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Mini Review

Mesenchymal stem cells for the treatment of inflammatory bowel disease: from experimental models to clinical application

Rhian Stavely, Samy Sakkal, Vanesa Stojanovska and Kulmira Nurgali *

College of Health and Biomedicine, Victoria University, Western Centre for Health Research & Education, VIC, Australia

Inflammatory Bowel Disease (IBD) is a highly debilitating and potentially fatal idiopathic disorder of the intestinal tract which is exceedingly prevalent in westernized society; however there is concern of an IBD epidemic in Asia due to increasing incidence rates. There is no cure for IBD with current treatments limited by their inefficacy, toxicity and adverse side-effects; thus necessitating the search for novel therapies. In the past decade mesenchymal stem cells (MSCs) have become attractive candidates for the cellular based therapy of IBD. MSCs are easily isolated and expanded from adult bone-marrow and adipose tissue; they possess unique therapeutic characteristics including the ability to home to sites of tissue damage and inflammation, facilitate tissue repair and modulate the immune system. The administration of MSCs in animal models of experimental colitis and clinical trials of fistulising and luminal Crohn's disease have yielded promising results, however an unequivocal therapeutic mechanism remains elusive. This review will explore the clinical application of MSCs in IBD and current evidence from experimental models of colitis elucidating their potential to ameliorate intestinal inflammation.

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*Correspondence should be addressed to:
Kulmira Nurgali, College of Health and Biomedicine, Victoria University, Western Centre for Health Research & Education, 176 Furlong Road, St Albans, 3021, VIC, Australia. Phone: +61-3-8395-8223, E-mail: kulmira.nurgali@vu.edu.au

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Introduction

Inflammatory Bowel Disease (IBD) is comprised of two main pathologies, Crohn's disease (CD) and ulcerative colitis (UC), which are characterised by the presentation of recurrent idiopathic intestinal inflammation. In UC inflammation is localised in the mucosa ascending continuously from the rectum to the colon. Conversely, inflammation in CD is transmural and manifests discontinuously

in skip lesions throughout the gastrointestinal tract with formation of granulomas[1].

All phenotypes of IBD greatly affect quality of life with symptoms including ulcerations, fistulae, strictures, perianal fissures, bloody stool, persistent diarrhea or constipation, abdominal pain and cramps[2]. Potential complications in IBD such as perforation, excessive bleeding from ulcerations, obstruction of the bowel and intestinal scarring resulting in malnutrition can lead to fatality. Furthermore, the risk of developing cancers including colorectal cancer[3] and lymphoma[4] are increased in IBD resulting in an indirect escalation in the mortality rate. Although IBD is predominantly a disease of westernized society, dramatic increases in the incidence of IBD have been observed throughout Asia[5] which may reach epidemic proportions[6].

The cause of IBD is unknown but concordant twin studies have revealed that the development of IBD is likely to require a multi-genetic predisposition and an environmental perturbation[7]. Numerous predisposing genes for CD and UC have been uncovered with around 30% of loci overlapping for both diseases suggesting similarities in pathological mechanisms[8]. Although the pathogenesis of IBD is unknown, it is predicted that antigens of commensal bacteria in the gut instigate an exaggerated immune response[9]. The role of epithelial permeability and leukocyte dysregulation in the exuberant antigen response is currently under investigation[10, 11].

Current treatment strategies do not provide a cure and are limited by their inefficacy, toxicity and adverse side-effects[12-14]; thus necessitating the search for novel therapies. One of the most promising treatments currently being investigated is mesenchymal stem cell (MSC) therapy. MSCs are easily isolated and expansively cultured from adult adipose tissue and bone marrow[15]. Furthermore, MSCs possess many unique properties making them an ideal candidate therapy for IBD. MSCs are immune evasive and can be transplanted between individuals and across species[16, 17]. Once administered, MSCs migrate through chemotaxis towards sites of inflammation; thus specifically targeting pathological manifestations[18]. After homing to the site of inflammation, MSCs facilitate tissue regeneration through secretion of pro-angiogenic and trophic factors which have been shown to promote endogenous repair mechanisms[19-21]. Moreover, MSCs are immunomodulatory and secrete anti-inflammatory factors suppressing the immune response and inflammation[22]. The clinical application of MSCs in CD and evidence for the possible mechanisms elucidating their potential to regenerate intestinal epithelium and reduce inflammation in experimental models of colitis will be reviewed.

Efficacy of MSCs in Clinical Trials

Clinical trials using MSCs for the treatment of CD fistulae and luminal inflammation have demonstrated that MSC therapy in IBD is both efficacious and feasible (summarised in Table 1). Predominantly, clinical trials of MSC therapy have focused on the treatment of fistulae caused by CD rather than CD manifestations as a whole. Primarily MSCs derived from adipose tissue (AT-MSCs) have been used for the treatment of fistulising CD and have resulted in the complete re-epithelialisation of rectovaginal, enterocutaneous and complex perianal fistulae in the majority of subjects. Fibrin glue was regularly used in conjunction with local MSC administration in fistulising CD, however evidence of a therapeutic benefit is not likely to be a result of fibrin glue alone[23, 24]. One clinical trial demonstrated that *in vitro* expansion is likely to be essential in harnessing the therapeutic potential of AT-MSCs rather than treating patients with the primary stromal vascular fraction of lipoaspirate[25]. The therapeutic outcome of MSC therapy in fistulising CD may be dose-dependent with greater efficacy achieved by doses of $2x10^7$ or $4x10^7$ MSCs/ml compared to $1x10^7$ MSCs/ml[26]. Long-term effects have been reported with CD and perianal activity index scores declining 12 months post treatment[27]. Furthermore, sustained closure of fistulae has been achieved in 88-100% of subjects 8-12 months after a course of MSC therapy[26, 28]; however these effects are relatively transient given that only 58% of subjects maintain closure after 3 years[29]. This suggests that repeated treatment may be required to maintain the therapeutic benefits of MSC therapy.

