lim et al., 2003). Beside the anti-inflammatory activity, more recently, it has been demonstrated that *Scutellaria* extracts have a potent antifibrotic effect (Nan et al., 2002; Park et al., 2005).

In conclusion, the concept of intestinal fibrosis in IBD has changed from being a static and irreversible entity to a dynamic and reversible disease, as also occur in other organs. Novel therapeutic strategies are under investigation to target specific molecules in the process of fibrogenesis with the aim to prevent, reduce or even reverse intestinal fibrosis in IBD.

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