gastrointestinal disorders

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Review

The Role of TGF-β, Activin and Follistatin in Inflammatory Bowel Disease

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Abstract: Inflammatory bowel disease (IBD) is an immune-mediated inflammatory condition predominantly affecting the gastrointestinal (GI) tract. An increasing prevalence of IBD has been observed globally. The pathogenesis of IBD includes a complex interplay between the intestinal microbiome, diet, genetic factors and immune responses. The consequent imbalance of inflammatory mediators ultimately leads to intestinal mucosal damage and defective repair. Growth factors, given their specific roles in maintaining the homeostasis and integrity of the intestinal epithelium, are of particular interest in the setting of IBD. Furthermore, direct targeting of growth factor signalling pathways involved in the regeneration of the damaged epithelium and the regulation of inflammation could be considered as therapeutic options for individuals with IBD. Several members of the transforming growth factor (TGF)-β superfamily, particularly TGF-β, activin and follistatin, are key candidates as they exhibit various roles in inflammatory processes and contribute to maintenance and homeostasis in the GI tract. This article aimed firstly to review the events involved in the pathogenesis of IBD with particular emphasis on TGF-β, activin and follistatin and secondly to outline the potential role of therapeutic manipulation of these pathways.

Keywords: TGF-β; activin; follistatin; inflammation; immune system; IBD

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1. Introduction

Inflammatory Bowel Diseases (IBD) are characterised by active, chronic inflammatory changes in the gastrointestinal (GI) tract [1]. IBD may be subtyped into typical forms (Crohn's disease (CD), ulcerative colitis (UC) or IBD-unclassified (IBDU)) and atypical subtypes (atypical UC, CD colitis) according to features such as disease location and the pattern of inflammation [1,2].

Although the pathogenesis of these conditions is not fully elucidated, the best accepted hypothesis is that gut inflammation begins in response to key environmental, dietary, microbial and immune responses in an individual at genetic risk [3,4]. In recent years, the complexity of the innate and acquired immune responses relevant to IBD has been investigated and understanding has expanded substantially.

One group of proteins that appear to play important roles in the inflammatory processes exhibited in IBD is the transforming growth factor (TGF)-β superfamily, which includes TGF-β and related proteins [5,6]. TGF-β is a multifunctional cytokine expressed by many types of leucocytes, particularly macrophages, with various immunomodulatory roles especially relevant to the GI tract (Figure 1). Activin, a small paracrine cytokine, and follistatin, an activin-binding and neutralising protein, also belong to the superfamily and both contribute to the development or maintenance of inflammation [7–9]. This review aimed to outline current understanding of the role and relevance of the abovementioned proteins in the setting of IBD and to also consider the potential therapeutic role of modulating these inflammatory pathways.

