Stem cell therapy in inflammatory bowel disease: which, when and how?

Deen, W.K. van; Oikonomopoulos, A.; Hommes, D.W.

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Welmoed K. van Deen[a,b], *Angelos Oikonomopoulos*[a], *and Daniel W. Hommes*[a]

Purpose of review
Stem cell therapy has emerged as a promising therapeutic strategy for inflammatory bowel diseases (IBDs). Currently, stem cell therapy is not part of the standard of care and is usually only performed as a part of clinical trials. In this review, clinical results, proposed underlying mechanisms, and future research directions will be discussed.

Recent findings
Administration of mesenchymal stem cells (MSCs) and hematopoietic stem cells (HSCs) has been evaluated for IBD treatment over the past years. MSC therapy is being explored as a treatment option for fistulizing Crohn's disease and for luminal Crohn's disease. It is shown to be well tolerated, but results on efficacy are inconsistent. HSC transplantation seems to be very effective, but serious adverse events are common. Therefore, future research should focus on improving efficacy of MSC therapy, and on improvement of safety of HSC therapy.

Summary
Both MSC and HSC therapy offer clinical potential, but currently are not routinely used for IBD treatment. MSC therapy seems well tolerated but results on efficacy are conflicting. HSC transplantation is shown to be effective but safety concerns remain. Nonetheless, for severe refractory IBD cases, stem cell therapy could well become the next-generation treatment option.

Keywords
hematopoietic stem cells, inflammatory bowel disease, mesenchymal stem cells

INTRODUCTION

Stem cell therapy is a field that has developed considerably in the past years. Stem cells have the capacity to generate different types of daughter cells, and thus have been proposed as a valuable tool for regeneration of damaged tissue. Hematopoietic stem cells (HSCs) are capable of regenerating immune cells [1], which creates the theoretical possibility to create a new immune system without autoimmunity in inflammatory bowel disease (IBD) patients [2▪▪]. Mesenchymal stem cells (MSCs) can differentiate into different mesenchymal cell lines and also exert immunosuppressive functions, which might be beneficial in IBD [3▪]. Several other adult stem cells have been explored in preclinical settings as well. In this article, the emphasis will be on HSCs and MSCs because those have been already used in clinical settings.

PATHOGENESIS OF INFLAMMATORY BOWEL DISEASES

The pathogenesis of IBD remains largely unclear even though considerable advances in this field have been made. IBD is thought to be caused by a dysregulated immune response against communal, nonpathogenic bowel antigens in a genetically susceptible individual. Both genetic and

[a]Department of Digestive Diseases, David Geffen School of Medicine, UCLA, Los Angeles, California, USA and [b]Department of Gastroenterology and Hepatology, Leiden University Medical Center, Leiden, The Netherlands

Correspondence to Welmoed K. van Deen, Center for Inflammatory Bowel Diseases, 10945 Le Conte Avenue #2114, Los Angeles, CA 90095, USA. Tel: +1 310 206 5785; fax: +1 310 206 9906; e-mail: wvandeen@mednet.ucla.edu

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KEY POINTS

- Both HSCT and MSC therapy are currently being evaluated for IBD treatment in trial settings.
- HSCT offers the opportunity to generate a new immune system free of autoimmunity. Currently, the main limitation of HSCT is safety, but efficacy has been shown consistently.
- MSCs have tissue regenerative properties and exert immunosuppressive functions, theoretically making them a valuable tool for IBD treatment. MSC therapy is well tolerated, but results on efficacy have been inconsistent. Further research needs to be aimed at improving MSC efficacy by optimizing isolation, expansion and stimulation procedures of MSCs.

environmental factors play a role in IBD development, mediated by changes in innate and adaptive immune function, epithelial barrier function and microbiome composition [4–6]. Genome-wide association studies (GWASs) start to shed more light on these processes, and the application of high throughput methods to analyze the expression of many factors involved in IBD pathogenesis will further improve our knowledge about IBD.