While autologous and allogeneic AT-MSCs have both demonstrated efficacy in the healing of fistulae, further evidence is required to determine long-term immune tolerance in patients with repeated allogeneic MSC exposure. Bacterial contamination has posed a problem in the expansion of autologous MSCs in the past causing delay in treatment[24, 30]. If allogeneic MSCs are determined to be equally efficacious, pre-prepared sources for treatment could prevent such setbacks.

One clinical trial has determined that MSCs were as effective in treating fistulae of cryptoglandular origin as they were for fistulae resultant of CD[23]. This result supports the view that the therapeutic value of MSCs can be attributed to

Table 1 Clinical trials of mesenchymal stem cell therapy in Crohn's disease

MSC			Targeted Pathology	Therapeutic Outcome	References
Homology	Source	Administration			
Autologous	AT	Injection into istulous tract wall or rectal mucosa	Treatment of fistulae	• Complete re-epithelialisation in 6/8 various types of fistulae 8 weeks after treatment	[30]
Autologous	AT	Injection into fistulous tract wall and sealed with fibrin glue	Treatment of complex perianal fistulae	• Fistula closure in 5/7 subjects treated with AT-MSCs and fibrin glue • Positive response to fibrin glue alone in 1/7 subjects	[23]
Autologous	AT	Injection into fistulous tract wall and sealed with cells suspended in fibrin glue	Treatment of enterocutaneous fistulae	• Complete healing with re-epithelialisation of the fistula opening in 3/4 treated subjects. Healing of the fistula with the stromal vascular fraction in 1/4 treated subjects	[25]
Autologous	AT	Injection into fistulous tract wall and sealed with cells suspended in fibrin glue	Treatment of fistulae	• Complete closure of various fistulae in 27/33 subjects administered with autologous AT-MSCs and fibrin glue after 8 weeks • Sustained closure in a one year follow up in 23/26 patients with previously healed fistulae	[28]
Autologous	AT	Injection into fistulous tract wall and mucosa of the opening and sealed with fibrin glue	Treatment of fistulae	• Partial closure in 3/3 treated subjects with $1x10^7$ • Complete healing in 2/3 treated subjects with $2x10^7$ MSCs/ml at week 8 after injection • Complete healing in 1/3 treated subjects with $4x10^7$ MSCs/ml • No reoccurrence 8 months after injection in 3/3 subjects with complete healing at 8 weeks	[26]
Autologous	BM	Injection into fistulous tract wall and lumen	Treatment of fistulae and disease activity index	• Closure of fistulae in 7/10 subjects • Statistically significant decrease in Crohn's disease and perianal activity indexes • Significant increase in mucosal and peripheral Tregs • Tregs remained significantly elevated at 1 year follow up	[27]
Allogeneic	AT	Injection into fistulous tract wall	Treatment of complex perianal fistulae	• Complete closure of the treated complex perianal fistula in 9/16 subjects after 24 weeks	[76]
Autologous Allogeneic	AT	Injection into fistulous tract wall	Treatment of rectovaginal fistulae	• Complete healing of rectovaginal fistulae in 3/4 women treated with AT-MSCs and fibrin glue • No healing in subjects treated with fibrin glue only • Only 4/8 women had fistulae resultant of Crohn's disease	[24]
Autologous	BM	Intravenous Injection	Refractory luminal Crohn's disease	• Positive clinical response in 3/9 subjects after 6 weeks • Worsening of disease in 3/9 subjects • Reduced endoscopic severity in 2/9 subjects • Reduction in $CD4^+$ T-cells	[32]
Allogeneic	BM	Intravenous Injection	Refractory luminal Crohn's disease	• Positive clinical response in 12/15 subjects after 4 weeks • Clinical remission achieved in 8/15 subjects after 4 weeks • Endoscopic improvement in 7/15 subjects	[33]

MSC, mesenchymal stem cell; AT, adipose tissue; BM, bone marrow; regulatory T-cell, Treg.

the release of trophic factors or the capability of MSCs to promote tissue regeneration via differentiation into stromal cells, at least specifically in the treatment of fistulae[30]. The cellular phenotype of MSCs post administration is poorly understood, however, differentiation into extraneous cell types has been rarely reported. Evidence from an *in vitro* study demonstrates that MSC differentiation into a myofibroblastic-like phenotype is subsequently reversible rather than committing, therefore MSCs are likely to possess a dynamic phenotype which is dependent on the *in vivo* signalling milieu[31].