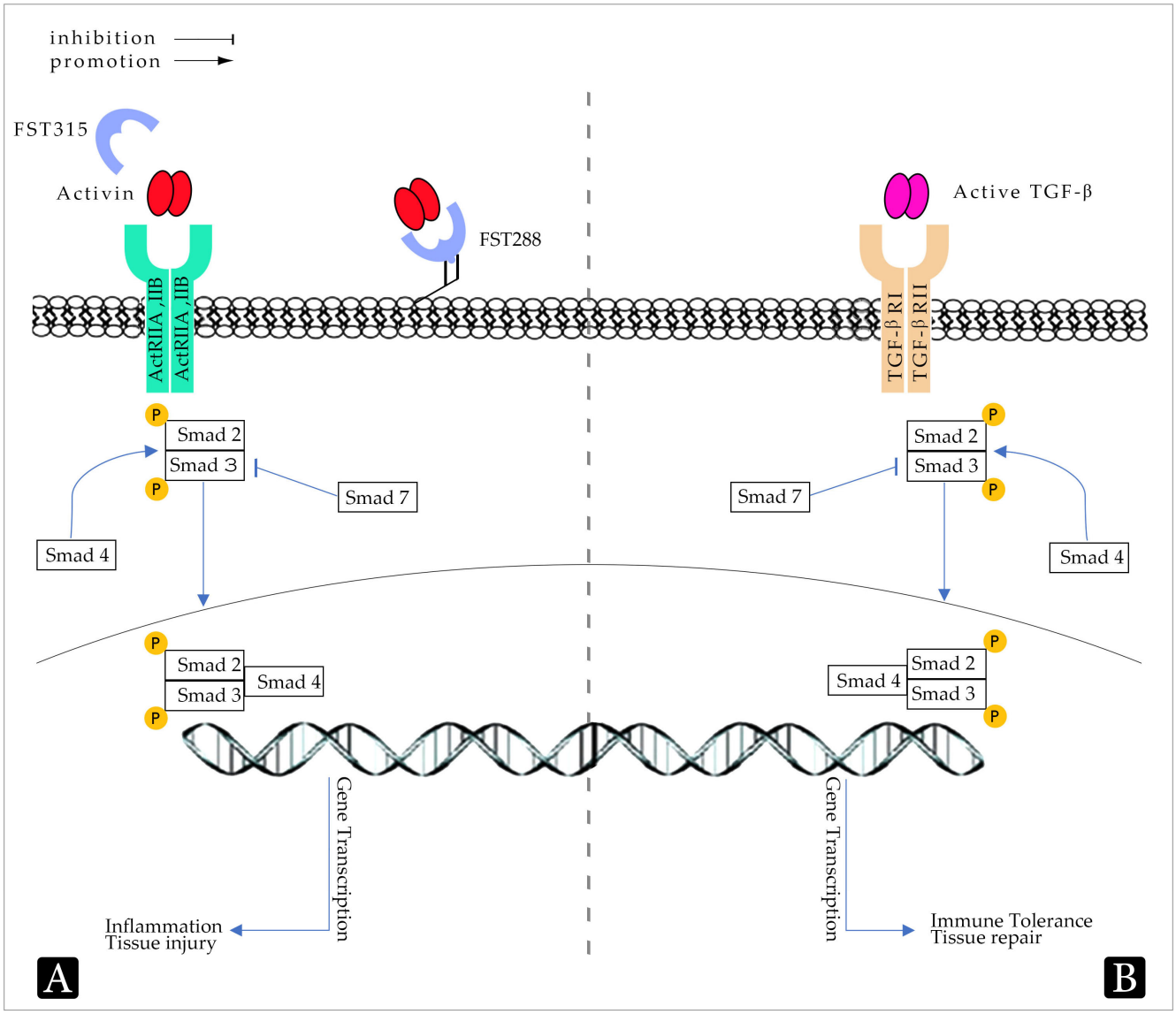

Figure 1. Depiction of key roles of TGF-β family members in the setting of gut inflammation with relevance to IBD. (**A**) Activin and follistatin pathways and (**B**) TGF-β pathways. FST = follistatin, TGF-B = transforming growth factor-β. IEL = intraepithelial lymphocytes, Th17 = T helper 17 lymphocytes.

2. TGF-β

2.1. General Aspects of TGF-β

The TGF-β family is comprised of three homologous peptides: TGF-β1, TGF-β2 and TGF-β3. Of these, TGF-β1 is predominant and plays a major regulatory role in the immune system [10,11]. In general, the TGF-βs are multifunctional cytokines that contribute to the regulation of several key cellular functions including cell proliferation, differentiation, migration, apoptosis and extracellular matrix production [12]. TGF-β is extensively expressed by epithelial cells, fibroblasts and immune cells [13]. It acts as a potent inhibitor of cell growth and stimulator of cell differentiation in intestinal epithelial cells [14]. TGF-β is a potent chemo-attractant for neutrophils and stimulates epithelial cell migration at wound sites, thus facilitating wound repair.

TGF-β also has an inhibitory role that diminishes subsequent inflammatory responses [15]. TGF-β promotes class-switching immunoglobulin (Ig)A in both human and mouse B cells and has an inhibitory activity upon the production of antibodies [16]. It stimulates resting monocytes, inhibits activated macrophages and acts as a chemoattractant for mono-

cytes [17]. Furthermore, it downregulates inflammatory cytokine production by monocytes and macrophages through the inhibition of nuclear factor (NF)-κB [18]. Overall, TGF-β maintains the immune balance of the GI tract in three key ways: enhancing mucosal defense, promoting immune tolerance and suppressing anti-inflammatory responses [19].

2.2. Biological Roles of TGF-β

The active TGF-β ligand binds to the TGF-β receptor (R)II and initiates TGF-βRII to form a complex with TGF-βRI with the assistance of accessory TGF-βRIII. The TGF-βR complex then mediates transduction of signals through canonical Smad (Smad 2, 3 and 4) and non-canonical pathways (Figure 2) [20]. In the Smad-dependent canonical pathway, phosphorylated Smad2 and Smad3 form a complex with Smad4 and enter the nucleus to regulate the transcription of target genes [11,21,22]. Smad7 is a downstream target of the TGF-β pathway that negatively regulates TGF-β signalling by competing for Smad3′s binding site on the TGF-β receptor, thereby blocking Smad3 activation [23–25].