Based on cytokine profiles observed in IBD patients, Crohn's disease is traditionally thought to be mainly mediated by a Th1-cell response, and ulcerative colitis by a Th2-like response [7▪,8]. Recently, it became clear that this separation is more nuanced. Crohn's disease seems to be mediated by a Th1 response combined with a Th17 response, both causing tissue injury. In ulcerative colitis the response is mediated by cytokines similar to those observed in a Th2 response, mediated by NK-cells causing direct and indirect tissue injury [8]. Evidence for the involvement of T-cells, NK-cells and dendritic cells in IBD pathophysiology has been confirmed in a large recent GWAS [9▪▪]. Other components of the adaptive immune response play a role in IBD pathogenesis as well. For example, bacterial recognition and antigen loading of bacterial fragments onto MHC molecules through the process of autophagy appear to be important [10]. In addition, increased expression of adhesion molecules, chemokines and mRNA involved in oxidative stress is observed in IBD patients [7▪], which indicates important roles for cell trafficking and responses to tissue injury in disease mechanisms.

HEMATOPOIETIC STEM CELL TRANSPLANTATION

Originally, hematopoietic stem cell transplantation (HSCT) was used as treatment for different malignancies [1]. The idea of using HSCT for autoimmune conditions emerged after the observation of positive effects in animal models. This concept was supported by the observation that human autoimmune diseases improved in patients undergoing HSCT for other indications [2▪▪]. The HSCT procedure starts with mobilization of HSCs from the bone marrow into the peripheral blood and harvesting of those HSCs. Before the transplantation, the patient undergoes a preparative treatment that ablates the immune system, after which the HSCs are administered intravenously [1]. The rationale behind HSCT for autoimmune and chronic inflammatory diseases, including IBD, is that this procedure can 'reset' the immune system. The preparative chemotherapy eliminates the immune system, including autoreactive T-cells. By subsequently performing autologous HSCT, hematopoietic precursors will generate a new tolerant T-cell population, mediated by T-cell selection processes in the thymus. This concept is supported by two studies analyzing immune reconstitution after autologous HSCT in patients with multiple sclerosis [11] and systemic lupus erythematosus [12], which showed increased thymic output after HSCT with the development of a new and diverse T-cell repertoire. Allogeneic transplantation offers the additional benefit of removing the genetic susceptibility of the immune system, and evidence for an additional beneficial effect of graft-versus-autoimmunity has been described as well. Allogeneic HSCT, however, is associated with higher complication and mortality rates and is therefore being discouraged as treatment for autoimmune diseases in current guidelines [2▪▪]. In IBD patients with severe refractory disease that are not eligible for surgery, autologous HSCT can be considered, using a relatively low-dose chemotherapy (compared with the regimens used in cancer treatment), usually consisting of cyclophosphamide and anti thymocyte globulin antibodies.

Currently, the most extensive experience in HSCT for IBD has been published by Burt *et al.* [13]. Twenty-four Crohn's disease patients with severe disease refractory to conventional therapy, including anti-TNFα antibodies, received autologous HSCT. Of these, 91% stayed in remission for 1 year after HSCT, 57% for 3 years and 19% for 5 years [13]. Recently, another group reported similar outcomes in a cohort of 10 refractory Crohn's disease patients, with remission rates of 80% after 1 year, 40% after 3 years, and 30% after 5 years. Also, out of four patients that suffered from fistula, three experienced closure of the fistula tract [14▪]. In our experience, two refractory Crohn's disease patients underwent autologous HSCT. Both patients

achieved disease remission after the transplantation procedure. One patient restarted medication after 12 months, and no luminal relapse was observed during the follow-up period of 6 years. The other patient restarted medication after 6 months, relapsed only after 5 years, and achieved remission again after switching medication [15]. These results are promising, but for a definite assessment of the efficacy of HSCT for IBD, the results of a randomized controlled phase III trial are warranted. Currently, a multicenter, prospective, randomized phase III study is being performed that analyzes the effect of autologous HSCT vs. stem cell mobilization alone in refractory Crohn's disease patients (ASTIC-trial) (http://www.nottingham.ac.uk/icr/astic). All patients have been recruited and preliminary data were presented recently. A median fall in the Crohn's disease activity index of 162 was observed in the HSCT group, compared with 82 in the group receiving mobilization alone. Patients that received HSCT also considerably improved endoscopically, while no improvement was seen in the control group. Though the results are preliminary, autologous HSCT seems to be an effective treatment for refractory Crohn's disease. Serious adverse events in both groups were common though, raising concerns about safety [16▪▪].