A clinical trial demonstrating the immunosuppressive effect of autologous bone marrow-derived MSCs (BM-MSCs) has been successful in the treatment of fistulising CD[27]. This study demonstrated that BM-MSC treatment of CD fistulae increased the proportion of mucosal and peripheral regulatory T-cells (Tregs) which remained significantly elevated after 12 months. Therapeutic effects of MSCs on luminal CD, rather than treatment of fistulae, have been assessed in two clinical trials[32, 33]. In a study

conducted by Duijvestein et al.[32] intravenous administration of BM-MSCs yielded equivocal results with a third of subjects demonstrating a clinical response and another third undergoing surgery due to worsening of the disease. Endoscopy revealed a reduction in mucosal inflammation in only 2/9 subjects, however biopsies of the inflamed mucosa revealed a decrease in $CD4^+$ T-cells. This suggests that MSCs suppressed the adaptive immune response; however subjects in this study suffered from refractory CD and were unresponsive to conventional treatment which may explain the poor results of the trial. Recently, Forbes *et al*.[33] successfully achieved clinical remission and endoscopic improvement in Crohn's colitis and ileocolitis in over half of subjects intravenously administered with allogeneic BM-MSCs. These favourable results indicate that MSCs could be a viable therapeutic option for intestinal inflammation; however experimental models are required to explore the mechanisms underlying therapeutic efficacy and safety of MSC therapy.

Route of MSC administration

MSCs have been predominantly administered by local injection directly into the fistulae or surrounding tissues in clinical trials (Table 1). Despite the success of this method in fistulising CD, alternative administration routes that facilitate homing to multiple inflammatory sites may be required to treat pathological manifestations of IBD in its entirety. Systemic administration of MSCs was used in clinical trials treating luminal CD[32, 33] and is predominant in studies of experimental colitis (Table 2). Whilst systemic administration is relatively non-invasive and regularly used in MSC research, it may result in inefficient targeting of the pathological site in IBD. Although MSCs can migrate to the site of inflammation, some studies in experimental colitis models have reported that systemically injected MSCs can accumulate in the lungs[34, 35] however other studies have not observed this phenomenon[36-38]. Additionally, it is likely that MSCs can become sequestered in other various tissues connected to the circulatory system when systemically injected[39]. The high first pass effect and difficulty of homing may account for large quantities of MSCs used in clinical trials. Administering the supernatant of MSCs, also termed "conditioned media", via enema could be a feasible solution to effectively target the site of inflammation and eliminate the use of live cells[40]. Although, this method could potentially be safer and more efficacious, the invasiveness of the procedure and distance of pathological manifestations from the rectum in some cases of IBD could pose limitations.

Safety considerations

Despite favourable results of limited clinical trials there is no consensus regarding the long-term safety of MSC therapy. Some adverse events and hospitalizations were reported, however, these were thought to be unrelated to MSC therapy. An allergic reaction after treatment was reported however this was suggested to be a result of dimethyl sulfoxide used for cryopreservation[32]. Dysplastic lesions were discovered in a subject during endoscopy 42 days post initial MSC treatment[33]. The subject was subsequently diagnosed with sigmoidal adenocarcinoma. A sigmoid mucosal biopsy revealed low-grade dysplasia upon entry into the study therefore it is unlikely that MSCs transformed into the dysplastic tissue in this case. The contribution of MSCs to the development of cancer is contentious and warrants further investigation. The limited self renewal capacity of MSCs combined with data from clinical trials and experimental models predicate a low probability of tumour formation and malignancy[41]. However, MSCs have been implicated in the progression of cancer by enhancing tumour growth and metastasis[42-44]. Furthermore, sarcoma development has been observed after MSC administration in mice[45] with other studies reporting spontaneous transformation of MSCs *in vitro* after long-term culturing[46, 47]. Therefore, screening before administration of MSCs and *in vitro* quality control need to be considered in the future as preventive measures.

Proposed Therapeutic Mechanism of Mesenchymal Stem Cells in Colitis

While clinical trials have demonstrated the efficacy and safety of MSC therapy in fistulising CD, the mechanisms of the therapeutic effects of MSCs in IBD are less understood. Animal models are relied upon to assess the feasibility of MSC treatment in colitis and provide an insight into potential mechanisms of action underlying the successful attenuation of colitis (summarised in Table 2).

Epithelial Integrity

The intestinal epithelium creates a distinct barrier protecting underlying tissues from pathogens in the gut lumen. Restoration of epithelial integrity has been predicted to ameliorate the excessive immune response in IBD by preventing interaction with foreign antigens[10, 48]. The

