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Molecular and Cellular Mechanisms Involved in Mesenchymal Stem Cell-Based Therapy of Inflammatory Bowel Diseases

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Abstract
Mesenchymal stem cells (MSCs) are promising resource for the therapy of inflammatory bowel diseases (IBDs) on the grounds of their differentiation capabilities and immuno-modulatory characteristics. Results of clinical studies indicate that local application of MSCs is a secure and beneficial approach for the treatment of perianal fistulas while systemic application of MSCs leads to the attenuation or aggravation of IBDs. Herein, we emphasized molecular mechanisms and approaches that should improve efficacy of MSC-based therapy of IBDs.

Keywords Mesenchymal stem cells · Chron's disease · Ulcerative colitis · Transplantation · Cell-based therapy

Introduction

Current therapy for inflammatory bowel diseases (IBDs), including Chron's disease (CD) and ulcerative colitis (UC), includes use of immunosuppressive drugs which encourage remission of intestinal inflammation and associated symptoms [1]. Medical treatment is only effective for achieving and maintaining remission as there is no therapeutic drug effective enough to completely invert colon inflammation process. Accordingly, non-responsive patients and patients that suffer from undesired side effects associated with standard therapy require novel therapeutic strategies, such as stem cell-based therapy [2].

Mesenchymal stem cells (MSCs) are promising resource for the therapy of IBDs on the grounds of their differentiation capabilities and immuno-modulatory characteristics [3]. Accordingly, in this review article, we emphasized molecular mechanisms and approaches involved in MSC-based therapy of IBDs. An extensive literature review was carried out in June 2017 across several databases (MEDLINE, EMBASE, Google Scholar, ClinicalTrials.gov), from 1990 to present. Keywords used in the selection were: "mesenchymal stem cells", "inflammatory bowel diseases", "Crohn's disease", "Ulcerative colitis". All journals were considered, and, initial search retrieved 229 articles. The abstracts of all these articles were subsequently reviewed by two of the authors (BSM and VV) independently to check their relevance to the subject of this manuscript. Eligible studies had to delineate molecular and cellular mechanisms involved in the MSC-based therapy of IBDs and their findings were analyzed in this review.

Etiology and Pathogenesis of IBDs

Etiology of IBDs is unknown [4]. According to recently published data, it seems that interaction of genetic, microbial, and environmental factors is responsible for the development and progression of IBDs [4, 5]. In countries which have acquired an industrialized lifestyle the incidence of IBDs has increased, pointing out the environmental factors influential

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in triggering the onset of the disease [4]. The episodes of the previous gastrointestinal infection (e.g. *Salmonella species, Shigella species, and Campylobacter species*) are usually seen in patients suffering from IBDs, suggesting that bacterial infection of the gut supposedly lead to changes in gut flora, and trigger the beginning of a chronic inflammatory process in genetically prone individuals [6]. Accordingly, colon-infiltrating immune cells, particularly macrophages, dendritic cells (DCs) and T lymphocytes have an important role in induction and progression of IBDs (Fig. 1).

Immune Cells: Central Players in the Development of IBDs

Colon-infiltrating macrophages have many crucial functions in the pathogenesis of IBDs. Pro-inflammatory M1 macrophages produce inflammatory cytokines: tumor necrosis factor alpha (TNF-α), interleukin (IL)-1β, IL-6, and nitric oxide (NO) and promote intestinal inflammation [7]. On the contrary, alternatively activated M2 macrophages may act as non-inflammatory scavengers of bacteria and may

