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Stem cell therapy for inflammatory bowel disease

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CHAPTER 1

Introduction

Adapted from *Journal of Crohn's and Colitis* 2008 Jun;2(2):99-106
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INFLAMMATORY BOWEL DISEASE

Inflammatory bowel disease (IBD) refers to chronic diseases that cause inflammation of the intestines. The most common inflammatory bowel diseases are ulcerative colitis (UC) and Crohn's disease (CD). These disorders have both distinct and overlapping pathologic and clinical characteristics. Ulcerative colitis is characterized by recurring episodes of inflammation limited to the mucosal layer of the colon. It almost invariably involves the rectum and may extend in a proximal and continuous fashion to involve other portions of the colon. Crohn's disease is characterized by chronic, relapsing transmural inflammation and ulceration, and may affect any part of the gastrointestinal tract from the oral cavity to the anus, but typically involves the ileum, colon or perianal region. Their pathogenesis remains incompletely understood.[1]

Epidemiology, clinical manifestation and diagnosis

Incidence rates range from 2.2 to 14.3 cases per 100 000 person-years for UC and 3.1 to 14.6 cases per 100 000 person-years for CD in North America.[2] In Europe, the overall incidence per 100 000 person-years is 5.6.[3] Prevalence rates range from 37 to 246 cases per 100 000 persons for UC and from 26 to 201 cases per 100 000 for CD. IBD can present at any age, although the peak incidence occurs between the ages of 15 and 30 years.[4] There is no gender specificity.

The major symptoms of IBD are abdominal pain, (bloody) diarrhea and generalized fatigue. Patients can also experience fever and weight loss. Frequent complications of CD are intestinal obstruction, fistula and abscess formation, and extra intestinal manifestations, such as cutaneous ulcerations, uveitis, and arthropathy. The diagnosis of IBD is established by the clinical features and can be confirmed by endoscopic, radiologic, and histopathologic examination.[5] Biopsy specimens from inflamed gut mucosa typically show inflammation, distorted crypt architecture, and crypt abscesses.

Treatment and prognosis

The choice of treatment of IBD depends on the location of the disease, its severity, and response to earlier therapy. Most clinicians initially treat patients with steroids, 5-aminosalicylic acid (5-ASA) agents, and antibiotics. Patients who are steroid dependent, and those with moderate to severe disease needing induction therapy with conventional corticosteroids, can be treated with azathioprine, mercaptopurine, and methotrexate. These so called immunomodulating drugs have been shown to be effective in inducing clinical remission, but their widespread use is limited by their toxicity. Infliximab and newer generation antibodies to tumor necrosis factor (TNF)-**α** (certulizomab, adalimumab) have been shown efficacious as well, but are not able to maintain remission in

most patients. Thereby, the treatment of IBD remains challenging for treating physicians.

In most patients with IBD, the course is chronic and intermittent. The disease responds less well to medical therapy with time and approximately 50-70% of the patients require surgical resection during the course of the disease. In the case of CD, surgery is accompanied by a high recurrence rate. On top of that, many patients have already had resections and are therefore at risk for developing short bowel syndrome. Consequently, there is an unmet need for more effective therapeutic strategies.

STEM CELL THERAPY

A classical definition of a stem cell is a cell that has the capacity for self-renewal and the ability to give rise to one or more types of differentiated progeny.[6, 7] Self-renewal is defined as the ability of a cell to proliferate while it maintains its proliferation and differentiation potential. Stem cells are known to exist in different tissues but their frequency, exact function and identity are generally not well understood. Both in animal models and in patients, it appears that bone marrow derived cells play a role in the healing process following intestinal injury[8] and that these cells may contribute to regeneration of various mucosal components.[9-11] The bone marrow contains at least two types of stem cells. One population consists of CD34 positive hematopoietic stem cells (HSC) committed to differentiate into all blood cell types, including the myeloid and lymphoid lineages. A second population of stem cells remains less well characterized. These non-hematopoietic stem cells are thought to support hematopoiesis and are variously known as mesenchymal stem cells, marrow stromal cells, and, more recently, mesenchymal stromal cells, all designated by the acronym MSC.[12]

Hematopoietic stem cell transplantation

Although more than fifty years ago hematopoietic stem cell transplantation (HSCT) was introduced as a treatment for injury, it is now principally used as treatment for hematologic and lymphoid cancers.[13] Evidence that HSCT is an effective treatment for autoimmune diseases comes from animal models[14, 15] and case reports from HSCT recipients with coexistent autoimmune diseases.[16] Ever since, autologous HSCT has been performed in more than 700 patients with autoimmune diseases,[17] the most frequent indications being systemic sclerosis, multiple sclerosis, rheumatoid arthritis and systemic lupus erythematosus.