A recent analysis assessing safety of HSCT in 70 patients with autoimmune diseases in the United Kingdom showed an overall survival of 87% after 1 year and 65% after 5 years following allogeneic HSCT. For autologous HSCT the survival was 85% at 1 year and 78% after 5 years. Age was strongly correlated with survival, with the highest survival in the 18–39 year age group (5 year survival 95%). The underlying autoimmune disease affected the outcome as well. The most common cause of death was infection after both autologous and allogeneic HSCT [17]. Another analysis of 900 autoimmune patients undergoing HSCT showed a 5-year survival rate of 85%. Half of the deaths were related to transplantation, with the main cause of death being infection. Outcomes were interestingly not dependent on the type of conditioning regimen, but were strongly correlated with the transplantation center [18]. Because of the inherent risks associated with HSCT, this therapy is only considered in severe refractory cases, in which the potential benefit weighs out against the risks. Currently, all trials have focused on severe refractory Crohn's disease patients. No trials analyzing HSCT for ulcerative colitis patients have been published yet, though improvement of ulcerative colitis after both allogeneic and autologous HSCT has been described in ulcerative colitis patients undergoing HSCT for other indications [19,20] and in a case of autoimmune anemia combined with ulcerative colitis [21].

To increase the applicability of HSCT for IBD, improvement of safety is a top priority. As mentioned above, infectious complications are the most common cause of treatment-related mortality due to prolonged lymphopenia after HSCT. To shorten the lymphopenic period after HSCT, different approaches have been sought to accelerate immune reconstitution after HSCT. In this context administration of keratinocyte growth factor, growth hormone and cytokines such as IL-2 and IL-7 has been tested, with the goal of stimulating thymic function and promoting lymphocyte survival [22▪,23▪]. Also, adoptive transfer of specific T cells [22▪], and ex-vivo expansion of hematopoietic precursor cells [24,25,26▪] are being explored to improve safety. To improve efficacy, allogeneic HSCT might be considered because the genetic susceptibility of the immune system will be permanently removed. Currently, a phase I trial assessing toxicity and efficacy of allogeneic HSCT in Crohn's disease is being performed (http://www.clinicaltrials.gov/ct2/show/NCT01288053).

MESENCHYMAL STEM CELL THERAPY

MSCs, also called mesenchymal stromal cells, are a heterogeneous group of stromal cells that have the capability to differentiate into different mesenchymal cell types and also exert immunosuppressive functions. The combination of those two properties makes the application of MSCs for tissue regeneration in inflammation-induced tissue injury a promising approach [3▪]. MSCs are isolated from stromal tissues based on their plastic adherence or using specific surface markers [27]. Originally, MSC research mainly focused on bone marrow-derived MSCs (bm-MSCs). Alternatively, adipose tissue-derived MSCs (ad-MSCs) can be isolated in high frequencies from liposuction aspirates. The immunophenotypes of ad-MSCs and bm-MSCs are more than 90% identical, and ad-MSCs have a similar or even enhanced immunosuppressive capacity compared with bm-MSCs. Since MSCs in liposuction aspirates are abundant, these cells can be used clinically without ex-vivo expansion, in contrast to bm-MSCs [28]. These properties mean that ad-MSCs can be a valuable, more accessible, and safe alternative to bm-MSCs.

In-vitro experiments have shown that MSCs are able to interfere with components of both innate and adaptive immune system. In the adaptive immune system MSCs interfere with complement, toll-like receptor signaling, macrophages, dendritic cells, neutrophils and NK cells. In the adaptive

immune system, MSCs inhibit T-cell function, shift T-cell balance and induce Treg cells. Inhibition of B-cells has also been suggested in different studies [29▪▪]. Before MSCs exert their immunosuppressive function they require priming by pro-inflammatory cytokines such as IFNγ, TNFα and IL-1β. The immunosuppressive effect that MSCs exert is mainly mediated by soluble factors, but certain MSC-immune cell interactions are contact-dependent [29▪▪]. Depending on the environment, MSCs can also acquire pro-inflammatory properties [29▪▪,30▪]. This suggests that in the event of active infection, MSCs might adopt a pro-inflammatory phenotype, whereas in the case of an exaggerated inflammatory response they will adopt an immunosuppressive phenotype (Fig. 1). In different mouse models, a positive effect of MSCs on experimental colitis is shown as well. MSCs are infused either intravenously [31] or intraperitoneally [32,33] and improve experimental colitis [31–33]. Our group confirmed *in vivo* that IFNγ-stimulated MSCs have enhanced immunosuppressive functions by showing reduced weight loss and lower histology scores in two mouse models of colitis [33].