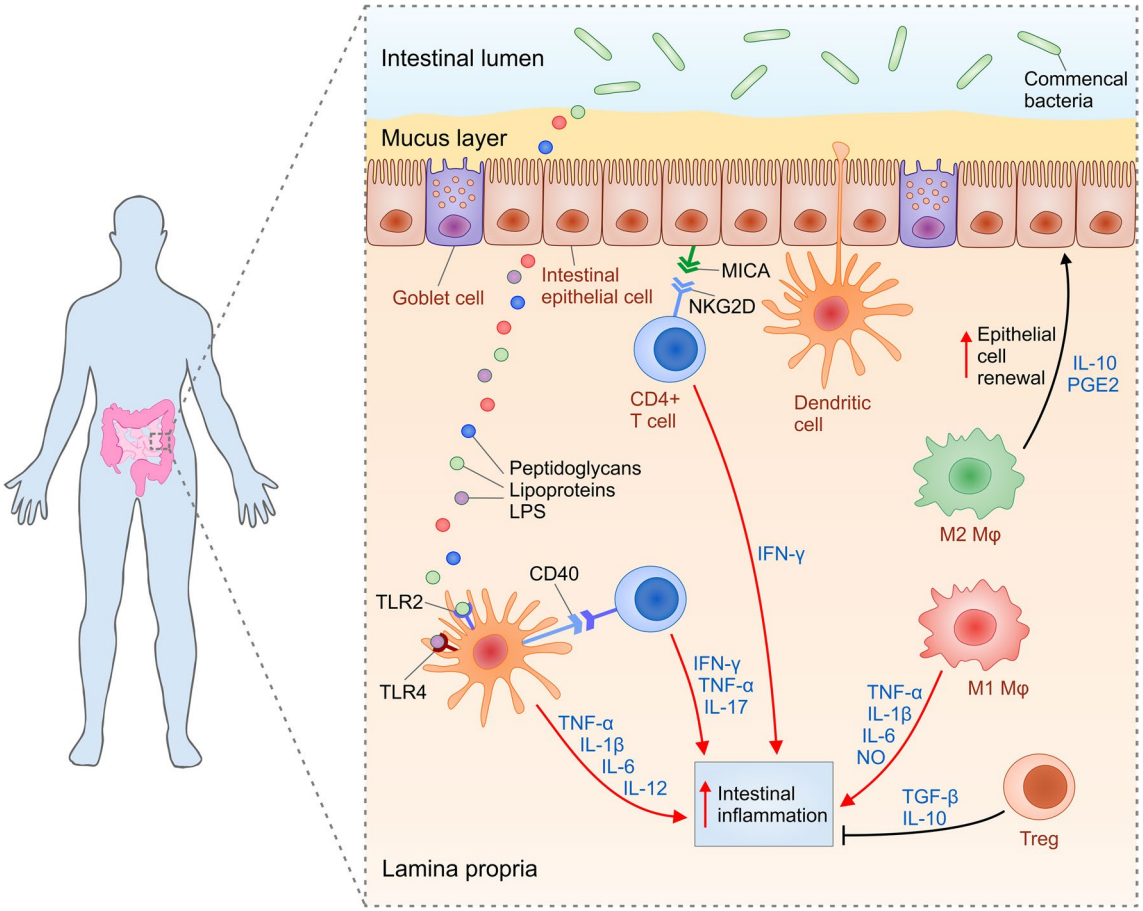

Fig. 1 Schematic diagram describing mechanism responsible for IBD pathogenesis in humans. Intestinal homeostasis involves the coordinated actions of epithelial, innate and adaptive immune cells. Barrier permeability permits microbial incursion, which is detected by the innate immune system, which then orchestrates appropriate tolerogenic, inflammatory and restitutive responses in part by releasing extracellular mediators that recruit other cellular components, including adaptive immune cells. Proinflammatory M1 macrophages produce inflammatory cytokines such as TNF-α, IL-1β, IL-6, and NO and promote intestinal inflammation. Alternatively activated M2 macrophages may act as non-inflammatory scavengers of bacteria and may promote epithelial cell renewal, via the production of IL-10 and PGE2. DCs interact with peptidoglycan and bacterial lipoproteins of pathogens through TLR-2 as well as with LPS through TLR-4 and promote the development of gut inflammation. Activated DCs release pro-inflammatory cytokines, particularly TNF-α, IL-1β, IL-6 and IL-12. Additionally, DCs express CD40 costimulatory protein involved in interaction with T cells and priming T-cell responses against bacteria. Activated CD4 + Th1 cells produce proinflammatory cytokines (IFN-γ, TNF-α and IL-17) and promote inflammation in the gut. Cytotoxic CD4 + NKG2D + T cells bind MHC class I polypeptide-related sequence A (MICA) molecule on injured intestinal epithelial cells and produce inflammatory cytokines which cause inflammation in the gut or have direct cytotoxic effects against epithelial cells. Tregs may suppress immune responses in inflamed gut by producing immunosuppressive IL-10 and TGF-β creating immune-tolerant microenvironment

promote re-epithelialization in IL-10 and prostaglandin E2 (PGE2)-dependent manner [8]. Thus, therapeutic strategies that suppress function of M1 macrophages and promote their conversion in M2 phenotype is expected to be effective in the therapy of IBDs.

Both myeloid and plasmacytoid DCs migrate into the inflamed gut of IBD patients. Colonic CD11c + DCs, isolated from IBD patients, express higher levels of toll-like receptor (TLR)-2 (receptor for peptidoglycan and bacterial lipoproteins), TLR-4 (interacting with lypopolisaharide (LPS)) and CD40 (involved in interaction with T cells) compared with healthy controls [9]. Through these receptors DCs recognize bacterial antigens and become activated. Activated DCs produce large amounts of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6 and IL-12), express high levels of co-stimulatory molecules (CD80 and CD86), and prime CD4 + T cells against colon-infiltrating bacteria and potentiate the development of gut inflammation [10]. On the contrary, infiltration of immature and tolerogenic DCs is remarkably reduced in inflamed colons of patients with active and remissive forms of IBDs, suggesting the importance of DC maturation and activation in progression and reactivation of IBDs. Thus, therapeutic approach that will prevent maturation of DCs and promote influx of tolerogenic DCs in inflamed colons will have beneficial effects in the therapy of IBDs.

CD4 + T cells, as an essential part of the adaptive immunity, play significant role in effector phase of gut inflammation [11]. It is believed that CD and UC are T-cell-driven diseases, developed as a consequence of improper cytokine production by CD4 + T-helper (Th) cells [5]. In this respect, a high number of interferon gamma (IFN-γ) and TNF-α producing helper CD4 + Th1 cells as well as cytotoxic CD4 + NKG2D + perforin + Th1 cells were increased in the mucosa of IBD patients, indicating that Th1 cells either produce inflammatory cytokines and promote inflammation in the gut or have direct cytotoxic effects against epithelial cells [12–15]. Since depletion of CD4 + T lymphocytes results with the attenuation of IBDs symptoms [16], therapeutic agents that can suppress activation and reduce influx of CD4 + Th1 cells in the inflamed gut will have beneficent effects in the therapy of IBDs.

CD4 + CD25 + FoxP3 + T regulatory lymphocytes (Tregs) have a crucial role in maintaining protection of the intestinal mucosa which is exhibited to a broad spectrum of foreign antigens, including bacterial flora and food antigens [17]. Attenuated number or dysfunction of Tregs was connected to a disruption of intestinal tolerance making contribution to the development of IBDs [18, 19]. Tregs inhibit intestinal inflammation by killing effector T cells and by producing immunosuppressive IL-10 and transforming growth factor beta (TGF-β) creating immuno-tolerant microenvironment in the colon. Therefore, therapeutic agents, which are able to promote expansion and infiltration of Tregs in inflamed colons could be effective in the therapy of IBDs.

Mesenchymal Stem Cells: New Agents in the Therapy of IBDs

MSCs are considered as new therapeutic agents in the treatment of immune-mediated diseases, including IBDs, particularly because of their potential to differentiate into gut epithelial cells and due to their pro-angiogenic and immunomodulatory characteristics (Fig. 2) [3].

MSCs Capacity to Differentiate into Gut Epithelial Cells

Cross-talk between epithelial and mesenchymal cells and their interaction with resident and recruited immune cells is particularly important for the maintenance of homeostasis in the gut [20]. Both cell types are able to modulate microenvironment of the gut by affecting recruitment and activation of immune cells, and at the same time, both cell types are responsive to the immune cell-derived cytokines and growth factors that modulate their own intrinsic function [20]. Accordingly, dysfunction of epithelial barrier is crucial for the development and progression of IBDs [4]. Bone marrow-derived mesenchymal stem cells (BM-MSC) represent a cellular source for epithelial repair since, under specific culture conditions (in the presence of Keratinocyte Growth Factor (KGF), Hepatocyte Growth Factor (HGF), Epidermal Growth Factor (EGF) and Insulin-like growth Factor-II), BM-MSCs can differentiate into epithelial cells in vitro [21]. However, mechanism of MSC-mediated repair of gut epithelium *in vivo* still remains unclear since Ferrand and co-workers showed that after engraftment in the gut, MSCs acquire epithelial characteristics through a fusion with resident intestinal epithelial cells and not by differentiation in epithelial cells [22].