HSCT includes conditioning (immune ablation with high dose chemotherapy, total body irradiation and/or anti-lymphocyte antibodies), in which the bone marrow cells of the host are completely eliminated, followed by the infusion of either autologous or allogeneic stem cells. The HSCs are either directly harvested from the marrow or mobilized from bone marrow or blood before being harvested by apheresis. High dose immune ablation is an intensive treatment with risks of severe complications which on rare occasions have been fatal. In autologous transplantation, the individual's own HSCs are harvested to be returned after conditioning. The graft is typically depleted of T-cells to avoid the

reinfusion of autoreactive T-cells. In allogeneic transplantation, the HSCs are harvested from a donor, usually a human leukocyte antigen (HLA) matched sibling. In addition to the complications associated with conditioning, allogeneic HSCT is associated with a much higher transplant-related morbidity, due to graft-versus-host disease (GvHD), aplastic anemia, and hematological malignancies, and also a higher mortality rate (15-25% vs. 3-5%[18]) due to a considerable risk on GvHD. In view of the risks related to allogeneic transplantation most patients treated for autoimmune disease have received autologous transplants.

Hematopoietic stem cell transplantation in IBD patients for other indications

The possibility that autologous HSCT could be an effective treatment for IBD was suggested by the improvement of the clinical course of disease in patients with CD that received autologous transplantation for other indications.[19-23] The first published abstract dates from 1993 and describes two year clinical remission in two patients with active IBD treated with autologous HSCT for breast cancer.[19] Long term clinical remission after autologous HSCT was reported in a patient with clinical disease control of CD up to seven years following transplantation for non-Hodgkin lymphoma.[21] Similar results were obtained in a 30-year-old patient with a ten year history of severe CD who developed Hodgkin's disease and remained in complete treatment-free remission of both diseases three years after autologous HSCT[24] and in an IBD patient who received HSCT for acute myeloid leukemia and had normal findings during ileo-colonoscopy at 1, 2, 3, and 5 years after transplantation.[23]

Similar to the first experience with autologous HSCT in IBD, the effect of allogeneic HSCT on IBD was initially described in patients treated for hematological malignancies. The first case report was published in 1998 and described a 35-year-old male free of symptoms and signs of CD eight years post allogeneic marrow transplantation for acute leukemia.[25] A second report in the same year described six patients that underwent allogeneic transplantation for leukemia between 1962 and 1982.[26] In this report, five out of six patients had active CD at time of transplantation. One patient died of septic complications 97 days after transplantation, the other five patients remained free of disease activity for more than one year post-transplantation. Only one out of these five patients relapsed during the follow up period of up to 15 years post-transplantation. Interestingly, the only patient that developed a mixed donor-host hematopoietic chimerism following allogeneic HSCT continued to have active CD. In a retrospective study by Ditschkowski et al., ten out of eleven patients remained free of symptoms following allogeneic HSCT for hematological malignancies with a median follow-up time of 34 months.[27] In another case report,[28] a 41-year-old man with CD underwent allogeneic HSCT for lymphoma. Following transplantation, his bowel symptoms ceased and he was able to stop all immunosuppressive drugs. Eighteen months after transplantation colonoscopy showed no evidence of CD activity. Remission of UC following allogeneic HSCT has also been described.[29, 30] Two patients, each with a long history of psoriasis and UC, received an allogeneic HSCT for leukemia and remained in full remission four and twelve years after transplantation.

The coincidental treatment of IBD with both autologous and allogeneic HSCT increased the interest in the possibility that stem cell transplantation could be of value in IBD treatment. Autologous HSCs are infused only to shorten the post-HSCT neutropenic interval, in contrast with allogeneic HSCT in which the recipient's immune and hematologic system is replaced with that of a healthy donor without the genetic predisposition to IBD. In this light it has been proposed that the risk of disease recurrence may be higher after autologous HSCT.[31]

Early studies on HSCT specifically for Crohn's disease

The first reports of autologous HSCT specifically given for the treatment of CD were published in 2003 and concerned five patients with severe disease activity refractory to conventional treatment and treatment with anti-TNFα antibodies, who received autologous HSCT.[32-34] No serious transplantation related complications were reported and all patients entered clinical remission. Some of the colonoscopies however, showed persistent mild inflammation up to one year post-transplantation. A larger phase I study on twelve patients with chronic active refractory CD also suggested that autologous HSCT can have a beneficial effect on CD activity. Besides fever, the autologous HSCT was well tolerated by the patients. Adverse effects included hematemesis from a Mallory-Weiss tear, a prolonged febrile course, clostridium difficile-induced diarrhea, and diarrhea after an upper respiratory tract infection. After fifteen months only one patient developed a recurrence of active CD. All others maintained in clinical and drug-free remission, but similar to the patients described in the reports above with persisting nonsymptomatic histologic and/or radiologic evidence of CD.[35] In **Chapter 2** of this thesis, the data on the clinical effect of HSCT in three patients with refractory CD can be found.

Potential risks of hematopoietic stem cell transplantation

HSCT may be an effective treatment for CD but is also associated with a high morbidity and mortality rate.[36] In 390 patients undergoing autologous HSCT for various autoimmune diseases, a mobilization associated mortality of 1.5% and an overall procedure related mortality of 9% were found.[37] Early toxicity is related to direct organ damage either from the agents used or due to infection and bleeding during the 10–12 days of bone marrow aplasia following the immunosuppressive conditioning period. Late toxicity relates to malignancy development due to the chemotherapy and/or radiation exposure. In addition, HSCT is associated with complications such as veno-occlusive disease of the liver and acute and chronic GvHD.[38] Although HSCT seems a reasonably successful treatment for CD it is clear that, given the considerable mortality rate of HSCT for autoimmune diseases, this treatment should only be considered in selected cases of CD. HSCT could be considered as a last resort in patients with debilitating disease refractory to all immunosuppressive drugs, including the different anti-TNFα compounds now available for treatment, and in patients in which surgery is not a treatment option.