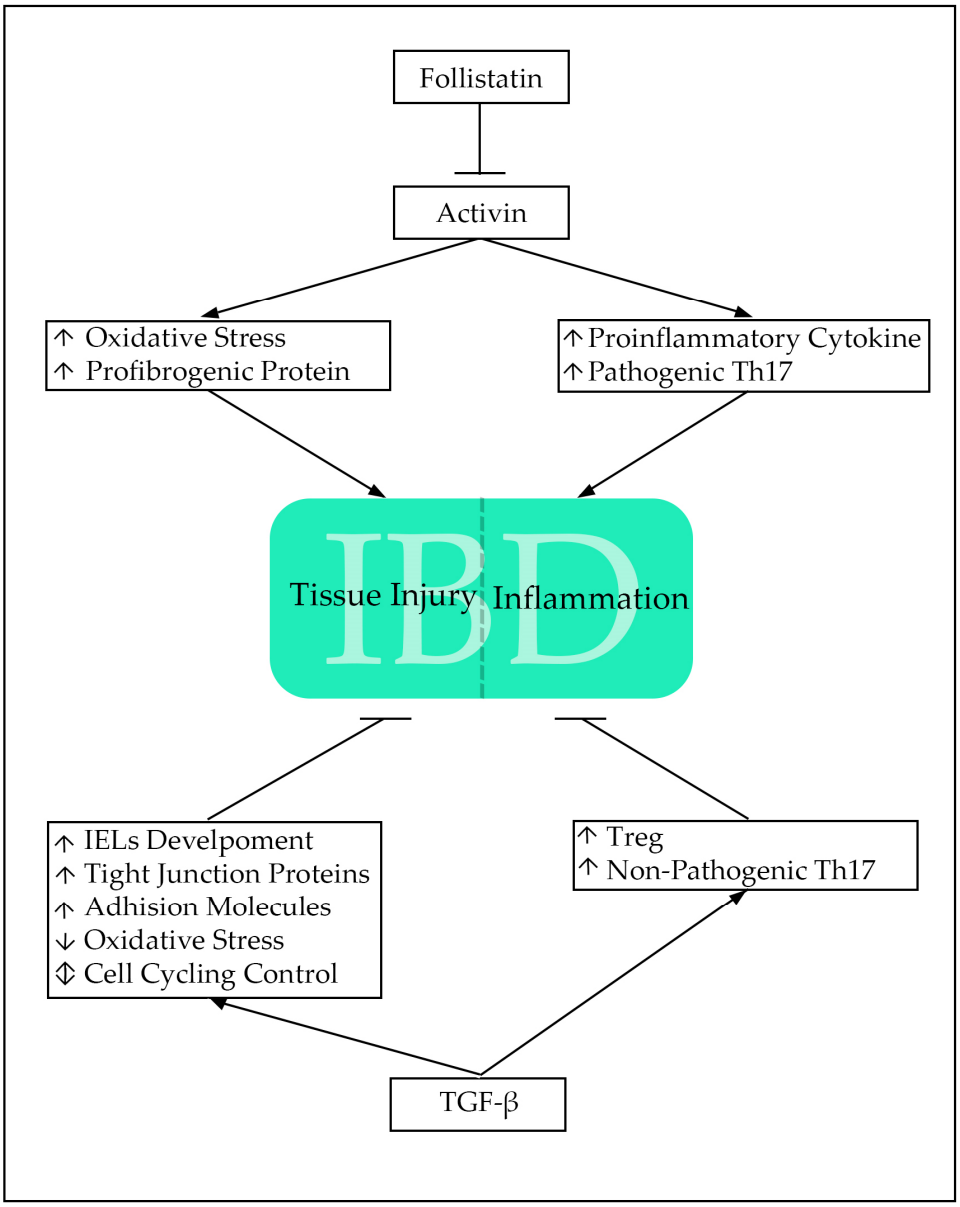

Figure 2. Signalling pathways of TGF-β, activin and follistatin. Promotion or inhibition indicated by up or down arrows (respectively).

Suppression of cytokine production by TGF-β is Smad2/3-dependent [26,27]. The Smad3/4 pathway is also an important mediator of TGF-β signalling in immune regulation. Disruption of Smad4, specifically in T cells, results in colitis and an increased susceptibility to spontaneous development of colorectal tumors [28]. It has been noted that Smad3 phosphorylation levels and Smad7 protein levels were markedly reduced and increased respectively in mucosal samples of patients with CD [29,30]. In these individuals there is marked over-expression of pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α and increased production of matrix-degrading enzymes by fibroblasts and macrophages. Simultaneously, endogenous healing pathways mediated by TGF-β are inhibited because mucosal inflammatory cells express Smad7 [31]. It has been demonstrated that Smad7 antisense therapy reduced Smad7 protein levels, increased levels of phosphorylated Smad3 and decreased levels of mucosal pro-inflammatory cytokines including TNF-α and interferon (IFN)-γ [29]. In addition to the canonical Smad pathway, other signalling pathways, including the extracellular signal-regulated kinases (ERK1/2), mitogen-activated protein kinase (p38), Src and phosphatidylinositol 3-kinase (PI3K) pathways, have been reported to mediate TGF-β effects in a context-dependent manner [32].

TGF-β, via Smad2/3, also induces Foxp3 (a master regulator of Tregs) in naïve T cells and inhibits the proliferation of immune cells as well as cytokine production via Foxp3-dependent and -independent mechanisms [22,33]. Foxp3 inhibits secretion of proinflammatory cytokines, (including interleukin (IL)-2, IFN-γ, IL-4 and IL-17), enhances expression of anti-inflammatory cytokines (such as IL-10 and TGF-β) and up-regulates cytotoxic T lymphocyte-associated protein (CTLA)4 [34]. TGF-β also induces $Foxp3^+$ expression in invariant natural killer T (iNKT) cells and allows them to acquire an immunoregulatory phenotype similarly to Tregs [35]. Tregs are a major source of TGF-β, and TGF-β is one of the effector molecules of Tregs upon activation by Treg/dendritic cell (DC) interaction [32]. Tregs are proposed to be involved in immune tolerance not just by secreting soluble TGF-β and IL-10 but also through a membrane-bound TGF-β-mediated cell–cell contact mechanism [36].

TGF-β also plays an important role in generating induced Tregs (iTregs) from naive T cells [32]. TGF-β has been implicated in the maintenance of Foxp3 in thymus-derived natural (n) Tregs [37]. Another function of TGF-β in T-cells is Th17 differentiation [38]. It has been suggested that the role of TGF-β in Th17 differentiation is the suppression of Th1 and Th2 differentiation (i.e., suppression of the production of IFN-γ and IL-4) since these cytokines strongly inhibit Th17 differentiation. Th17 cells produce IL-17 and IFN-γ, which support mucosal defense against bacteria, but tend to promote intestinal inflammation [38,39]. IL-17 production leads to expression of chemokines and other proinflammatory cytokines (such as IL-6 and TNFα) [40].