The Role of MSCs in Angiogenesis

MSCs have at least three functions that can enhance angiogenesis and modulate immune response during gut regeneration: (1) homing to the site of injury, (2) producing pro-angiogenic cytokines and growth factors, (3) trans-differentiating into functional endothelial cells (ECs). Since systemically infused MSCs engraft in the injured gut only at low rates, it seems that the beneficial pro-angiogenic effects of transplanted MSC are often mediated by transient, paracrine mechanisms comprising the secretion of MSC-derived soluble factors without requiring presence of MSCs in the gut.

MSCs may promote angiogenesis through the production of several pro-angiogenic factors (Vascular

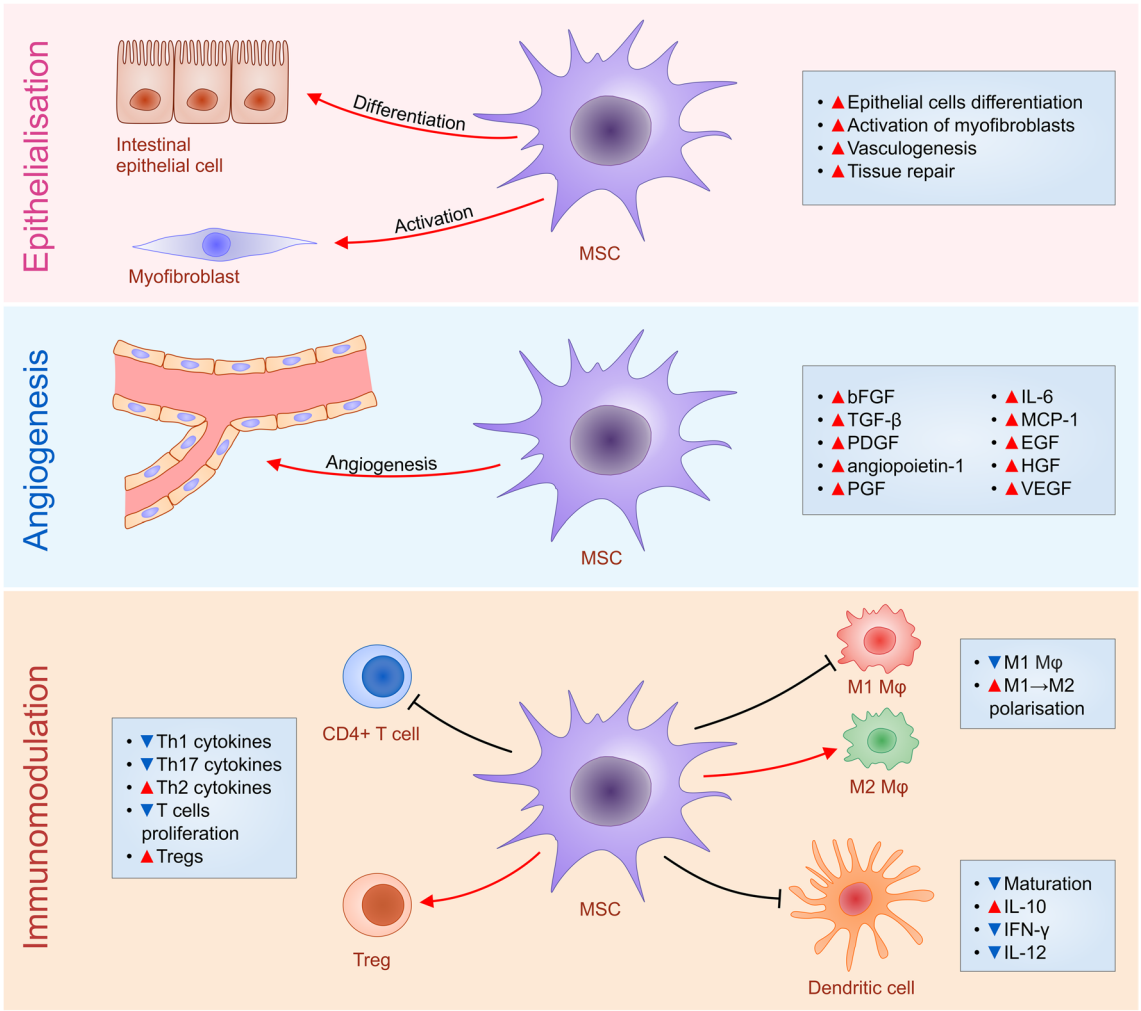

Fig. 2 Differentiation ability, pro-angiogenic and immune-modulatory characteristics of MSCs. MSCs could serve as an effective therapeutic agent for tissue repair. Transplanted MSCs can contribute to tissue repair either by forming epithelial cells (differentiation) or activated myofibroblasts (vasculogenesis). MSCs also enhance their expression of α-smooth muscle actin (a-SMA), and are able to heal epithelial injuries (upper panel). Additionally, MSCs promote angiogenesis through the production of several pro-angiogenic factors (bFGF, TGF-β, PDGF, angiopoietin-1, PGF, IL-6, MCP-1, EGF, HGF, VEGF), which facilitate tissue regeneration (middle panel). In cell to cell contact and through the production of soluble factors, MSCs can alter the function of all immune cells that play crucial role in the pathogenesis of IBDs. MSCs suppress inflammatory M1 macrophages and promote their conversion in alternative, M2 phenotype. MSCs can inhibit maturation of DCs and alter their secretion profile resulting in decreased production IFN-γ and IL-12 and increased production IL-10 which leads to attenuated activation of T cells. Also, MSCs can directly decrease production of Th1 and Th17 cytokines and increase production of Th2 cytokines. MSCs can inhibit proliferation of T cells and increase the number of Tregs which suppress the immune response and inflammation (lower panel)

endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), TGF-β, platelet-derived growth factor (PDGF), angiopoietin-1, placental growth factor (PGF), IL-6, monocyte chemotactic protein-1 (MCP-1), EGF, HGF), which facilitate tissue regeneration by inducing proliferation of ECs and by promoting neo-vascularization [23]. Moreover, under specific culture conditions MSCs have the capacity to differentiate into ECs and to create a capillary network in vitro and in vivo [24, 25].