The first clinical successes with MSCs in inflammatory conditions were achieved in severe graft versus host disease (GvHD). Several encouraging results are obtained from clinical trials assessing allogeneic MSCs for the treatment [34] and prevention [35] of GvHD. Significant improvement of severe GvHD was observed after administration of MSCs compared to a control population [34], and significantly fewer patients developed severe GvHD after HSCT if MSCs were co-infused simultaneously with the graft [35]. However, a randomized placebo-controlled trial using industrially manufactured allogeneic MSCs for GvHD treatment failed to show a durable complete response for ≥28 days [36].

In the IBD field MSCs have been tested in clinical trials for two indications, namely fistulizing disease and luminal disease (Table 1) [37,38,39▪,40,41▪,42▪,43,44▪,45▪]. For luminal disease, the rationale for the use of MSCs lies mainly in their immunosuppressive capacity. In fistulizing disease, the differentiation potential of MSCs is thought to be of crucial importance as well to achieve fistula tract closure. Several phase I/II trials have been performed analyzing ad-MSCs for fistulizing disease, using autologous [37,38,39▪] or allogeneic [41▪] MSCs. One study evaluated the effect of bm-MSCs on fistulizing Crohn's disease [40]. The outcomes of these trials are promising and the procedure seems safe. A placebo-controlled trial evaluating the efficacy of ad-MSCs compared with fibrin glue for Crohn's disease related and unrelated fistula found 70% healing in the treatment group, which was four-fold higher than in the control group [38]. After 4 years of follow-up, however, only 33% remained healed in the treatment group vs. 15% in the control group [39▪]. Recently the results of the FATT1 trial were published, a phase III randomized controlled trial assessing the efficacy of autologous ad-MSCs for

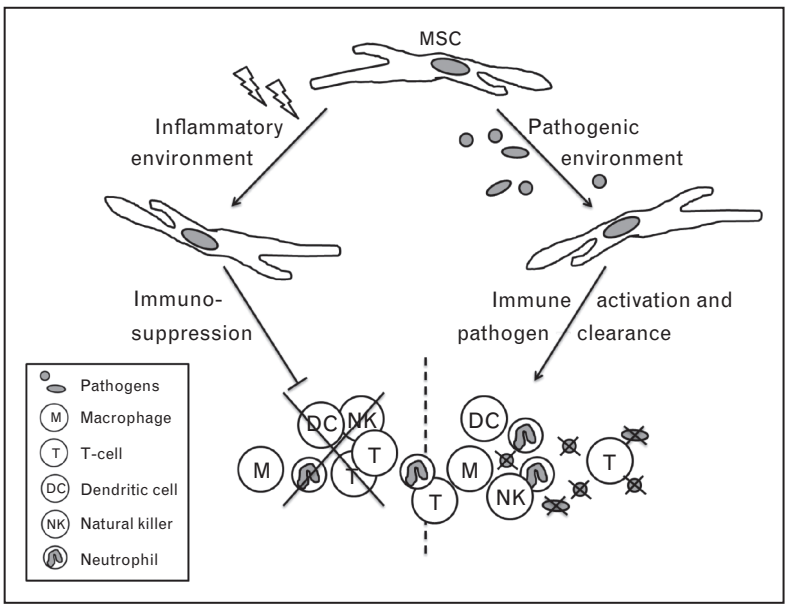

FIGURE 1. The two faces of mesenchymal stem cells (MSCs). It is thought that under inflammatory conditions, MSCs can acquire an immune suppressive phenotype (left), suppressing among others T-cells, macrophages, dendritic cells, natural killer cells, and neutrophils. Under influence of an active infection, MSCs might acquire a phenotype that activates the immune response and support pathogen clearance (right).

Table 1. Published trials analyzing mesenchymal stem cell treatment for different fistulizing diseases and for luminal inflammatory bowel disease

	Indication	MSC source	Results
Fistulizing disease			
Garcia-Olmo *et al.* [37]	CD	ad-MSC (auto)	6/8 healed, 2/8 improvement (8 wk)
Garcia-Olmo *et al.* [38] Guadalajara [39▪]	CD and non-CD	ad-MSC (auto)	71% ad-MSC ↔ 16% fibrin glue (8 wk) 33% ad-MSC ↔ 15% fibrin glue (4yr)
Ciccocioppo *et al.* [40]	CD	bm-MSC (auto)	7/10 healed, 3/10 improved (1 yr)
de la Portilla *et al.* [41▪]	CD	ad-MSC (allo)	14/20 closure (24 wk)
Herreros *et al.* [42▪]	Non-CD	ad-MSC (auto)	42% ad-MSC ↔ 39% fibrin glue (24 wk)
Luminal IBD			
Duijvestein *et al.* [43]	CD	bm-MSC (auto)	Improvement 3/10, no remission
Liang *et al.* [44▪]	CD and UC	bm/uc-MSC (allo)	Remission 5/8
Forbes *et al.* [45▪]	CD	bm-MSC	Improvement 12/14, remission 8/14