MSC Transplantation

HSCT is thought to result in clinical remission in CD due to the combination of the immunosuppressive conditioning regimen and the replacement of the derailed lamina propria immune cells that maintain the disease. A novel emerging stem cell treatment may offer the benefit of immunosuppression without the need for conditioning chemotherapy, even when given as allogeneic transplant.

Mesenchymal stromal cells (MSC) reside in almost every type of connective tissue. Friedenstein and colleagues were the first to identify an adherent, fibroblast-like population of cells in the bone marrow.[39] Once isolated, these cells adhere to plastic, are capable of developing colony forming-units, and proliferate in vitro. In addition, MSCs are multipotent cells capable of differentiating into multiple lineages of the mesenchyme, including fat, bone, and cartilage tissue. MSCs consist of a heterogeneous population of cells and thus far no unique marker has been identified that allows reproducible isolation of precursors with predictable developmental potential. The isolation and characterization of these cells therefore still relies on their ability to adhere to plastic and their expansion potential. Isolated and expanded MSCs express CD73, CD90 and CD105 and are negative for hematopoietic stem cell markers (CD14, CD34, CD45), thereby distinguishing them from the hematopoietic stem cells.[40] Furthermore, MSCs do not express major histocompability complex (MHC) class II or co-stimulatory molecules and are poor antigen presenting cells that do not elicit a proliferative response in allogeneic lymphocytes, which suggests that MSCs are of low immunogenecity. Although MSCs are present in virtually all tissues, our current knowledge is based on MSCs isolated from accessible tissues (e.g. bone marrow, adipose tissue, and umbilical cord blood).

Immunomodulatory capacities of MSCs

The immunomodulatory functions of MSCs were examined in vitro by coculturing them with purified subpopulations of immune cells. It has been shown that MSCs suppress several functions of naïve and memory T-cells,[41-43] B-cells,[44] and natural killer cells[45, 46] as well as the differentiation, maturation, and function of dendritic cells (DCs).[47] Furthermore, expanded MSCs alter cytokine secretion profiles of DCs, naïve and effector T-cells, and natural killer cells to induce a more anti-inflammatory or tolerant phenotype.[48] Even though cell-cell contact plays a role in the interaction between MSCs and other immune cells, immunosuppressive mechanisms of MSCs are mainly mediated through soluble factors. MSCs constitutively produce transforming growth factor-β1 (TGF-β1), hepatocyte growth factor (HGF), interleukin (IL)-10, prostaglandin E_2 (PGE_2), soluble HLA-G5, and IL-6 (Figure 1). The latter inhibits monocyte differentiation towards DCs and MSC-DC interaction directs DC maturation towards an anti-inflammatory or regulatory phenotype, thereby decreasing their stimulation capability on T-cells.[47] Other factors, such as the enzyme indoleamine 2,3-dioxygenase (IDO), are released upon stimulation with inflammatory cytokines such as IFNγ and TNFα. IDO metabolizes tryptophan to kynurenin, which causes depletion of local tryptophan and accumulation of toxic breakdown products, thereby suppressing both CD4 and CD8