Table 2 Mesenchymal stem cell treatment in experimental colitis models

Model	MSC homology	MSC tissue source	Administration	Major findings	
Rat (TNBS)	Allogeneic	BM (rat)	Intravenous injection	• MSC over-expressing CXCR4 ameliorated colitis	[77]
Rat (TNBS)	Allogeneic	BM (rat)	Submucosal injection	• ↓ Clinical and histopathological severity of colitis • ↓ Lesion size • Intravenously injected MSCs accumulated in lungs • Unexpanded bone marrow cells did not ameliorate colitis • MSCs differentiate into interstitial cells, fibroblast and myofibroblast *in vivo* • MSCs expressed VEGF and TGF-β1 *in vivo* through immunostaining	[34]
Rat (TNBS)	Allogeneic	AT (rat)	Submucosal injection	• ↓ Histopathological severity of colitis • MSCs secrete trophic factors *in vitro* • ↑ Proliferation of colonic epithelium • ↓ Neutrophil infiltration in the colon • MSCs selectively localized to inflamed ulcerated areas • Repaired colonic ulcers	[49]
Rat (TNBS)	Allogeneic	AT (rat) BM (rat)	Intraperitoneal injection Intravenous Injection	• Intravenously administered MSCs did not migrate to the colon • Intraperitoneally administered MSCs migrated to areas of colonic inflammation (submucosa and muscular layer) • AT-MSCs and BM-MSCs reduced the endoscopic and histopathological severity of colitis, collagen deposition, and epithelial apoptosis	[50]
Rat (DSS)	Allogeneic	BM (rat)	Intravenous injection	• ↓ Clinical and histopathological severity of colitis • ↓ Pro-inflammatory cytokines and trophic factors in the colon • MSC co-cultured monocytes activated by LPS ↓ monocytic TNF-α secretion • MSCs localized to inflamed tissue, predominantly the lamina propria and crypts	[36]
Rat (DSS)	Allogeneic	BM (rat)	Intravenous injection	• Dose-dependent therapeutic effect on body weight • Reduced epithelial injury • Prevented loss of mucin secreting cells • Myogenic lineage differentiation of MSCs *in vivo*	[52]
Rat (TNBS and DSS)	Allogeneic	Conditioned media	Enema	• Treatment with MSC supernatant/conditioned media • Enema ↓ clinical and histopathological severity of colitis • Intraperitoneal and intravenous injection had no therapeutic effect • ↓ Epithelial injury • Secreted pleiotropic gut trophic factors	[40]
Rat(DSS and DSS+BM hypoplasia)	Allogeneic	BM (Rat)	Intravenous injection	• Poor MSC migration to colon in DSS colitis • No therapeutic effect in DSS colitis • ↑ Tight junction proteins, claudin 2, 12 and 15 in DSS bone marrow hypoplasia model	[54]
Rat (acetic acid)	Allogeneic	BM (rat)	Intravenous injection	• ↓ Histopathological severity of colitis • ↑ Mucin secreting cells	[51]
Mouse (TNBS)	Allogeneic	BM (mouse)	Intravenous injection	• Green fluorescent protein labelled MSCs homed to the inflamed colon • ↓ Clinical and histopathological severity of colitis • ↓ Colonic expression of Th1 and Th17 related cytokines. • ↓ Colonic expression of Th1 and Th17 markers T-bet and RORγt • ↑ Colonic expression of Th2 and Treg related cytokines. • ↑ Colonic expression of Th2 and Treg marker GATA-3 and Foxp3 • ↑ Proliferation of intestinal epithelial cells • ↑ Differentiation of intestinal stem cells	[53]
Mouse (TNBS)	Xenogeneic	UC (human)	Intravenous injection	• ↓ Clinical and histopathological severity of colitis • ↓ Neutrophil infiltration in the colon • ↓ Pro-inflammatory cytokines in the colon • ↓ Th17 marker RORγt in the colon • Transwell MSC co-culture with LPMCs ↓ IFN-γ and ↓ IL-17 suggesting immune suppression of Th1 and Th17 in paracrine fashion	[35]
Mouse (TNBS)	Xenogeneic Allogeneic Syngeneic	AT (human) AT (mouse) AT (mouse)	Intravenous injection	• ↓ Clinical and histopathological severity of colitis • ↓ Pro-inflammatory cytokines ↑ IL-10 in the colon • ↓ TNF-α in LPMCs isolated from MSC treated mice • Co-cultures of MSCs or 'conditioned media' with colitic macrophages significantly ↓ TNF-α and IL-12 production • *Ex vivo* mesenteric lymph nodes co-cultured with MSCs or 'conditioned media' ↓ IL-2/ IFN- γ and ↑ IL-10 secreting T-cells • ↑ Treg in MLN of MSC treated colitic mice • Isolated T-cells from MSC treated mice ameliorate experimental colitis	[37]