ad-MSC, adipose tissue derived MSC; allo, allogeneic; auto, autologous; bm-MSC, bone marrow derived MSC; CD, Crohn's disease; IBD, inflammatory bowel disease; UC, ulcerative colitis; uc-MSC, umbilical cord derived MSC; wk, week; yr, year.

perianal fistulas that were not related to Crohn's disease. Disappointingly, no significant difference between the different treatment groups was shown [42▪]. To assess the efficacy of MSCs for Crohn's disease-related fistula, the ADMIRE-Crohn's disease study is currently being performed (http://www.clinicaltrials.gov/ct2/show/NCT01541579). Several trials for luminal Crohn's disease have been performed as well. A phase I trial we performed using autologous bm-MSCs for luminal Crohn's disease, designed primarily for safety and feasibility, showed improvement in three out of 10 patients, of which none achieved remission [43]. Another group treated four Crohn's disease and three ulcerative colitis patients with allogeneic MSCs. Five patients achieved remission, of which two stayed in remission for over 2 years [44▪]. Recently, preliminary results of a phase II trial analyzing the use of allogeneic bm-MSCs for refractory luminal Crohn's disease were presented. Improvement was observed in 12 out of 14 included patients, remission in eight and endoscopic improvement in seven [45▪]. These studies were not designed to assess efficacy, but did demonstrate safety of intravenous (i.v.) infusion of MSCs. This notion is supported by a recent meta-analysis analyzing safety of i.v. MSC infusion for many different indications. MSC infusion is associated with a transient fever, but not with acute infusion toxicity, organ system complications, infection, malignancies, or death [46▪▪].

MSC therapy for IBD represents a promising strategy but results have been inconsistent. The comparison between different studies is challenging because different isolation, selection and expansion protocols are being utilized. The development of uniform protocols is warranted in order to achieve reproducible and consistent results. To improve efficacy, robust priming of MSCs is probably of crucial importance. In the currently performed trials, no priming of MSCs has been performed. Therefore, determining the optimal protocols to prime MSCs before administration might improve the clinical results. Second, MSCs are a heterogeneous cell population and it has been described that different subsets of these cells have different functional capacities [27]. Identifying and isolating a subpopulation of MSCs with enhanced immunosuppressive properties might be a promising strategy to improve clinical efficacy. Lastly, notable differences in immunosuppressive capacity between MSCs from different donors have been found [47▪]. Therefore, a careful selection process of the right donor might be favorable for outcomes too.

EMERGING STEM CELL THERAPIES

Several other stem cells have been explored in preclinical settings as well. Induced pluripotent stem cells (iPSCs) are pluripotent cells derived from terminally differentiated cells by dedifferentiation. The generation of iPSCs creates the possibility of generating tissue specific cells, but also HSCs or MSCs [48]. Intestinal stem cells also carry a strong therapeutic potential to enhance mucosal healing. After administration of ex-vivo expanded colonic stem cells, engraftment of the cells in, and healing of, colonic mucosa has been shown in mice [49▪▪]. Lastly, specific cellular therapies are offering clinical potential as well. For example, administration of ex-vivo expanded Treg cells

has been shown beneficial in a GvHD mouse model [50].

CONCLUSION

Treatment of refractory IBD patients remains a challenge. HSCT and MSC therapy are both promising strategies to improve disease control in this patient group. HSCT is effective, but is also accompanied by a high complication rate. Improving safety will increase the applicability of this therapy in IBD patients. MSC therapy seems promising but results have been inconsistent. Optimization of this therapeutic strategy is therefore strongly warranted. For both therapies results of phase III studies for IBD are still lacking but are expected in the near future and will shed more light on the efficacy. Next to those two clinically investigated therapies, several other stem cell and cellular therapies are being explored in preclinical settings. Some of those have a promising clinical potential, but further research needs to be performed to explore safety and feasibility of those approaches.

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Conflicts of interest

There are no conflicts of interest.

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