Overall, TGF-β through the inhibition of effector Th cell differentiation, induction of naive T cells into regulatory T cells, inhibition of the proliferation of T cells and B cells, inhibition of effector cytokine production (such as IL-2, IFN-γ and IL-4) and suppression of macrophages, DCs and natural killer (NK) cells regulates immune responses [32]. TGF-β contributed to the development of an "inflammatory energy" macrophage phenotype, which is characterised by a lack of proinflammatory cytokine production under inflammatory stimuli but retention of phagocytic and bactericidal activity [18].

TGF-β production and signalling by T-cells are also important steps in the development of regulatory intraepithelial lymphocytes (IELs) in the intestinal epithelium [41]. TGF-β modulates the barrier function of the epithelium by regulating the expression levels of tight junction proteins and adhesion molecules [42]. Moreover, TGF-β is capable of degrading toll-like receptor (TLR)2 on epithelial cells [43]. TGF-β inhibits mast cell growth and downregulates the expression of membrane Ig-E receptors to attenuate the release of histamine, cysteinyl leukotrienes and TNF-α [35]. TGF-β also functions as a negative autocrine feedback regulator to prevent tissue injury caused by excessive nitric oxide (NO) [44,45]. Furthermore, it also regulates cell proliferation by controlling the expression of cell cycle regulators [46].

2.3. Role of Transforming Growth Factor-β in IBD

Stimulation of colonic epithelial cells with pathogenic bacteria and/or cytokine results in the up-regulation of a distinct array of pro-inflammatory cytokines such as TNF-α, IL-1β and IL-6 that are key elements in the pathophysiology of IBD [47,48]. Once the inflammatory response has been initiated, efficient and prompt resolution of inflammation is important in order to minimise tissue injury and restore the integrity of the mucosal barrier [49]. The main function of TGF-β is expansion and survival of Treg cells that control the immune response and tolerance [50]. A reduction in Treg differentiation has been reported as a result of impaired TGF-β signalling in patients with IBD [51].

Tissue TGF-β levels are increased in the setting of IBD, but this may not be sufficient to counteract the ongoing inflammation [51]. This event is attributable to the remarkable upregulation of Smad7, blocking TGF-β-mediated IκBa transcription, in conjunction with the effects of TGF-β and IL-6, leading to the increased production of Th17 cells [52].

Pre-incubation of normal lamina propria mononuclear cells with TGF-β could prevent TNF-α-induced NF-κB activation [53]. This phenomenon results from an elevated pSmad3, which promotes IκBa transcription and reduces NF-κB entering into the nucleus, thus down-regulating inflammatory cytokine expression [54]. Biopsy specimens obtained from individuals with a normal (un-inflamed) colon that were exposed to anti-TGF-β antibodies exhibited downregulation of T-cell apoptosis and an increase in the production of proinflammatory cytokines including interferon-γ, TNF-α, IL-2, IL-6, IL-8 and IL-17 [55].

2.4. Therapeutic Approaches Related to TGF-β

Several therapeutic approaches involving TGF-β have been considered for the treatment of IBD. These involve direct administration of TGF-β and use of foods with enhanced or high content of TGF-β. However, preservation of the biological activity of TGF-β during the passage through the length of the GI tract has to be considered.

Conway et al. [56] worked on the encapsulation of all-trans retinoic acid (ATRA) and TGF-β separately and then oral administration of loaded microspheres alone or in combination in a murine model of IBD. The authors reported that the activity of encapsulated TGF-β was fully preserved and had optimal therapeutic effects on gut inflammation through local induction of regulatory T-cells. In another study, Hammer et al. [57] investigated the oral administration of TreXTAM (a combination of encapsulated TGF-β along with ATRA) in rats. A reduction in local and systemic baseline levels of active TGF-β was observed. According to the known effects of TGF-β on fibrosis [7], this negative feedback suggests that encapsulated TGF-β may have a potential role in the prevention of IBD-associated fibrosis.

A number of reports have evaluated the delivery of TGF-β within foods, in enteral formulae or directly via gavage in animal models with mucosal inflammation (Tables 1 and 2). Feeding with an enteral diet containing TGF-β2 resulted in improvement in body weight, lower pathological scores and lower Serum Amyloid A (SAA) levels in an IL-10 knockout mouse model of IBD [4]. Maheshvari et al. [58] reported that enteral supplementation of TGF-β2 protected mice from an experimental necrotising enterocolitis (NEC)-like injury by suppression of macrophage cytokine production and mucosal inflammatory responses in the developing intestine. In another animal study, gavaged TGF-β1 protected the immature gut from the development of NEC via suppression of immune effects on the intestinal epithelium along with a reduction in systemic IL-6 and IFN-γ levels [59]. Furthermore, diet enriched with TGF-β2 prevented mucosal injury, enhanced p-ERK and β-catenin induced enterocyte proliferation, inhibited enterocyte apoptosis and improved intestinal recovery in rats with experimentally methotrexate-induced intestinal mucositis [60].

Modulen IBD (Nestlé Health Science, Vevey, Switzerland) is a polymeric enteral formula rich in TGF-β that has been evaluated in a number of animal and clinical studies. In a rat model, for example, the administration of this formula provided protection against weight loss, hypoalbuminemia, acidosis, and GI mucosal damage [61]. Santactiv Digest powder (https://www.pharmacypanayiotou.com (accessed on 30 March 2023)) is another

polymeric product featuring colostrum-derived TGF-β2 supplemented with probiotics (*Bacillus coagulans*) and n-3 fatty acids.