Immuno-modulatory Characteristics of MSCs

In cell to cell contact and through the production of soluble mediators, MSCs can alter the function of all immune cells that have essential role in the pathogenesis of IBDs. MSCs suppress inflammatory M1 macrophages and promote their conversion in alternative, M2 phenotype [26]. MSCs may suppress maturation of DCs and alter their secretion profile towards tolerogenic phenotype resulting in

decreased production of pro-inflammatory IFN-γ and IL-12 and increased production of anti-inflammatory IL-10 which leads to the attenuated activation of T cells [27]. The MSCs can also directly alter the cytokine profile of CD4 + T cells by decreasing production of Th1 and Th17 cytokines (IFN-γ and IL-17) and by increasing production of Th2 cytokines such as IL-4 and IL-10 [28]. MSCs can inhibit proliferation of effector T cells in programmed death 1 (PD-1), TGF-β or IL-10-dependent manner and may increase infiltration of Tregs in the gut, which suppress the immune response and inflammation [29].

Different Types of MSCs

For a long time, bone marrow (BM) has been considered as the main source for the isolation of MSCs. BM-MSCs have many advantages for therapeutic use, such as: easy acquisition, short doubling time in vitro, minor risk for immunological rejection, long-term coexistence in the host, maintenance of differentiation ability after repeated passages and ease of transfection [30]. Nevertheless, isolation of BM-MSCs involves harvesting of BM that is a highly invasive procedure and the number of obtained BM-MSCs significantly decline by aging [31]. Therefore, alternative sources of MSCs have been strongly pursued, including umbilical cord blood (UCB) and adipose tissue (AT) [32, 33].

UCB-derived MSCs (UCB-MSCs) are easy to obtain by non-invasive and safe procedure, able to produce large yields, have significant immunosuppressive characteristics, which make them useful for allogeneic transplantations [32].

AT is another alternative source of MSCs. AT-MSCs can be easily isolated from liposuctions. Total number of obtained AT-MSCs is usually significantly higher than after BM harvesting [30]. AT-derived MSCs have differentiation capacity similar to BM-MSCs [33].

BM-, UCB- and AT-MSCs are plastic adherent stem cells with multi-lineage differentiation potential that display a variety of cell surface markers (Table 1) [34]. There is no significant difference in the morphology between BM-, UCB- and AT-derived MSCs [35]. Differences could be observed in the success rate of isolation, proliferation capacity and clonality of these cells. In contrast to BM-MSCs and AT-MSCs, UCB-MSCs could not differentiate into adipocytes and are not able to suppress B cells, but have the highest growing rate, clonality and express low levels of senescence markers [35, 36].

Molecular and Cellular Mechanisms Responsible for MSC-Mediated Modulation of IBDs

Molecular and cellular mechanisms responsible for MSC-mediated attenuation of murine colitis involve: promotion of angiogenesis and regeneration of damaged epithelium, suppression of colon inflammation and induction of regulatory mechanisms that lead to the enhanced healing process in injured colon (Fig. 2). Transplantation of MSCs significantly increases expression of vascular growth factors in inflamed colons and promotes trans-differentiation of engrafted MSCs into ECs, myofibroblasts and fibroblasts, contributing to the colon regeneration [37, 38]. Additionally, transplanted MSCs decrease expression of inflammatory cytokines and chemokines in the colon, reduce infiltration of inflammatory cells, attenuate detrimental Th1 and Th17 immune responses [39, 40], and promote conversion of colon infiltrated T cells and macrophages in regulatory and anti-inflammatory phenotypes [41–43].

Similar mechanisms are responsible for therapeutic effects of BM-MSCs, UCB-MSCs and AT-MSCs in modulation of IBDs (Fig. 3).

BM-MSC as New Agents in Cell-Based Therapy of IBDs

In several animal models of UC, BM-MSCs managed to efficiently regenerate colon epithelium either by promoting proliferation of epithelial cells or by inducing angiogenesis in injured colons [37, 38, 44]. In rats suffering from dextran sodium sulphate (DSS)-induced colitis, intravenously injected allogeneic MSCs restored expression of proteins

Table 1 Different types of MSCs

Type	Metod of isolation	Media	Supplement serum	Cell surface markers	
				positive	negative
BM-MSCs [35]	Ficoll density gradient method Novel marrow filter device	DMEM/F12	FBS	CD73, CD90, CD105 STRO-1	CD14, CD34, CD45 HLA-DR
hUCB-MSCs [35]	Digestion method	DMEM-HGDMEMC-MRL1660	FBS	CD29, CD44, CD73, CD90 CD105	CD34, CD45
AT-MSCs [35]	Digestion method Membrane filtration method	DMEM-LG	FBS FCS	CD73, CD090, CD29 CD44, CD71, CD105 CD13, CD166, STRO-1	CD14, CD31, CD34, CD45