Model	MSC homology	MSC tissue source	Administration	Major findings	
Mouse (DSS)	Allogeneic	BM (mouse)	Intravenous injection	• ↓ Clinical and histopathological severity of colitis • MSCs increased expression of phosphorylated TGF-βR1 and downstream target SMAD2 in colon • TGF-βR1 inhibition abrogated therapeutic effect of MSC • Mφ2 major source of TGF-β1 in colon	[56]
Mouse (DSS)	Allogeneic	BM (mouse)	Intravenous injection	• ↓ Clinical and histopathological severity of colitis • ↓ Pro-inflammatory cytokines in the colon	[38]
Mouse (DSS)	Xenogeneic	UC (human) BM (human)	Intraperitoneal injection	• UC-MSCs ameliorated DSS-induced colitis • UC-MSCs modulated Treg/Th17 cells in the spleen and mesenteric lymph nodes • UC-MSCs inhibited LPMCs *in vitro*	[78]
Mouse (DSS)	Xenogeneic	Gingiva (human) BM (human)	Intraperitoneal injection	• ↓ Clinical and histopathological severity of colitis • Suppress $CD4^+$ T lymphocyte infiltration • Promoted Treg infiltration • ↓ Pro-inflammatory cytokines in colon • ↑ Anti-inflammatory cytokines in colon	[58]
Mouse (DSS)	Xenogeneic Allogeneic Syngeneic	AT (Human) AT (mouse)	Intraperitoneal injection	• ↓ Clinical and histopathological severity of colitis • ↓ Neutrophil infiltration in the colon • ↓ Pro-inflammatory cytokines in the colon • ↓ Pro-inflammatory cytokine production of mononuclear cells *ex vivo* • MSC and monocytes or dendritic cell co-cultures progressively reduced T-cell proliferation and IFN-γ secretion suggesting APCs may have a significant role in further suppressing pro-inflammatory T-cells • IL-10 blockade partially reversed this effect • ↓ Production of IFN-γ, ↑ IL-10 and no effect on IL-4 in *ex vivo* stimulated MLNs • ↑ Treg in ex vivo MLN • Implantation of T-cells isolated after MSC treatment ameliorated colitis mediated by Tregs • Abolishment of IL-10 and Tregs *in vivo* negated therapeutic effect of MSCs	[63]
Mouse (TNBS and DSS)	Syngeneic	AT (mouse)	Intraperitoneal injection	• Mouse MSCs ↓ clinical and histopathological severity of colitis • Human MSC co-cultured with macrophages ↑ markers associated with regulatory macrophages • Human MSC induced Mφ2 ↑ TGF-β1, ↑ IL-10 and ↓ IL-12 • Human MSC stimulated macrophages ↓ splenocyte proliferation *in vitro* • Effect was diminished in IL-10 knockouts • Human MSC stimulated macrophages ↓ clinical and histopathological severity of colitis	[65]
Mouse (TNBS and DSS)	Xenogeneic	CB (human)	Intraperitoneal injection	• CB-MSCs reduced colitis severity • Higher anti-inflammatory properties in NOD2 stimulated CB-MSCs	[79]
Mouse (TNBS and DSS)	Allogeneic Xenogeneic	BM (mouse) BM (human)	Intraperitoneal injection	• IFN-γ stimulated MSCs • ↓ Clinical and histopathological severity of colitis	[80]

TNBS, 2,4,6-trinitrobenzenesulfonic acid; DSS, dextran sodium sulphate; AT, adipose tissue; BM, bone marrow; CB, cord blood; UC, umbilical cord; LPS, lipopolysaccharide; LPMC, lamina propria mononuclear cells; MLN, mesenteric lymph nodes; APC, antigen presenting cell.

attenuation of gross morphological damage through MSC treatment is regularly demonstrated in experimental colitis. Macroscopically, MSCs home to inflamed tissues, reduce ulceration and prevent fibrosis[34, 49, 50]. Histopathologically, MSCs prevent the loss and discontinuity of the surface columnar epithelial lining and derangement of the crypts[51]. A protective effect on intestinal mucin secreting cells has also been observed[51, 52]. It has been suggested that MSCs have a regenerative effect by promoting the proliferation of intestinal epithelial cells and the differentiation of intestinal stem cells[49, 53]. Moreover, it has been reported that MSCs stimulate endogenous mechanisms of intestinal epithelial repair[21]. MSC-conditioned medium decreases epithelial damage in colitis highlighting the significance of the MSC secretome[40]. Regeneration of intestinal epithelium has been attributed to the secretion of angiogenic and trophic factors including vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF) and adiponectin detected *in vitro* MSC culture[40, 49]. Reduction in the levels of VEGF and HGF has been reported in the inflamed colon after MSC administration[36], however MSCs have been observed to localize to the basal crypts[36] and express VEGF and

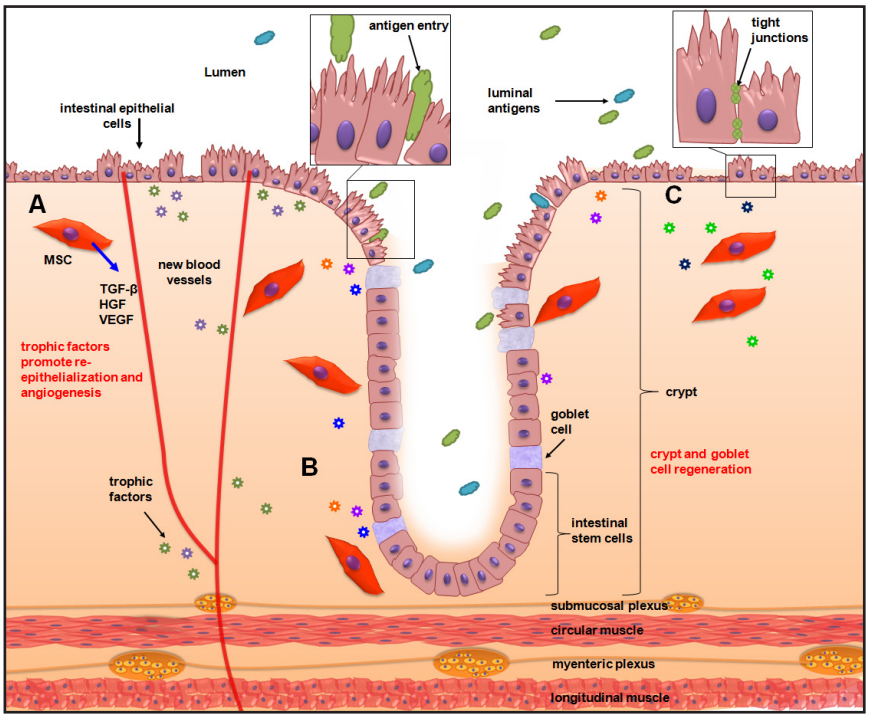

Fig.1 Effects of mesenchymal stem cells on epithelial integrity in intestinal inflammation