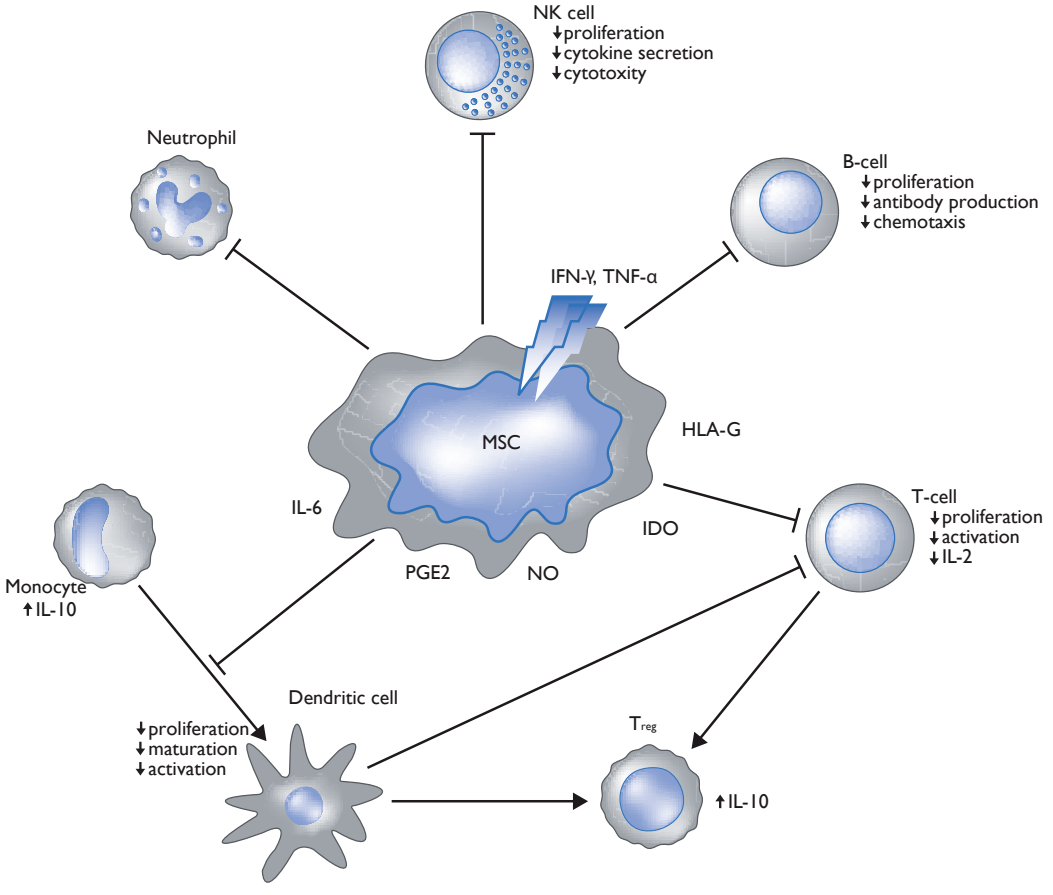

Figure 1. Schematic illustration of the immunomodulatory effects of MSCs on different cells of the immune system. After activation, MSCs secrete various factors, such as PGE2, IDO, and HLA-G. These factors regulate the function and proliferation of NK cells, B-cells, and T-cells. Constitutively secreted IL-6 plays a role in the inhibition of alloantigen-induced generation and maturation of DCs and neutrophil activation. DC: dendritic cell; HLA-G: human leukocyte antigen-G; IDO: indoleamine 2,3-dioxygenase; IL: interleukin; IFN-γ: interferon-γ; MSC: mesenchymal stromal cell; NO: nitric oxide; PGE2: prostaglandin E2; NK: natural killer; Treg: regulatory T cell; TNF-α: tumor necrosis factor-α.

T-lymphocytes proliferation with mitogens and specific antigens.[49, 50] Although the involvement of the enzyme IDO has been consistently reported, IDO alone is not responsible for inhibition of T-cell proliferation, as IDO inhibitors were not able to restore proliferation of peripheral blood mononuclear cells (PBMCs). Moreover, MSCs lacking both IFNγ receptor 1 and IDO still exert immunomodulatory activity.[51] In mice, MSCs express very little IDO and is the induction of inducible nitric oxidase synthase (iNOS) essential in T-cell proliferation inhibition.[52] Available data on the interaction between MSCs and B-cells is controversial. MSCs have been shown to inhibit as well as stimulate B-cell proliferation, depending on dose, source, and test system.[53] For example, MSCs decrease proliferation and immunoglobulin secretion of B-cells in a 1:1 ratio,[44] whereas in a lower concentration MSCs stimulates B-cell antibody secretion.[54]

Therapeutic applications of MSCs

Animal studies on models for tissue damage and autoimmune disease indicate that, similar to their immunosuppressive capacities in vitro, MSCs also display immunosuppressive capacities in vivo. For example, in a T-cell mediated experimental model of multiple sclerosis (MS) in mice, murine MSCs have been used to successfully treat experimental MS through the induction of peripheral T-cell tolerance.[55] Systemically infused MSCs first show a wide-ranging distribution followed by homing to injured tissues,[56] including the gut,[57] where they may participate in tissue repair. However, only a fraction of the systemically infused MSCs are traceable, and the fate of the remainder of the cells remains unknown. That intravenous infused MSCs entrapped as emboli are activated to secrete the anti-inflammatory protein TSG-6[58] and that MSC derived molecules have anti-inflammatory properties,[59] suggests that specific homing of MSCs to damaged tissues is not required for an effect.

In humans, the safety and feasibility of both local and systemic MSC administration has been studied in a variety of phase I and phase II trials. MSCs are low immunogenic and not restricted by MHC, therefore MSCs do not have to be human leukocyte antigen (HLA) matched to the recipient and can be infused without the need for conditioning chemotherapy, not even when given as allogeneic transplant. Both allogeneic and autologous MSCs are therefore currently under investigation. A great advantage of the allogeneic MSCs is their immediate availability. Furthermore, the age and fitness of the donor is controlled. MSC number and functionality decreases with age[60, 61] and an ongoing discussion is whether MSCs might contribute to or if the MSCs are affected by the underlying disease. For instance, MSCs from patients with systemic lupus erythematosus yield low cell numbers and are difficult to grow in culture.[62] Moreover, MSCs from patients with multiple myeloma are functionally defective and possibly contribute to the pathogenesis of the disease.[63,64] Rationale for autologous application comes from data that MSCs may under certain conditions also be subject to immune rejection. In a nonmyeloablated host, allogeneic MSCs are able to mount a T-cell memory response and consequently are eliminated.[65] Comparable loss of immune privilege has been reported by others.[66]

The initial clinical trials were in patients with osteogenesis imperfecta[67], followed by trials in which the immunosuppressive effects of the MSCs were used either to reduce the incidence of GvHD after allogeneic HSCT[68] or as treatment of active disease, including GvHD of the gut.[69] Based on their ability to moderate T-cell response, MSCs are currently under evaluation in a range of (autoimmune) diseases (Table 1).