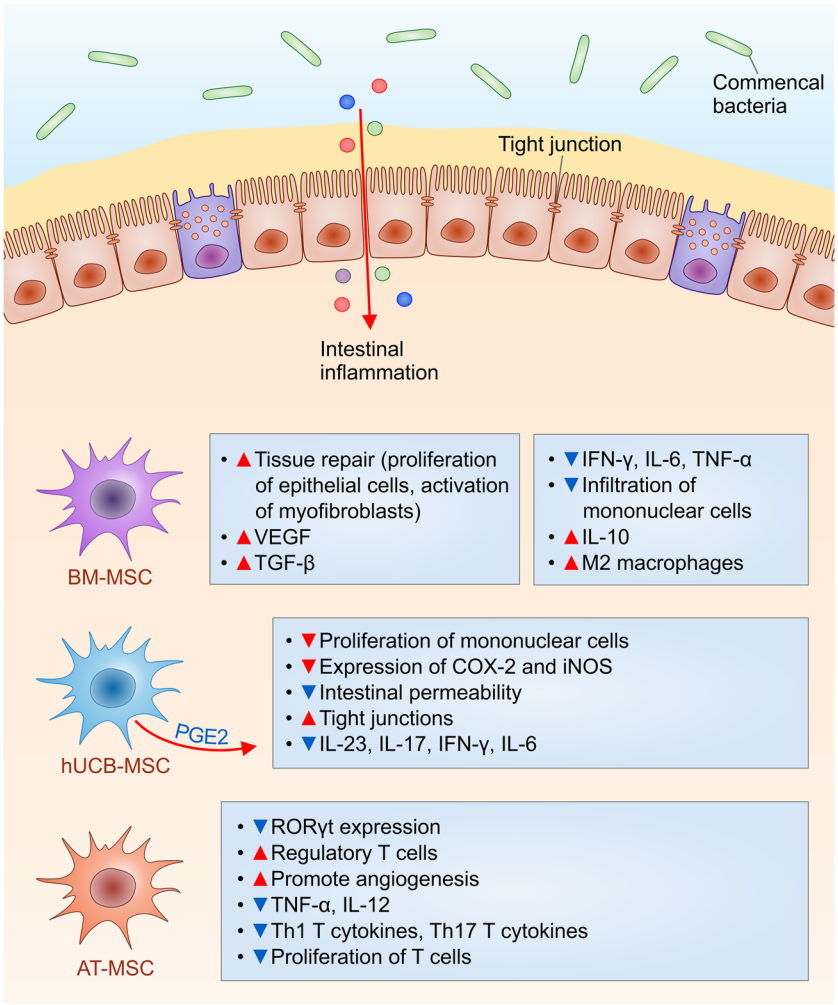

Fig. 3 Molecular mechanisms responsible for MSCs-mediated modulation of IBDs. Similar, but slightly different mechanisms are responsible for therapeutic effects of BM-MSCs, UCB-MSCs and AT-MSCs in modulation of IBDs. BM-MSCs regenerate epithelial cells by promoting vasculogenesis in VEGF dependent manner. Moreover, they are capable to reduce proinflammatory cytokines (IFN-γ, IL6, and TNF-α), attenuate infiltration of mononuclear cells in injured colons and promote the expansion of alternatively activated IL-10 producing M2 macrophages. hUCB-MSC-derived PGE2 is the main factor in reducing the inflammation locally (in colon tissue), whereas systemic immune suppression was mediated by the attenuation of Th1/Th17 immune response. hUCB-MSCs markedly decreased the expression of COX-2 and iNOS in the injured colons. The levels of Th1 cytokine (IFN-γ) and Th17 cytokines (IL-17 and IL-23) are decreased after administration of hUCB-MSCs. Administration of hUCB-MSCs decrease intestinal permeability and restored the expression of tight junction proteins enhancing defensive mechanisms of epithelium. AT-MSCs modulate immune response in colon by affecting conversion of Th17 and Th1 cells into IL-10 producing regulatory phenotype and by promoting angiogenesis and colon regeneration. AT-MSCs down-regulate RORγt expression and decrease production of Th1 and Th17 cytokines in colon infiltrating T lymphocytes

involved in maintaining epithelial barrier of the gut (claudin-2,-12,-15) which resulted with enhanced regeneration of damaged epithelium [45]. In animal model of (2,4,6-Trinitrobenzenesulfonic acid solution, TNBS)-induced colitis, intravenous application of murine BM-MSCs significantly increased proliferation of intestinal epithelial cells and promoted differentiation of intestinal stem cells towards epithelial cells, indicating that MSCs may promote epithelialization of the injured gut through the cross-talk with intestinal stem cells [46]. MSC-mediated self-renewal of gut epithelium and restoration of epithelial barrier prevents invasion of intestinal bacteria into subepithelial tissue and consequent progression of colitis. It is well known that microbiota regulates maturation and activation of colon infiltrating immune cells, playing crucial role in the maintenance of intestinal homeostasis and tolerance [47]. Accordingly, it was recently shown that normal microbiota is required for the therapeutic effects of BM-MSCs in DSS-induced colitis. BM-MSCs derived from germ free (GF) mice did not manage to attenuate DSS-induced colitis and had reduced

capacity to induce apoptosis of T cells [48]. Importantly, colonization of GF mice with specific-pathogen-free (conventionalized) microbiota completely restored immunosuppressive potential of BM-MSCs, indicating the importance of intestinal microbiota for MSC-based modulation of immune response in the gut [48].

MSC-mediated modulation of angiogenesis can significantly contribute to the regeneration of gut epithelium, as well. By using DSS-induced colitis, Khalil and co-workers demonstrated that murine CD34-negative BM-derived stem cells engrafted in the damaged colons, differentiated into ECs and promoted neo-vasculogenesis in a paracrine manner which leads to the regeneration of gut epithelium [37]. Similar as in DSS-induced colitis, MSCs promote angiogenesis and regenerate colon epithelium in TNBS-induced colitis, as well [38]. BM-MSCs were shown as an important source of VEGF in colon tissue and some of engrafted MSCs differentiated into ECs and interstitial lineage cells significantly contributing to the healing process.

In addition to regenerative mechanisms, BM-MSCs affect phenotype and function of colon-infiltrating macrophages, modulating early phase of gut inflammation.

MSCs produce Galectin-3 (Gal-3), a protein that is important for proliferation, adhesion, and migration of immune cells [49]. We recently showed that pharmacological inhibition of Gal-3 enhances capacity of BM-MSCs to promote alternative activation of macrophages in vitro and in vivo, increased production of IL-10 in colon-infiltrating macrophages, that resulted with elevated serum levels of IL-10 and attenuation of DSS-induced colitis [43].

The application of BM-MSC-derived tumor necrosis factor-induced protein 6 significantly decreased serum levels of inflammatory cytokines (IFN-γ, IL6, and TNF-α), reduced infiltration of leukocytes in injured colons and promoted the expansion of alternatively activated IL-10 producing M2 macrophages that created immuno-tolerant microenvironment in the gut and resulted with attenuation of colitis [50].