Mesenchymal stem cells (MSCs) facilitate the repair of the intestinal epithelial barrier via the (A) secretion of trophic factors including vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF) and transforming growth factor (TGF)-β which promote re-epithelialisation and angiogenesis. (B) MSCs home to the basal crypts where they induce endogenous repair mechanisms by promoting intestinal stem cell migration and differentiation and aid in the regeneration of mucin secreting goblet cells. (C) Additionally MSCs promote the expression of tight junction proteins between epithelial cells which may attenuate inflammation-induced permeability. The regeneration of intestinal epithelium and promotion of epithelial barrier integrity prevents luminal antigens/pathogens from invading into the mucosa and submucosa; thus averting pathogen-associated molecular pattern activation of innate leukocytes and antigen presentation to T-cells which elicit the inflammatory response.

transforming growth factor-β1 (TGF-β1) *in vivo*[34], therefore local paracrine signalling may still play a role. In addition to facilitating epithelial regeneration, MSCs promote the expression of tight junction proteins, claudin 2, 12 and 15[54], which may prevent inflammation-induced increase in epithelial permeability; and thus avert antigenic insult (Fig. 1).

Immunomodulation

The immunomodulatory properties of MSCs in models of experimental colitis have been well documented. MSCs reduce *in vivo* levels of pro-inflammatory cytokines and increase the production of anti-inflammatory cytokines in the gut and serum (Table 3). Conditioned medium has been shown to ameliorate the effects of colitis suggesting that the therapeutic value of MSCs is harnessed from secreted factors present in the secretome. TGF-β1 secreted by MSCs *in vitro*[55] is elevated in the intestinal homogenate after *in vivo* MSC administration[53]; inhibition of TGF-β1 signalling abrogates the therapeutic effect of MSC in experimental colitis[56]. Therefore TGF-β1 may be a common link between the therapeutic effect of conditioned medium and administered MSCs *in vivo*. Additionally, interleukin (IL)-10, which is theorized to be a target in the amelioration of enterocolitis[57], was elevated in the intestine and serum after MSC treatment of experimental colitis[37, 53, 58]. However, there is no evidence of IL-10 secretion by unstimulated MSCs in culture[59, 60]. It has been established that MSCs can be potentiated by tumour necrosis factor (TNF)-α, interferon (IFN)-γ and toll-like receptor (TLR) activation to induce an anti-inflammatory phenotype[61, 62]; therefore the possibility that the inflammatory microenvironment or the gut flora could upregulate anti-inflammatory factors including IL-10, indoleamine 2,3-dioxygenase (IDO) and prostaglandin E2 (PGE2) cannot be disregarded.

MSCs instigate leukocytes to mediate their immunomodulatory effects in gastrointestinal inflammation (Fig. 2). The innate immune system is critical in IBD pathology and mass neutrophil infiltration is utilized in CD diagnosis. In experimental colitis, MSCs prevent neutrophil invasion and thus damage from cytotoxic granules[35, 49, 63] therefore ameliorating a key pathological marker of CD. Other cellular components of the innate immune system such as monocytes and macrophages have been postulated to be responsive to MSC secreted factors; this is highly plausible given that macrophages are highly receptive to both pro and anti-inflammatory signals. MSCs decrease the secretion of pro-inflammatory cytokines TNF-α and IL-12 from monocytes and macrophages *in vitro*[36, 37]. Bain *et al*.[64] suggested that resident macrophages in

Table 3 Effects of MSC treatment on *in vivo* levels of cytokines, chemokines and trophic factors in experimental colitis