Safety issues and concerns

In the limited number of patients treated with MSCs in the last decade, few adverse events have been attributed to MSC administration. Although acute toxicity appears low, little is known about long-term unwanted side effects. Also, as mentioned before, safety concerns remain concerning immunogenecity and the dysfunction of MSCs due to the underlying disease. Moreover, potential hazards include the possibility of malignant transformation, ectopic tissue formation and the possible xenogenic transmission of disease and antibody formation when fetal bovine serum (FBS) is added to the culture medium. In fact, reports showed that extensively in vitro expanded stem cells may be prone to malignant transformation.[70] It has been demonstrated by some groups that MSCs stimulate the growth of cancers and promote metastasis in rodents,[71-73] although an increased risk of tumor formation has never been confirmed in humans.[74] Moreover, two main works reporting transformation of human MSCs in culture were recently retracted as obtained data were based on tumor cell contaminated MSC cultures.[75] Although reassuring, safety issues remain important and it is therefore essential to carefully characterize MSCs passaged in vitro to maximize safety for the recipient. Furthermore, patients should be thoroughly screened before MSC administration as the cells might enhance the growth of unknown cancer.

MSCs for IBD

Data from experimental colitis models

MSCs have been studied in both dextran sulfate sodium (DSS) and trinitrobenzene sulfuric acid (TNBS) colitis, in mice as well as in rats

Autoimmune diseases	Orthopedics	Neurology	Cardiology
• Diabetes mellitus, type 1 and 2	• Fractures	• Amyotrophic lateral sclerosis (ALS)	• Myocardial ischemia
• Systemic sclerosis (SSc)	• Arthrosis and arthritis	• Ischemic cerebral stroke	• Dilated cardiomyopathy
• Systemic lupus erythematosus (SLE)	• Chondral and meniscal lesions	• Multiple sclerosis (MS)	• Chronic ischemic left ventricular dysfunction secondary to myocardial infarction
• Primary Sjögren's syndrome (pSS)	• Articular cartilage defects	• Parkinson's disease	

Table 1. Overview of indications for MSC therapy. Registered trials were found using the International Clinical Trials Registry Platform (ICTRP), available on www.who.int/ictrp.

(Table 2). Different sources of MSCs have been used; i.e. bone marrow (bmMSCs), adipose tissue (atMSCs) and, though not commonly used, gingiva (gMSCs). Interestingly, MSCs obtained from both syngeneic and allogeneic sources have been applied and also human MSCs were studied in wild type mice (xenogenic). Systemic route of administration was either via the tail vein (rat) or intraperitoneally (i.p.) in mice. The latter prederred in mice as MSCs, due to their large size, frequently entrap in the pulmonary circulation which can cause acute death due to asphyxia.[76] Local administration was also studied by injecting the MSCs into the colonic submucosa.[77]

The first two articles published in 2008 showed beneficial effects of the bmMSC in both DSS[78] and TNBS[77] colitis in rats. These data were further supported by two articles from the same group on atMSCs. In their first article, the authors showed that systemic infusion of MSCs obtained from adipose tissue ameliorated the clinical and histopathologic severity of TNBS colitis, abrogating body weight loss, diarrhea and inflammation, and increasing survival.[79] A second paper supported these data by showing that systemic infusion of atMSCs protects against experimental DSS colitis and sepsis. The therapeutic effect was associated with down-regulation of the Th1-driven inflammatory responses.[80] Zhang et al. nicely demonstrated that also MSCs from human gingiva, a tissue source easily accessible from the oral cavity, have similar immunomodulatory and anti-inflammatory properties as bmMSCs. In addition, they showed that a comparable therapeutic effect was mediated in the acute model of DSS colitis. This effect was achieved by the suppression of inflammatory infiltrates and inflammatory cytokines/mediators, by the increased infiltration of regulatory T-cells, and by the expression of anti-inflammatory cytokine IL-10 at the colonic sites.[81]

Recent data suggest that resting MSCs do not have significant immunomodulatory activity, but that the immunosuppressive function of MSCs has to be elicited by interferon-gamma (IFN-γ).[49, 50] In **Chapter 5**,

Hematology
- Myelodysplastic syndromes
- Graft-versus-host disease (GvHD)

Dermatology
- Epidermolysis bullosa
- Burn injury
- Diabetic foot

Nephrology
- Diabetic chronic kidney disease
- Lupus nephritis
- Chronic allograft nephropathy
- Allograft rejection after renal transplantation

Other
- Osteogenesis imperfecta
- Liver cirrhosis
- Periodontitis
- Inflammatory bowel disease (IBD)