Ferreira et al. [62] assessed the effects of nutritional supplementation with and without Modulen for 3 months in individuals with active CD. The patients receiving the TGF-β2-enriched formula showed an improvement in histologic parameters and reduced C-reactive protein (CRP) levels. Beaupel et al. [63] reported the preoperative administration of Modulen to be beneficial in high-risk patients with complicated CD, with reduced postoperative morbidity. In another study, 29 adults with active CD received Modulen IBD for 4 weeks [14]. Clinical improvement was noticed in 69% of the patients. In addition, nutritional parameters and inflammatory markers improved within the four weeks of the pilot study.

Davanço et al. [64] observed that the intake of whey protein supplemented with TGF-β by patients with CD resulted in improved lean body mass, while concurrently reducing the fat percentage. Triantafillidis et al. [65] compared the results of the administration of Modulen IBD with those of mesalazine in a group of patients with CD for six months. At the end of the trial, no significant differences were observed in remission between two groups. They did report that high density lipoprotein (HDL) levels increased while low density lipoprotein (LDL) levels decreased in the patients receiving formula. This is relevant, as HDL can modulate LDL oxidation, LDL-induced cytokine production and inflammation [66]. In another study, Triantafillidis et al. [67] reported complete remission of scleritis and psoriasis in a patient with CD after treatment with Modulen IBD for five weeks.

Two different polymeric formulae rich in TGF-β were used in two studies for 8 weeks as exclusive diets [68,69]. The first of these was a small study of patients with CD mainly affecting the small bowel while the second one included patients with different disease locations. The TGF-β formulae were effective in inducing remission and mucosal healing. Biochemical markers of inflammation, such as erythrocyte sedimentation rate (ESR) and C reactive protein (CRP), normalised while serum levels of albumin increased with treatment. Colonic histological scores also improved significantly. It is of interest that there was a reduction in the mRNA levels for IL-1, IL-8 and IFN-γ; however, endogenous TGF-β increased.

In summary, a number of positive outcomes of the use of formula enriched with TGF-β have been shown, as illustrated with these selected studies. None of the available studies have conclusively shown that these outcomes are related solely to the TGF-β. Other elements in the formula, the exclusion of normal diet during these studies or other factors may contribute to the observed findings. However, the data arising from the clinical studies are concordant with those of the animal studies. Further, these clinical reports have not indicated any adverse outcome or safety issues related to the TGF-β.

Table 1. Outcomes of the oral administration of TGF-β in various animal models of IBD. TGF-β = transforming growth factor-β, ATRA = all trans retinoic acid, NEC = necrotising enterocolitis, SAA = Serum Amyloid A, LPS = lipopolysaccharide, MUC2 = mucin 2.

Product	Route of Administration	Effect	Model	Ref.
TGF-β/ATRA-loaded microspheres	Gavage	Dose-dependent effects. Encapsulated TGF-β prevented weight loss, reduced average disease score, SAA levels, colon weight-to-length ratio and histological score. Encapsulated TGF-β/ATRA reduced gut inflammation, slowed disease progression and prolonged survival with no increased lung and intestinal fibrosis. Significant expression of Foxp3 in colonic lamina propria CD4+ CD25+ T-cells.	Mouse CD4 + CD25- T cell transfer	[56]

Table 1. *Cont.*

Product	Route of Administration	Effect	Model	Ref.
TreXTAM® (a combination of microencapsulated (ATRA), and encapsulated TGF-β (TPX6001)	Gavage	Significant reduction in serum and tissue TGF-β levels. TPX6001 only reduced weight loss transiently. TreXTAM or TPX6001 was safe and well tolerated at the highest doses.	Rat, Mouse CD4 + CD25-T cell transfer	[57]
TGF-β2	enteral supplementation	Protection against NEC-like intestinal injury	transgenic mice with defect in TGF-β signalling platelet-activating factor and LPS induced	[58]
TGF-β1	Gavage	Activation of Smad2 in intestinal epithelium—decrease in NEC severity and incidence. Inhibition of NF-kB activation, maintainance of Ikβα expression, reduction of proinflammatory cytokine production (IL-6 and IFN-ϒ)	NEC Neonatal Rat	[59]
TGF-β2	Supplemented in diet	Reversed intestinal damage, stimulated intestinal recovery, enhanced cell proliferation and inhibition of apoptosis (up-regulation of BCL-2 and downregulation of BAx expression). Significant increase in bowel and mucosal weight, DNA and protein content, pERK, IL-1β and b-catenin protein levels in intestinal mucosa.	Rat methotrexate-induced	[60]
Modulen	Enteral diet	More weight gain, no diarrhoea or prolapse, lower pathological scores, SSA and TNFα; higher hematocrit.	Mouse IL-10-/-	[4]
Modulen	Supplemented in diet	Protection against histologic damage, weight loss, hypoalbuminemia, acidosis and GI damage.	Rat methotrexate-induced	[61]
Casein based diet	Supplemented in diet	Lower inflammatory score, mucosal thickness, IFN-ϒ mRNA expression, leukocytosis and acute phase response. Improved diarrhoea, increased fecal dry matter, MUC2 protein expression and the muscle catabolic response. Restoration of immune homeostasis.	Rat HLA-B27	[70]
Modulen IBD	Supplemented in diet	Lower clinical and inflammatory score. Lower myeloperoxidase, inhibited intestinal angiogenesis. Significant decrease in the numbers of T cells, natural killer cells, dendritic cells and significant decrease in cytokine expression.	Mouse DSS-induced	[71]

Table 2. Selected studies demonstrating the outcomes of oral administration of TGF-β enriched formula in patients with IBD. EEN = exclusive enteral nutrition, ESR = erythrocyte sedimentation ratio, CRP = C-reactive protein, CD = Crohn's disease, UC = ulcerative colitis, PCDAI = pediatric Crohn disease activity index, BMI = body mass index, BCL-2 = B cell lymphoma 2, HLA = human leukocyte antigen, DSS = dextran sulfate sodium, HDL = high density lipoprotein and LDL = low-density lipoprotein.