	Gut			Serum		
Factors	Effect	MSC source	References	Effect	MSC source	References
IL-10	↑ ↑ ↑ ↑ N.D.	AT (human) BM (human) Gingiva (human) BM (mouse) BM (human)	[37] [58] [58] [53] [80]	↑	BM (mouse)	[53]
IL-4	↑	BM (mouse)	[53]	↑	BM (mouse)	[53]
TGF-β1	↑	BM (mouse)	[53]			
IL-8	↓	AT (rat)	[49]			
TNF-α	↓ ↓ ↓ N.D.	AT (human) BM (rat) BM (mouse) BM (human)	[37] [36] [38, 53, 56] [80]	↓ ↓ ↓	AT (human) UC (human) BM (mouse)	[37] [35] [38, 53]
IL-6	↓ ↓ ↓ ↓ ↓ N.D.	AT (human) BM (human) UC (human) Gingiva (human) BM (mouse) BM (human)	[37] [58] [35] [58] [53, 56] [80]	↓ ↓	AT (human) BM (mouse)	[37] [53]
CCL5	↓	AT (human)	[37]			
MIP-2	↓	AT (human)	[37]	↓	AT (human)	[37]
IL-1β	↓ ↓ ↓	AT (human) BM (rat) BM (mouse)	[37] [36] [38]	↓ ↓	AT (human) BM (mouse)	[37] [53]
IL-12	↓	AT (human)	[37]			
IFN-γ	↓ ↓ ↓ ↓ ↓ N.D.	AT (human) BM (human) UC (human) Gingiva (human) BM (mouse) BM (human)	[37] [58] [35] [58] [53, 56] [80]	↓	BM (mouse)	[53]
IL-23	↓	UC (human)	[35]			
IL-17	↓ ↓ ↓ ↓ N.D.	BM (human) UC (human) Gingiva (human) BM (mouse) BM (human)	[58] [35] [58] [53, 56] [80]	↓ ↓	UC (human) BM (mouse)	[35] [53]
IL-2	↓	BM (mouse)	[53]	↓	BM (mouse)	[53]
bFGF	↓	BM (rat)	[36]			
VEGF	↓	BM (rat)	[36]			
HGF	↓	BM (rat)	[36]			

↑ - increase, ↓ - decrease, N.D.- no difference, AT - adipose tissue, BM - bone marrow, UC - umbilical cord, IL - interleukin, TGF-β1 - transforming growth factor β1, TNF-α - tumour necrosis factor-α, CCL5 - chemokine ligand 5, MIP-2 - macrophage inflammatory protein-2, IFN-γ - interferon-γ, bFGF - basic fibroblast growth factor, VEGF - vascular endothelial growth factor, HGF - hepatocyte growth factor.

the small and large intestines progressively acquire anti-inflammatory characteristics including decreased TLR sensitivity to gut flora and increased secretion of anti-inflammatory IL-10 and PGE2. It was also reported that this process is arrested in a murine model of IBD resulting in an accumulation of phenotypically pro-inflammatory macrophages (Mϕ1) from the same precursor[64]. MSC and macrophage co-cultures induce a regulatory phenotype of anti-inflammatory macrophages (Mϕ2), characterised by the secretion of TGF-β and IL-10[65]. When administered into an experimental colitis model Mϕ2 successfully attenuated the clinical and histopathological severity of colitis[65]. Furthermore, Mϕ2 are the major source of TGF-β1 in MSC-ameliorated colitis suggesting that Mϕ2 may partially mediate the anti-inflammatory properties of MSCs[56]. Monocyte or dendritic cell co-cultures with MSCs reduce T-cell proliferation and IFN-γ secretion mediated partly by IL-10[63, 65]. Therefore, polarisation of antigen presenting cells into a regulatory phenotype may have a further anti-inflammatory role through the production of antagonizing

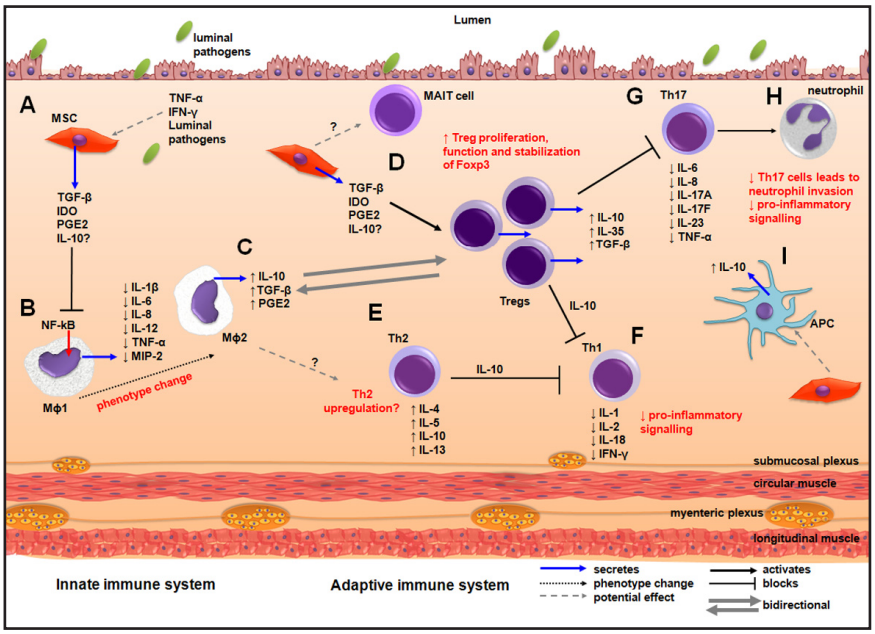

Fig. 2 Immunomodulatory effects of mesenchymal stem cells in intestinal inflammation