Ref.	Species	Colitis model	Donor source	Tissue source	Cell number	Route	Timing	Sarifice	Outcome of MSC treatment
81	C57BL/6	7 days 3% DSS	human	bm and g	2×10^6	i.p.	one day after DSS initiation	day 10	Amelioration of colitis. Suppression of inflammatory infiltrates and inflammatory cytokines/mediators. Increased infiltration of regulatory T cells and the expression of anti-inflammatory cytokine IL-10 at the colonic sites.
80	C57Bl/6	acute colitis: 7 days 5% DSS	human, allogeneic and syngeneic	at	10^5–5×10^6	i.p.	day 2	day 5-14	Amelioration of the clinical and histopathological severity of colitis. Less weight loss, diarrhoea and inflammation, and increase in survival. Downregulation of the Th1-driven inflammatory responses.
	C57Bl/6	chronic colitis: two cycles of 7 days with 3% DSS, followed by a 10-day period without DSS supplementation		at	1×10^6	i.p.	day 7 each cycle	day 10 or 26	
91	male Lewis rats	DSS colitis with bone marrow hypoplasia: busulphan i.p. day 0, 1% DSS day 5 - 10	SDTG (CAG-EGFP) rat	bm	2×10^4/g	tail vein	day 7	day 10	Less severe colitis due to restoration of epithelial barrier integrity, no changes in cytokine expression
78	male Lewis rats	7 days 4% DSS	syngeneic	bm	5×10^6	tail vein	day 0, 2, and 4	day 7	Reduction in bloody stools, weight loss, colon shortening, and microscopic injuries. Decrease in mRNA expression of TNF-alpha, IL-1beta, and COX-2 in the rectum of MSC treated rats. Suppression of VEGF, HGF, and b-FGF. Green-fluorescent-labeled MSCs in lamina propria of inflamed regions.

Ref.	Species	Colitis model	Donor source	Tissue source	Cell number	Route	Timing	Sarifice	Outcome of MSC treatment
79	BALB/c	3 or 5 mg TNBS in 50%EtOH intrarectally	human, allogeneic and syngeneic	at	10^5–10^6 cells	i.p.	12 hours after TNBS installation	day 3 and 10	Protection against colitis, reduction in histopathologic signs and infiltration of macrophages, lymphocytes, and neutrophils. Reduced levels of inflammatory cytokines (TNF-α, IFN-γ, IL-6, IL-1β, and IL-12) and chemokines. Abrogation of established colitis. Increased levels of the anti-inflammatory/regulatory cytokine IL-10. Reduction of disease recurrence.
	BALB/c	4 or 5 mg TNBS in 50%EtOH intrarectally	human	at	1×10^6	i.p.	2 consecutive days starting day 6	day 14	
	BALB/c	1.5 mg TNBS day 0 and 9	human	at	1×10^6	i.p.	12 hours after the first infusion of TNBS	day 14	
77	male Sprague-Dawley rats	0.15 M TNBS in 35% EtOH	syngeneic	bm	1×10^7	injected into the colonic submucosa	immediately after the TNBS-induced colon injury	day 6	The engrafted MSCs survived and accelerated healing of TNBS-induced colitis. After the implantation, the MSCs became potential sources of VEGF and TGF-β1, angiogenic and immunomodulating factors, in colon tissues.

Table 2. Published studies with mesenchymal stromal cells in experimental models of colitis. A literature search in Pubmed was performed using the following keywords, alone or in combination: 'mesenchymal stem cell', 'mesenchymal stromal cell', 'colitis', 'crohn's disease' and 'inflammatory bowel disease'.

the effects of IFN-γ prestimulation of MSCs (IMSCs) was assessed in vitro and in animal models of colitis, demonstrating an enhanced therapeutic activity of MSCs after IFN-γ exposure.

Clinical trials in patients with Crohn's disease

Currently, multiple trials on MSCs for the treatment of CD are registered in the public registries for clinical trials (Table 3). The indication is either active luminal disease, for which MSCs are injected intravenously, or fistulizing disease, for which MSCs are injected locally in fistula tracts. Cells are isolated from bone marrow or from adipose tissue and from either the patient itself (autologous) or from a healthy donor (allogeneic). At least three companies are currently investigating the application of MSCs in Crohn's disease, i.e. Anterogen (Korea), Cellerix (Spain) and Osiris (USA).

Completed trials

Safety of local application of atMSCs in the treatment of fistulizing CD was demonstrated in a phase I clinical trial in which in total nine fistulas in four patients were inoculated with autologous atMSCs. Although the results are preliminary and follow-up is short, they are interesting as after 8 weeks 75 percent of these fistulas were considered healed and no adverse effects were observed in any of these patients.[82] This phase I study was followed by a multicenter, randomized, controlled trial sponsored by Cellerix to evaluate the efficacy and safety of atMSCs. Forty-nine patients with complex perianal fistula from cryptoglandular disease (n=35) or CD (n=14) were included. Patients received fibrin glue or 20 million cells plus fibrin glue intralesionally. Fistula healing was evaluated at 8 weeks. If not healed, a second dose of fibrin glue or 40 million cells plus fibrin glue was administered, with healing evaluated 8 weeks later. Healing was defined as absence of drainage and complete reepithelization of the external openings. The proportion of patients whose fistulas were healed was significantly higher with atMSCs than with fibrin glue alone in the CD as well as the non CD patients.[83] Osiris Therapeutics uses Prochymal™, MSCs obtained from the bone marrow of healthy adult volunteer donors. Although a significant decrease of the Crohn's disease Activity Index (CDAI) score was observed in the phase I trial, [84] the company recently terminated a phase III trial because of a high placebo response rate. Unfortunately, the results have not yet been published in peer reviewed journals. In **Chapter 3** the feasibility and safety of the intravenous application of autologous MSCs obtained from the bone marrow (bmMSC) of CD patients, was assessed in a phase I trial.[85]

Ongoing studies

The Royal Perth Hospital (Australia) has just launched a multicenter phase II trial in 20 patients to evaluate the safety and efficacy of weekly intravenous infusion for 4 weeks with allogeneic bmMSCs. The University Hospital La Paz in Madrid is performing a phase I/IIa trial on allogeneic atMSCs in the local treatment of recto-vaginal fistula in CD. The Leiden University Medical Center (LUMC) is currently investigating the safety and preliminary efficacy of allogeneic bmMSCs in the induction of response for active fistulizing CD in a dose escalation study.