Product	Route of Administration	Effect	Subjects	Ref.
CT3211	Polymeric formula as EEN	Mucosal healing and down regulation of mucosal pro-inflammatory cytokine mRNA. Increase in ileal TGF-β mRNA. Improved clinical remission. Decreased PCDAI and CRP. Increased weight standard deviation score.	Active CD (29 children)	[68]
AL110	Polymeric formula as EEN	Increased serum albumin and weight gain. Improvement in endoscopic appearance and mucosal healing. Reduction in mucosal inflammation, Lloyd Still index, ESR and CRP.	Active CD (7 children)	[69]
AL004	Polymeric formula as EEN	Clinical remission and improvement in quality-of-life scores.	Active CD (26 children)	[72]
Modulen	Polymeric formula as EEN	Clinical remission, inflammatory remission, decrease in endoscopic and histological scores.	Active CD (32 children)	[73]
Modulen IBD	Enteral nutrition as EEN	Improvement in remission of CD, height and weight z scores. Decreased inflammatory parameters (62%) of patients with UC had improved clinical remission and laboratory values	Active UC and CD (73 children)	[74]
Modulen IBD	Supplementary formula	Decreased ESR, CRP and PCDAI normalisation of weight and height z scores, continuing remission	Active CD (28 children)	[75]
Modulen IBD	Polymeric formula as EEN	Improved ESR, CRP, albumin and platelet count; improved growth; induction of remission	Active CD (27 children)	[76]
Modulen IBD	Polymeric formula as EEN	Improved ESR, CRP and albumin; decreased PCDAI; increased weight gain induction and maintenance of remission	Active and recently diagnosed CD (19 children)	[77]
Modulen IBD	Polymeric formula as EEN	Induction clinical remission; normalisation of inflammatory markers, improvements in weight/BMI *Z*-scores.	Active CD (114 children)	[78]
Modulen IBD	Systematic review of EEN	Improvements in growth and development, CRP, ESR and albumin	Active CD (147 children)	[79]
Modulen IBD	Polymeric formula as EEN	Improved ESR, CRP, hemoglobin, platelets and albumin; decreased PCDAI, induction and maintenance of remission, increased weight and height.	CD (106 children)	[80]
Modulen IBD	Polymeric formula as EEN	Clinical improvement in 69% patients. Reduced CRP and ESR. Improved nutritional parameters, anthropometric parameters, weight, and general wellbeing	Active CD (29 adults)	[14]

Table 2. *Cont.*

Product	Route of Administration	Effect	Subjects	Ref.
Modulen IBD	Nutritional supplement	Improved histologic parameters and significant reduction in CRP.	Active CD (38 adults)	[62]
Modulen IBD	Polymeric formula as EEN	Decreased postoperative morbidity. Discontinuation of steroids, decreased postoperative complications in patients at low or high risk.	preoperative CD (35 adults)	[63]
whey protein with TGF-β	Nutritional supplement	Increased lean body mass.	Active CD (22 adults)	[64]
Modulen IBD	Nutritional supplement	Improvement in all anthropometric and many nutritional and biochemical parameters. Improvement in HDL and reduction in LDL serum level. Maintenance of remission in quiescent CD.	mild to moderately active CD (32 adults)	[65]
Modulen IBD	Polymeric formula as EEN	Remission of severe scleritis and psoriasis in one patient	Active CD (one adult)	[67]

3. Activins

3.1. General Aspects of Activins

Activins, which are also members of the TGF-β superfamily, have been shown to regulate a number of different cellular functions, including cell proliferation, differentiation and apoptosis (Figure 1) [81,82]. Activins are expressed in almost every cell type, including epithelial cells, macrophages and fibroblasts [83,84]. Several different forms of activin are recognised: activin A is a homodimer of two activin A subunits, activin B is a homodimer of two activin B subunits; and activin AB is a heterodimer of these two subunits. Activin C, D, and E subunits also occur in mammals [85,86].

3.2. Biological Activity of Activins

Activins bind to one of two specific type 2 activin receptors (ACVR2A or ACVR2B) on the cell surface, which dimerise with a type 1 activin receptor serine/threonine kinase (activin receptor-like kinase, ALK) [87,88]. This leads to phosphorylation of the intracellular Smad proteins 2 and 3, which then form a heteromeric transcription factor with Smad4 [89] and translocate to the nucleus [90]. The downstream effects of Smad2/3 signalling depend on the cell type and cofactors associated with the Smad2/3-4 complex. This is the same transcription factor cascade activated by TGF-β (Figure 2). Furthermore, activins promote additional signalling pathways via TRAF6 and downstream activation of the MAP kinases, p38 MAPK, JNK and ERK1/2 [91–93]. The activity of activin is modulated by the synthesis and expression of activin receptors and levels of inhibin, an antagonist, and follistatin, a neutralising binding protein that binds activins with high affinity and blocks their function [85,86].