(A) Mesenchymal stem cells (MSCs) migrate into intestinal tissue and secrete anti-inflammatory paracrine factors including transforming growth factor (TGF)-β, prostaglandin E2 (PGE2), indoleamine 2,3-dioxygenase (IDO) and possibly interleukin (IL)-10. Pro-inflammatory cytokines tumour necrosis factor (TNF)-α and interferon (IFN)-γ or pathogens within the microenvironment may stimulate MSCs to upregulate the production of these anti-inflammatory factors (B) which polarise pro-inflammatory macrophages (Mφ1) into a (C) regulatory phenotype of anti-inflammatory macrophages (Mφ2). (D) Furthermore, the anti-inflammatory factors secreted by MSCs induce the polarisation and mitogenesis of anti-inflammatory regulatory T-cells (Tregs). MSCs may also modulate resident populations such as mucosa-associated invariant T (MAIT) cells, however this has not been investigated. (E) Mφ2 and Tregs secrete anti-inflammatory factors further promoting Mφ2 and Treg development which coordinates the change from pro-inflammatory to an anti-inflammatory microenvironment. (F) T helper (Th)1 cells are suppressed directly by Tregs and possibly Th2 commonly mediated through an IL-10 dependant mechanism which may become upregulated via Mφ2 secretion. (G) Additionally, Tregs antagonise pro-inflammatory Th17 secretion and function which is responsible for the recruitment of neutrophils; thus further reducing pro-inflammatory signalling. (I) Resident antigen-presenting cells may contribute to maintaining gut homeostasis after potentiation by MSCs and the anti-inflammatory microenvironment.

cytokines suppressing pro-inflammatory T-cells in colitis.

While MSCs may polarise the immune response through modulation of the innate immune system; their direct or indirect effect on adaptive immunity is vital in suppressing intestinal inflammation. Reduced infiltration of $CD4^+$ T-cells parallels decreased inflammation in MSC attenuated colitis[58]. Subsequent to MSC therapy, the expression of cytokines and T-cell markers in the colon suggests a decrease in pro-inflammatory T helper (Th)1 and Th17 cells and an increase in Th2 and Treg cells[35, 53]. Co-cultures of MSCs with lamina propria mononuclear cells in transwell plates decrease IFN-γ and IL-17 suggesting immune suppression of Th1 and Th17 in a paracrine fashion. Furthermore, *ex vivo* mesenteric lymph node co-cultures with MSCs decrease the production of pro-inflammatory IFN-γ and IL-2, have no effect on the Th2 cytokine IL-4, and promote IL-10 secreting T-cells suggesting the induction of Tregs[37, 63]. Increased Tregs are observed after the amelioration of experimental colitis via MSC treatment[53, 58]. Additionally, mucosal and peripheral Tregs are elevated after MSC therapy in human fistulising CD[27]. Implantation of T-cells after MSC conditioning ameliorates experimental colitis[37, 63]. This suggests that the anti-inflammatory effect of MSCs in colitis is mediated, at least in part, by Tregs. Recent evidence has suggested that an additional T-cell subset, mucosa-associated invariant T (MAIT) cells, may be implicated in the pathophysiology of experimental colitis and IBD[66, 67]; however the influence of MSCs on MAIT phenotypes is yet to be explored.

Evidence suggests IL-10 secreting antigen presenting cells prevent exaggerated Th1 and Th17 responses to bacteria in murine colitis mediated through the induction of Tregs[68]. MSCs may initially polarise macrophages into Mφ2 and induce Treg development via PGE2 and IDO[69-71]. Additionally, TGF-β1 is pivotal for the induction of Tregs and furthermore antagonises nuclear factor κB (NF-κB) intracellular signalling pathways, thus negatively regulating pro-inflammatory cytokine production[72, 73]. The polarisation of Mφ2 and Tregs may result in an anti-inflammatory cascade mediated by IL-10 and TGF-β1 further promoting their development. Anti-inflammatory cytokines and Tregs antagonise pro-inflammatory Th1 and Th17 phenotypes, thus shifting the microenvironment into an anti-inflammatory state. Th17 cells are responsible

for neutrophil recruitment[74], therefore Treg suppression of Th17 may coincide with decreased neutrophil invasion observed after MSC treatment of experimental colitis[35]. Additionally, Tregs have been demonstrated to promote anti-inflammatory properties in neutrophils[75]. Thus, Tregs may further suppress the inflammatory response by directly acting on the innate immune system.

Future Outlook

Currently there are more than 200 ongoing clinical trials testing MSCs as a viable treatment option, however, only a few clinical trials tested MSCs in IBD patients. These trials demonstrated that MSC treatment is effective for refractory fistulising and luminal CD after local and systemic administration. Clinical data combined with studies in experimental models are indicative of the potential of MSC therapy to ameliorate IBD. These findings have been attributed to immunomodulation and restoration of epithelial barrier integrity through paracrine trophic factors and cytokines secreted by MSCs.

Outstanding questions include assessing the role of MSCs in tumour formation and progression; this remains contentious and further studies are warranted. Evidence of MSC entrapment in filtering organs after systemic injection necessitates the need for further research into more efficient methods of administration. Future studies should comparatively assess administration techniques to effectively target gastrointestinal inflammation. Additionally, long-term immune tolerance to MSCs received from donors needs to be discerned. The effects of MSC treatment on inflammation-induced damage to the enteric nervous system embedded in the gastrointestinal wall has not been studied, but is important for understanding pathophysiology of disease reoccurrence and severity. Thus, therapeutic mechanisms of MSCs need to be elucidated taking into account the complexity of the intestinal microenvironment.

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Conflict of interests

None

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