UCB-MSC-Dependent Attenuation of IBDs

Therapeutic effects of UCB-MSCs in IBDs are mainly the consequence of UCB-MSC-dependent modulation of adaptive immunity (suppression of T and B lymphocytes) [42, 51–53].

Human UCB-MSCs (hUCB-MSCs) attenuate colitis in mice by modulating inflammation in PGE2-dependent manner [42, 51, 52]. Engrafted hUCB-MSCs markedly decreased the expression of cyclooxygenase 2 (COX-2) and inducible nitric oxide synthase (iNOS) locally, in the injured colon tissue, and suppress systemic Th1/Th17 immune response. Significantly lower levels of Th1 cytokine (IFN-γ) and Th17 cytokines (IL-17 and IL-23) were noticed in the sera of the hUCB-MSC-treated mice [42].

UCB-MSCs may promote proliferation and immunosuppressive characteristics of peritoneal CD5 + regulatory B cells [53]. Upon co-culture with hUCB-MSCs, capacity of CD5 + B regulatory cells to produce IL-10 and inhibit proliferation and activation of T cells is increased. Moreover, the adaptive transfer of hUCB-MSCs-primed CD5 + B regulatory cells managed to significantly attenuate colitis in mice in a similar manner that was achieved after transplantation of hUCB-MSCs [53].

In addition to immuno-modulation, transplantation of hUCB-MSCs significantly decreased intestinal permeability and restored the expression of tight junction proteins (occludin, claudin-1 and zona occludens protein-1) enhancing defensive mechanisms of epithelium, as well [52].

Molecular Mechanisms Responsible for AT-MSCs-Mediated Modulation of IBDs

AT-MSCs modulate immune response in colon by affecting conversion of Th17 and Th1 cells into IL-10-producing regulatory phenotype and by promoting angiogenesis and colon regeneration.

Transplantation of AT-MSCs attenuates Th17 immune response in miR-1236-dependent manner, resulting with significant amelioration of TNBS-induced colitis [54]. Zhang and co-workers showed that miR-1236 binding to the 3′- untranslated region of ROR-γ resulted in inhibition of ROR-γ, transcriptional factor responsible for differentiation of naive CD4 + T lymphocytes in Th17 cells [54].

It seems that AT-MSCs stimulate expansion of IL-10-producing regulatory T cells by promoting conversion of Th17 cells into FoxP3 + regulatory phenotype, resulting with an increase of Treg/Th17 cell ratio and suppression of colon inflammation [54]. IL-10 and TGF-β -producing T regulatory cells maintain intestinal homeostasis by suppressing Th1 and Th17 immune response to resident commensal microbes [55, 56]. In vivo depletion of IL-10 or Tregs partially reversed the beneficial action of AT-MSCs, demonstrating the importance of AT-MSCs: Tregs cross-talk in MSC-mediated attenuation of colon inflammation [57].

Similar as described in UC, AT-MSCs treatment significantly attenuate CD in mice as demonstrated by increased survival rate and reduced histopathologic changes. AT-MSCs promote angiogenesis in VEGF-dependent manner and down-regulate production of inflammatory TNF-α and pro-Th1 cytokine (IL-12) in antigen presenting cells that significantly attenuated polarization of naive CD4 + T lymphocytes into Th1 cells. Additionally, transplantation of AT-MSCs significantly increased presence of immunosuppressive IL-10 in gastrointestinal tract leading to the suppression of immune response and attenuation of CD [58].

It seems that engrafted AT-MSCs are also able to directly, without affecting function of DCs, inhibit production of

inflammatory cytokines in T cells and to reduce their proliferation. These inhibitory effects were partially reversed when T cells and AT-MSCs were separated by a semi-permeable transwell membrane, suggesting partial cell–cell contact dependence for this effect of AT-MSCs [41].

IFN-γ-Mediated Priming: Method for Obtaining Optimal Immune-Suppressive Characteristics of MSCs

Both mouse and human MSCs need priming to obtain their optimal immuno-suppressive characteristics [51]. This is usually achieved by IFN-γ, combined with inflammatory cytokines: TNF-α, IL-1α, or IL-1β [59, 60]. IFN-γ-primed MSCs (IMSCs), but not IFN-γ non-primed MSCs, managed to completely attenuate colitis in mice [40]. Survival rate, IBD clinical score and weight gain were significantly improved in IMSCs-treated mice compared with mice that received IFN-γ non-primed MSCs. IMSCs inhibit Th1 inflammatory response in colon by reducing proliferation, activation and production of IFN-γ in Th1 cells that was followed by attenuated activation of colon infiltrated macrophages.

These results indicate that in vivo efficacy of transplanted MSCs may depend on their priming by pro-inflammatory cytokines which are produced during the early phase of gut inflammation [61]. In line with these findings is observation that AT-MSCs increase survival rate and weight gain and attenuate colon inflammation in DSS-induced colitis when injected 2 days after the onset of colitis, but were non-effective when injected 1 day before colitis induction, when pro-inflammatory cytokines were present at low levels [41].

Clinical Trials Using Different Types of MSCs

Currently there are two routes for the administration of MSCs in IBDs patients: the local administration as a therapeutic approach for patients with perianal fistulazing CD and systemic (intravenous) administration for the systemic control of intestinal inflammation in luminal CD and UC.

Local Administration of MSCs

Administration of autologous or allogeneic BM-MSCs and AT-MSCs achieved significant clinical efficacy in patients with fistulazing CD by down-regulating local immune response and inducing wound healing (Table 2) [62–71].