3.3. Inflammatory and Physiological Roles of Activins

Activins regulate the activity of many cell types involved in inflammation, immunity and fibrosis through regulation of fundamental cellular functions such as proliferation, differentiation and apoptosis in diverse cell systems including epithelial cells [94,95]. In animal experiments, an early rise in systemic activin A levels after lipopolysaccharide (LPS) injection was illustrated [95,96].

Activin A has the potential to antagonise the local actions of IL-6 and IL-1 [97]. Failure of activin A to modulate the actions of these cytokines is linked to the persistently elevated levels of acute phase proteins seen in chronic disease states such as secondary amyloidosis [98]. Activin A is released early in the cascade of circulatory cytokines in systemic

inflammatory processes, roughly coinciding with TNF-α and before IL-6 and follistatin are elicited [8]. Interestingly, monocyte secretion of activin A is enhanced upon contract with antigen-specific T cells [99] and following antigen sensitisation in the lung [100]. Activin A, via stimulation of quiescent macrophages, promotes the production of proinflammatory cytokines [101,102] and also promotes pro-inflammatory Th2-, Th9- and Tfh-related responses [103–105].

Activin A promotes inflammation by stimulating production of inflammatory mediators including TNF-α, IL-6 and IL-1β, and nitric oxide, whereas follistatin reduces oxidative stress and modulates the inflammatory process in tissue repair by neutralising activin A [105–107]. Following burns and in wound healing, activin A stimulates inflammation and production of pro-fibrogenic proteins such as endothelin and TNFα [108,109]. Clinical and animal studies have shown that activin A levels increase in both acute and chronic inflammation and correlate with the severity of illness [110–112]. Moreover, inhibition of activin function is shown to reduce inflammation, damage, fibrosis and morbidity/mortality in various disease models. In this regard, follistatin prevents fibrosis through inhibition of activin produced by cells in response to TGF-β [100,113].

3.4. Activins and T Helper 17 Cells

Th17 cells are known to exert both pathogenic and non-pathogenic functions. Pathogenic-Th17 cells express high amounts of pro-inflammatory molecules including GM-CSF and IL-23R, while non-pathogenic-Th17 cells express high amounts of IL-10 and CD5L that contribute to tissue homeostasis [114–118].

During autoimmune inflammation, Activin A drives pathogenic Th17 cell differentiation through an Activin-A-ALK4-ERK axis [119]. Thus, this axis could be considered as a therapeutic target for pathogenic-Th17-cell-related diseases including IBD, rheumatoid arthritis and asthma [112,120–122].

3.5. Roles of Activin in Inflammatory Bowel Disease

Activins appear to have various roles in the maintenance of intestinal homeostasis and in gut inflammation, with particular relevance to IBD [123]. Dignass et al. [124] showed the presence of activin receptors I and II throughout the GI tract of patients with IBD. Hubner and colleagues [125] demonstrated upregulation of activin A mRNA in the gut of individuals with IBD; in contrast, levels were undetectable in samples from healthy controls. Furthermore, activin A mRNA correlated positively with histological severity and IL-1 levels. Similarly, activin AB was also expressed only in the intestinal tissue from those with IBD. In contrast, activin B mRNA levels in most specimens from inflamed areas were only slightly higher than those in control tissue samples [124]. Animal studies have also demonstrated increased tissue and systemic levels of activin during experimental colitis [126].

3.6. Activin Signalling as an Emerging Target for Therapeutic Interventions

Activins are emerging as important diagnostic tools and therapeutic targets in inflammatory and fibrotic diseases. In particular, activin signalling contributes to the etiolopathogenesis of a variety of inflammatory diseases. Accordingly, activin inhibition may have clinical benefits in these settings [84].

There are a number of inhibitors of the activin receptor signalling pathways (IASPs), which have differing modes of action [127]. Firstly, ActRIIA or ActRIIB ectodomains fused with the Fc portion of human immunoglobulin (Ig)-G1 can act as soluble decoy receptors that trap ligand extracellularly [128,129]. Secondly, various antibodies, peptibodies or adnectins act to neutralise activin [130–132]. Thirdly, antibodies directed towards ActRIIA or ActRIIB (or both) prevent ligand–receptor interaction [133,134]. Finally, endogenous TGF-β superfamily antagonists, such as growth and differentiation factor (GDF)-associated serum protein [135] or follistatin (FST), can also interrupt activin receptor signalling [136,137]. Activin antagonists such as follistatin, an endogenous activin-binding protein, offer consid-

erable promise as therapies in conditions as diverse as sepsis, liver fibrosis, acute lung injury, asthma, wound healing and ischaemia–reperfusion injury. IASPs have also been considered in muscle wasting conditions [137–141] and various inflammatory disorders [142–144].

Numerous IASPs have been developed and evaluated in different settings [145–170]. One such example is Bimagrumab, an anti-ActRIIA/IIB antibody, which appears to have a beneficial role in muscle disorders [156–159]. This agent appears to also affect sex hormone profile, likely secondary to the effect of activin upon the gonadotropin-releasing hormone [171,172]. Whilst these agents have not yet been evaluated in the setting of IBD, their underlying mechanisms of action and their distribution in the gut suggest potential roles in IBD.