Main trial ID	Title	Indication	Cell type and source	Dose	Delivery route	Time of delivery	Primary endpoint	Phase and design	Patient number	Site/company
Recruiting										
NCT01011244	A Phase II Clinical Study of ADIPOPLUS (Autologous Cultured Adipose-derived Stem Cell) for the Treatment of Crohn's Fistula to Evaluate Safety and Efficacy	Fistula	autologous/at		in fistula site		1) Efficacy: more than half closure of fistula (week 8) 2)Safety: Clinically measured abnormality of laboratory tests and adverse events	Phase II, uncontrolled		Anterogen Co., Ltd.
NCT01090817	A Multicentre Australian Phase 2 Study to Evaluate Safety and Efficacy of Mesenchymal Stromal Cells for Treating Biologic Refractory Crohn's Disease	Luminal	allogeneic/bm	$2x10^6$/kg recipient weight	intravenous	weekly for 4 weeks	Clinical response to MSC: Reduction of Crohn's disease Activity score by 100 points or more at six weeks post start of therapy	Phase II, non-randomized, historical controls	20	Royal Perth Hospital
NCT00999115	Clinical Trial in Phase I-IIa to Study the Feasibility and Security of the Allogenic Use of Adipose-derived Stem Cells for the Local Treatment of Recto-vaginal Fistula in Crohn´s Disease	Recto-vaginal fistula	allogeneic/at	$20x10^6$ cells	intralesional injection	at baseline with a possible second administration of $40x10^6$	Percentage of subjects in whom the external openings of the treated rectovaginal fistula have closed (12 weeks)	Phase I-IIa	10	Biomedica del Hospital Universitario la Paz
NCT01020825	A Prospective Study for the Assessment of the Long-term Safety and Efficacy of Cx401 in Patients Taking Part in the FATT-1 Trial	Perianal fistula	autologous/at	20 and $40x10^6$	intralesional injection		Cumulative incidence of adverse events (clinical or laboratory) attributed to the study therapy in the preceding FATT-1 randomized trial (CX401 or fibrin glue) (6 months)	Prospective, observational	150	Cellerix
NCT01144962	Dose-escalating Therapeutic Study of Allogeneic Bone Marrow Derived Mesenchymal Stem Cells for the Treatment of Fistulas in Patients With Refractory Perianal Crohn's Disease	Perianal fistula	allogeneic/bm	10, 30, $90x10^6$ or placebo	intralesional injection	baseline	1) The number of adverse and serious adverse events and 2) a reduction in the number of draining fistulas (12 weeks)	Phase I-II, dose escalation	21	Leiden University Medical Center
Active, not recruiting										
NCT00482092	A Phase III, Multicenter, Placebo-Controlled, Randomized, Double-Blind Study to Evaluate the Safety and Efficacy of PROCHYMAL[tm] (ex Vivo Cultured Adult Human Mesenchymal Stem Cells) Intravenous Infusion for the Induction of Remission in Subjects Experiencing Treatment-Refractory Moderate-to-Severe Crohn's Disease	Luminal	allogeneic/bm	600 or 1200 $x10^6$ or placebo	intravenous	over 4 infusions in 2 weeks	Disease remission (CDAI at or below 150) (28 days)	Phase III, multicenter, placebo-controlled, randomized, double-blind	270	Osiris Therapeutics
Completed										
NCT00543374	A Phase III, Multicenter, Placebo-controlled, Randomized, Double-blind Durability and Retreatment Study to Evaluate the Safety and Efficacy of PROCHYMAL™ (ex Vivo Cultured Adult Human Mesenchymal Stem Cells) Intravenous Infusion for the Maintenance and Re-induction of Clinical Benefit and Remission in Subjects Experiencing Treatment-refractory Moderate-to-severe Crohn's Disease	Luminal	allogeneic/bm	NA	NA	NA	1) Duration of clinical benefit 2) Re-induction of clinical benefit (6 months)	Observational, double blind, randomized, placebo-controlled		Osiris Therapeutics
NTR1360	Bone Marrow Derived Mesenchymal Stem Cells for the Treatment of Refractory Crohn's Diseas	Luminal	autologous/bm	$2x10^6$/kg body weight	intravenous	2 infusions, one week apart	1) Safety: rate of (serious) adverse events in the study population 2) Feasibility: determination of the number of expanded MSCs in relation to the amount of bone marrow collected, number of passages required and time to reach study target doses (week 6)	Phase I, open label	10	Leiden University Medical Center
NCT00294112	A Phase II, Open-Label, Randomized Study to Evaluate the Safety and Efficacy of PROCHYMAL™ IBD (ex Vivo Cultured Adult Human Mesenchymal Stem Cells) Intravenous Infusion for the Treatment of Subjects Experiencing Moderate-to-Severe Crohn's Disease That is Refractory to Steroids and Immune Suppressants	Luminal	allogeneic/bm	2 or $8x10^6$/kg body weight	intravenous	2 infusions, one week apart	Crohn's disease activity index (28 days)	Phase I, open label, randomized	10	Osiris Therapeutics
NCT00992485	A Phase I Dose Escalation Clinical Study of ADIPOPLUS (Autologous Cultured Adipose-derived Stem Cell) for the Treatment of Crohn's Fistula to Evaluate Safety and Efficacy	Fistula	autologous/at	Escalating doses	in fistula site		1) Efficacy: closure of fistula (week 8) 2) Safety: Clinically measured abnormality of laboratory tests and adverse events	Phase I, dose escalation	9	Anterogen Co., Ltd.
NCT01144962	Dose-escalating Therapeutic Study of Allogeneic Bone Marrow Derived Mesenchymal Stem Cells for the Treatment of Fistulas in Patients With Refractory Perianal Crohn's Disease	Perianal fistula	allogeneic/bm	10, 30, $90x10^6$ or placebo	intralesional injection	baseline	1) The number of adverse and serious adverse events and 2) a reduction in the number of draining fistulas (12 weeks)	Phase I-II, dose escalation	21	Leiden University Medical Center