BM-MSCs Local administration of **autologous BM-MSCs** showed promising effects in the therapy of fistulazing CD [62]. Ten patients with fistulazing CD that were refractory to or unsuitable for current available therapies received four

Table 2 Clinical trials of MSCs in inflammatory bowel disease therapies

Disease	Phase	Number of patients	Stem cell source	Dosage	Route	Outcome
Crohn's fistula [63]	I	10	BM-MSCs (auto-BM)	2–5 monthly injections of $15–30\times10^6$	intrafistula	improved
Perianal fistulizing Crohn's disease [64]	IIa	21	BM-MSCs (allo-BM)	10, 30 or 90×10^6	intrafistula	improved
Crohn's fistula [66]	I	4	AT-MSCs (auto-adipose)	$3–30\times10^6$	intrafistula	improved
Complex perianal fistula [67]	II	14	AT-MSCs (auto-adipose)	20×10^6 (with fibrin glue or placebo) repeated with 40×10^6 if incomplete closure at week 8	intrafistula	improved
Complex cryptoglandular perianal fistula [68]	III	200	AT-MSCs (auto-adipose)	20×10^6	intrafistula	improved
Crohn's fistula [69]	II	43	AT-MSCs (auto-adipose)	$30–60\times10^6$/cm, with fibrin glue, repeated with 1.5 times more cells if incomplete closure at week 8	intralesional	improved
Crohn's fistula [70]	I	10	AT-MSCs (auto-adipose)	10, 20 or 40×10^6/ml in proportion to the size of the fistula	intrafistula	improved
Complex perianal fistula [71]	I/II	24	AT-MSCs (allo-adipose)	20×10^6 40×10^6 At week 12 if incomplete closure	intralesional	improved
Complex perianal fistulas [71]	III	212	AT-MSCs (allo-adipose)	120×10^6	intralesional	improved

local (intra-fistular) injections of 20×10^6 autologous BM-MSCs, at 4-week intervals [62]. BM-MSC-based therapy managed to heal rectal mucosa in all patients, improved both Crohn's disease activity index (CDAI) and perianal disease activity index (PDAI). Fistulas were totally closed in 70% of patients without any side events [62]. The effectiveness of **allogeneic BM-MSC-based therapy** of refractory perianal fistulazing CD was assessed in the phase IIa trial, in which complete healing was noticed in 7 of 15 patients, 12 weeks after injection of BM-MSCs into the fistula's wall [63]. Patients received injections of 10, 30 or 90×10^6 BM-MSCs or placebo. Interestingly, better results were noticed in the 10×10^6 and 30×10^6 BM-MSCs-treated groups compared with the 90×10^6 BM-MSCs-treated group, indicating that dose of transplanted BM-MSCs did not directly correlate with their effects in the treatment of fistulazing CD [62].

AT-MSCs The benefits of **autologous AT-MSCs-based therapy** for treatment of fistulas in CD patients were confirmed by several studies [64–69]. Garcia and co-workers reported successful healing of recto-vaginal fistula in a patient with CD that received autologous AT-MSCs [64]. Garcia-Olmo and co-workers demonstrated that single intra-fistular injection of $3–30\times10^6$ autologous AT-MSC completely healed fistulas in 75% of CD patients 8 weeks after AT-MSC transplantation without any observed adverse events during 22-month follow up [65]. Therapeutic potential of AT-MSCs was confirmed in the phase II clinical trial, sponsored by Cellerix, where 14 patients with fistulizing CD were successfully treated by local application of autologous AT-MSCs [66]. In another clinical study that recruited 200 patients, 20×10^6 AT-MSCs as well as combination of 20×10^6 AT-MSCs and fibrin glue showed no serious adverse effects and achieved healing rates of more than 50% at 1-year follow-up [67]. These findings were confirmed in studies conducted by Lee and colleagues and Cho and co-workers [68, 69], demonstrating the tolerability, safeness, and effectiveness of autologous AT-MSCs for the healing of fistulazing CD. In study conducted by Cho et al., autologous AT-MSCs (10×10^6, 20 or 40×10^6 cells) were injected in perianal fistulas of 10 patients suffering from CD [69]. Eight months follow up revealed complete healing in 30% of patients, while partial closure with no drainage was observed in all other AT-MSC treated patients [69]. In Phase II trial, conducted by Lee et al. [68] the number of autologous AT-MSCs that were transplanted in fistulas of 43 patients was proportionate to the size of the fistulas (30×10^6 or 60×10^6 cells). Injection of AT-MSCs, with 1.5 times more cells, was repeated 8 weeks after first application if fistula closure was not complete. Complete fistula healing was observed in 82% patients without reported side effects [68].

Similar as transplantation of autologous AT-MSCs, intra-fistular injection of **allogeneic AT-MSCs** did not cause any adverse events in 24 patients with fistulazing CD [70]. Significantly reduced number of draining fistulas was noticed in almost 70% of patients while complete closure of fistulas was observed in more than 50% of AT-MSCs-treated patients, 24 weeks after their application [70]. Recently published phase III clinical study revealed that 120×10^6 allogenic AT-MSCs, injected in tissue adjacent to fistulas tracts and openings, significantly improved PDAI and effectiveness of surgical closure of fistulas. Application of allogeneic AT-MSCs was well tolerated since adverse events related to injection of AT-MSCs were observed in only 17.5% patients [71].

Accordingly, from the results obtained in all these clinical trials [62–71] it can be concluded that local application of autologous or allogeneic BM-MSCs and AT-MSCs is safe and effective therapeutic approach for the treatment of perianal fistulas in CD patients.

Systemic Administration of MSCs

Effects of systemic application of autologous or allogeneic MSCs have been estimated in clinical studies in which MSCs were intravenously injected in patients suffering from luminal CD or UC. Only two trials, with a small number of recruited patients, investigated effects of intravenously injected autologous MSCs, while systemic application of allogeneic MSCs has been evaluated in a significant number of large clinical trials.

Autologous BM-MSCs Therapy Systemic administration of autologous BM-MSCs appears to be a feasible procedure for the therapy of refractory CD since no severe side effects were noticed during isolation and application of BM-MSCs [72]. In a study reported by Duijvestein and co-workers, nine patients intravenously received two doses of $1–2\times10^6$ BM-MSCs/kg body weights and the only observed adverse effect was a mild allergic reaction that was noticed in one patient, which probably happened due to the cryopreservant Dimethyl sulfoxide [72]. However, the therapeutic effects of BM-MSCs were not promising since three patients showed good clinical response, while three patients needed surgical intervention due to disease worsening [72].

Similar results were noticed in another clinical trial in which twelve CD patients intravenously received autologous 2×10^6, 5×10^6 or 10×10^6 BM-MSCs//kg body weights [73]. Beneficent effects and attenuation of CD was noticed in five patients, disease worsening was observed in five patients and two BM-MSC treated patients had severe adverse events linked with the MSCs injection.