4. Follistatin

4.1. General Aspects of Follistatin

Follistatin (FST), another member of the TGF-β superfamily, is a multifunctional regulatory protein secreted by many tissues [173]. FST has a characteristic multidomain structure. Different isoforms can be found, consequent of alternative splicing of the corresponding gene and post-translational modification [174,175]. FST288 comprises 288 amino acids and is a locally acting form of FST characterised by a high affinity for heparin, a key component of the extracellular matrix and cell surface glycoproteins [174,176,177]. In contrast, FST315 consists of 315 amino acids and has a significantly reduced affinity for heparin because of the presence of an additional acidic C-terminal domain [178,179]. It represents the major circulating FST isoform. Another isoform, FST303, is likely produced by proteolytic cleavage of FST315 [102].

FST binds to and neutralises TGF-β superfamily members including activins, growth differentiation factor 8 (GDF8, myostatin) and GDF11 with high affinity (Figure 2). Neutralisation is achieved extracellularly when two FST molecules surround and sequester the receptor-binding sites of these ligands [175,179,180]. Follistatin is expressed on the surface of the target cells. FS288 is more potent than FST 315 and binds to activin A with very high affinity (50 pM) and activin B with a 10-fold lower affinity [176,179,181]. Activins trapped irreversibly by FST 288 are endocytosed into the cell and inactivated by proteolysis [181].

4.2. The Role of Follistatin in Inflammation and Tissue Repair

Follistatin has regulatory roles in several inflammatory and immune processes. In sheep, after in vivo challenge with LPS, up-regulation of FST follows that of activins [95]. In mice, blocking the actions of activin A following exposure to LPS with FST alters the induced cytokine cascade, attenuating the rise of TNFα and altering the levels of other inflammatory cytokines (e.g., IL1β and IL-6) [182].

Furthermore, FST is shown to reduce oxidative stress and modulate the inflammatory process in tissue repair by neutralising activin A [106,107]. In general, the activin/follistatin system contributes to tissue regenerative processes [183,184]. Neutralisation of activin in rats by FST promoted regeneration of hepatocytes after partial hepatectomy [185,186] and tubular epithelial regeneration after renal ischaemia [187]. A single administration of FST into the portal vein was much more effective than several administrations of hepatocyte growth factor in promoting liver regeneration and DNA was synthesised several hours earlier than in control [188,189].

FST attenuated radiation-induced fibrosis in a murine model [109] and diminished bleomycin-induced pulmonary fibrosis in mice by attenuating the proinflammatory and profibrotic actions of activin A [113]. In this regard, FST prevented fibrosis through inhibition of the activin produced by cells in response to TGF-β [100,113]. However, activin inhibition through FST did not affect TGF-β-induced growth suppression, suggesting that activin inhibition may specifically inhibit pro-oncogenic TGF-β functions while preserving its growth suppressive functions [190].

4.3. The Role of Follistatin in Intestinal Inflammation

Follistatin also appears to have particular roles in inflammatory processes in the GI tract. Administration of FST resulted in improvements in three distinct murine models of colitis [127]. In one model of colitis induced by administration of the hapten trinitrobenzene sulfonic acid (TNBS), FST pretreatment improved survival and reduced pro-inflammatory proteins. Rescue treatment with FST also resulted in improved histological scores in this model. Further to this, these researchers also demonstrated that FST administration reduced the severity of colitis in mice exposed to dextran sulfate sodium (DSS) and in the IL-10 knockout murine model [127].

These findings were supported by the results of a second study. Zhang et al. [191] demonstrated similar beneficial effects of FST using the DSS model and mice with a mdr1a knockout. Follistatin also appeared to enhance epithelial cell growth with consequent tissue repair and reduced apoptotic rate in mice exposed to DSS, reversing the adverse effects of activins upon the epithelium. A recent study showed that FST also strongly reduces macrophage trafficking in the epidermis [192]. Given the role of macrophages in these murine models of gut inflammation, this may be an additional component of the anti-inflammatory outcomes seen with FST.

FST-based protein therapeutics also comprise an emerging class of pharmaceuticals for the treatment of muscular diseases. The first FST-based drug in clinical development [138,161]; ACE-083, upon intramuscular injection, acts as ligand trap for different cytokines of the TGF-β superfamily and thus interferes with ActRIIA and ActRIIB activation [138,161]. FollistatinΔHBS-Fc, based on follistatin 315, has been evaluated as a systemic therapy for muscle injury and osteoporosis in preclinical disease models [126,193]. Whilst these agents have not yet been assessed in the setting of IBD, the in vitro data and the understanding of the underlying mechanisms of action suggest that they may also have applications for IBD.

5. Conclusions

IBD is characterised by chronic intestinal inflammation and impaired epithelial repair. While the traditional treatment strategy for patients with IBD has focused on decreasing inflammation, current therapies are suboptimal and not curative. Recent advances suggest that growth factors may be potential tools for modulation of intestinal inflammation and enhanced repair that could provide safe and effective treatment for patients with IBD. The TGF-β superfamily growth factors (particularly TGF-β, activin and follistatin) appear to have putative roles.

Manipulation of the activity of these growth factors may provide safe and effective individualised treatment for patients with IBD. Whilst there is biological rationale to consider this, the balance between efficacy and adverse effects (including growth stimulation in dysplastic lesions) needs to be considered. Accordingly, further data from pre-clinical and clinical studies are required to fully delineate the role of these potentially beneficial therapies.

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