Interestingly, BM-MSCs obtained from patients with CD showed morphology, phenotype, doubling time and immunosuppressive characteristic analogous to BM-MSCs

isolated from healthy controls [72, 73]. Moreover, functional characteristics of BM-MSCs were not different among the patients, indicating that the microenvironment of the gut in which BM-MSCs were engrafted after intravenous injection had a crucial role in polarizing BM-MSCs towards pro- or anti-inflammatory phenotype which resulted with attenuation or progression of CD [73]. This could be explained by the fact that MSCs either suppress or promote inflammation according to the inflammatory milieu to which they are subjected [61]. When MSCs are transplanted in the tissue with high levels of inflammatory cytokines, MSCs develop an immuno-suppressive phenotype and modify maturation of DCs, promote conversion of macrophages in anti-inflammatory M2 phenotype and suppress generation, activation and expansion of T lymphocytes, natural killer (NK) and natural killer T (NKT) cells. When MSCs are engrafted in the microenvironment with low levels of inflammatory mediators, they obtain pro-inflammatory phenotype, produce large amounts of pro-inflammatory cytokines and chemokines that stimulate activation and migration of neutrophils and T cells and increase inflammation [61].

Allogeneic BM-MSCs Therapy As reported by Onken and co-workers [74], an intravenous injection of 2×10^6 or 8×10^6 allogeneic BM-MSCs/kg body weight managed to increase quality of life and to decrease CDAI in all of nine patients with moderate to severe CD that received BM-MSCs. Total clinical remission was observed in one patient while adverse events were noticed in five patients [74]. Results obtained by Liang et al., during the 6-month follow up period, showed that combination of standard therapy and single intravenous injection of 1×10^6/kg allogeneic BM-MSCs induce complete remission in five of eight BM-MSC-treated patients, reduced CDAI in all patients without reported serious adverse effects. Significantly reduced number of lamina propria-infiltrated lymphocytes and attenuated inflammation in the gut has been observed in standard therapy + BM-MSCs-treated patients compared to patients that received only standard therapy, indicating that BM-MSCs enhanced immunosuppressive effects of standard therapy [75].

Another multicenter, phase II clinical study included 16 patients with active luminal CD who intravenously received 2×10^6 allogeneic BM-MSCs/kg body-weights. BM-MSCs were administered for 4 weeks (one infusion per week) [76]. After each MSCs infusion, an improvement in mean quality of life scores was observed. Endoscopic examination revealed improvement in 7 of 15 BM-MSC-treated patients, complete clinical remission was observed in eight patients and CDAI was reduced in 12 patients. Two dysplasia-associated lesions were noticed in one patient, but, as concluded by clinicians, this probably was not caused by BM-MSCs [76].

Discouraging results were obtained in clinical trial conducted by Pfizer which investigated therapeutic potential of allogeneic stem cells obtained from adult BM and non-embryonic tissue sources since no clinical benefit was seen in patients with moderate to severe UC that received these cells [77].

Clinical trial that recruited 270 patients with active form of CD who did not respond to standard therapy was initiated by Osiris Therapeutics 10 years ago. In this randomized study, patients received total number of 600×10^6 or 1200×10^6 allogenic BM-MSCs (2×2 infusions, 2 weeks apart) or placebo. As estimated, results of this study will be published during 2018 [78].

Since exogenous application of MSCs may have detrimental effects [72–74], possible therapeutic use of endogenous, circulating stem cells could be further tested as new therapeutic approach for the treatment of IBDs. Most recently, Marlicz et al. [79] and Boltin and co-workers [80] reported that significant number of stem cells circulated in peripheral blood of patients suffering from active CD. Although it is still unknown whether this phenomenon reflects an intrinsic mechanism for regenerating intestine, these findings reveal the possibility about the use of these endogenous stem cells for the treatment of IBDs.

Challenges Towards Clinical Use of MSCs in the Therapy of IBDs

Although MSCs are currently used in clinical trials, there are still several challenges that should be addressed with aim to improve their therapeutic potential in the therapy of IBDs.

First, immuno-suppressive characteristics of MSCs should be thoroughly analyzed and considered in future clinical trials in order to avoid potential undesirable interactions with the immuno-modulatory drugs that are used as standard therapy in IBD treatment. In line with these observations are findings recently reported by Lindsay and co-workers [81] showing increased number of serious adverse events in patients who received immunosuppressive drugs just before stem cell treatment. Majority of patients who received cyclophosphamide and stem cells within in a short time frame developed infection and reported respiratory and gastrointestinal symptoms [81].

Second, optimal number of transplanted MSCs and route of their application should be clearly defined with aim to find the right balance between safeness and effectiveness of MSC-based therapy. Interestingly, MSC-dependent effects do not strictly correlate with their dosage. As reported by Molendijk and co-workers [63], better improvement in IBD clinical scores were noticed in patients that received 30×10^6 MSCs when compared to patients that received 90×10^6 MSCs.

Additionally, possible malignant transformation of transplanted MSCs as well as their potential to induce

neo-vascularization that may promote tumor growth and metastasis are still major concerns regarding safety of MSC-based therapy [82] and should be further analyzed in ongoing and future clinical trials.

Finally, since there are conflicting results regarding safety and efficacy of intravenously injected MSCs, protocols describing their isolation and application should be uniformed in a way to increase reproducibility and consistency of data obtained in clinical trials.

Conclusions

Because of their differentiation potential, as well as due to their pro-angiogenic and immuno-modulatory properties, MSCs are considered as new therapeutic agents for the treatment of IBDs. Results obtained in a large number of clinical trials suggest that local application of autologous as well as allogeneic BM-MSCs or AT-MSCs is a safe and beneficial therapeutic approach for the healing of perianal fistulas in CD patients. Safety of MSC-based therapy, after systemic application of MSCs, still need to be explored since several clinical trials reported aggravation of CD or UC in patients that intravenously received BM-MSCs. To address this concern, the optimal origin, number and routes of MSC application, should be defined. Future clinical studies must be focused on resolving these issues with aim to utterly exploit the promising therapeutic potential of MSCs in the treatment of IBD.

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Compliance with Ethical Standards

Conflict of Interest The authors declare no potential conflicts of interest.

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