Table 3. Registered clinical trials on mesenchymal stromal cells in Crohn's disease. Both recruiting and completed trials are listed (sources: clinicaltrials.gov and trialregister.nl).

Safety issues and concerns

In the case of autologous MSCs, an ongoing discussion is whether MSCs are affected by or may contribute to the underlying disease. For instance, MSCs from patients with systemic lupus erythematosus are difficult to expand in culture and yield low cell numbers[62] and those from patients with multiple myeloma have been shown to be impaired and possibly contribute to the pathogenesis of the disease.[86] **Chapter 3** therefore evaluates MSCs obtained from refractory CD patients, focusing on growth potential, yield, and functional properties.

Previous studies showed that immunosuppressive drugs can be harmful to hematopoietic stem cells or endothelial progenitor cell proliferation, thereby affecting their functional capacities.[87-90] We hypothesized that likewise, immunosuppressive agents might have an effect on MSC function and could, therefore, change the outcome of MSC therapy and affect safety. **Chapter 4** investigates the interaction between MSCs and immunosuppressive drugs frequently used in the treatment of IBD.

REFERENCES

1. Sandler RS. Epidemiology of Inflammatory Bowel Disease. In: Targan SR and Shanahan F, eds. Inflammatory Bowel Disease; from bench to bedside. 1994 ed. Baltimore: Wiliams & Wilkins, 2007:5-30.
2. Loftus EV, Jr., Schoenfeld P, Sandborn WJ. The epidemiology and natural history of Crohn's disease in population-based patient cohorts from North America: a systematic review. Aliment Pharmacol Ther 2002;16:51-60.
3. Shivananda S, Lennard-Jones J, Logan R, Fear N, Price A, Carpenter L, van Blankenstein M. Incidence of inflammatory bowel disease across Europe: is there a difference between north and south? Results of the European Collaborative Study on Inflammatory Bowel Disease (EC-IBD). Gut 1996;39:690-697.
4. Abdullah BA, Gupta SK, Croffie JM, Pfefferkorn MD, Molleston JP, Corkins MR, Fitzgerald JF. The role of esophagogastroduodenoscopy in the initial evaluation of childhood inflammatory bowel disease: a 7-year study. J Pediatr Gastroenterol Nutr 2002;35:636-640.
5. Glickman RM. Inflammatory Bowel Disease: Ulcerative Colitis and Crohn's Disease. In: Fauci AS, Braynwald E, Isselbacher KJ, Wilson JD, Martin JB, Kasper DL, Hauser SL, and Longo DL, eds. Harrison's Principles of Internal Medicine. 14th edition ed. McGraw-Hill, 2007:1633-1646.
6. Morrison SJ, Shah NM, Anderson DJ. Regulatory mechanisms in stem cell biology. Cell 1997;88:287-298.
7. Prockop DJ. Marrow stromal cells as stem cells for nonhematopoietic tissues. Science 1997;276:71-74.
8. Okamoto R, Yajima T, Yamazaki M, Kanai T, Mukai M, Okamoto S, Ikeda Y, Hibi T, Inazawa J, Watanabe M. Damaged epithelia regenerated by bone marrow-derived cells in the human gastrointestinal tract. Nat Med 2002;8:1011-1017.
9. Brittan M, Hunt T, Jeffery R, Poulsom R, Forbes SJ, Hodivala-Dilke K, Goldman J, Alison MR, Wright NA. Bone marrow derivation of pericryptal myofibroblasts in the mouse and human small intestine and colon. Gut 2002;50:752-757.
10. Peppelenbosch MP, van Deventer SJ. T-cell apoptosis and inflammatory bowel disease. Gut 2004;53:1556-1558.
11. Komori M, Tsuji S, Tsujii M, Murata H, Iijima H, Yasumaru M, Nishida T, Irie T, Kawano S, Hori M. Involvement of bone marrow-derived cells in healing of experimental colitis in rats. Wound Repair Regen 2005;13:109-118.
12. Horwitz EM, Le Blanc K, Dominici M, Mueller I, Slaper-Cortenbach I, Marini FC, Deans RJ, Krause DS, Keating A. Clarification of the nomenclature for MSC: The International Society for Cellular Therapy position statement. Cytotherapy 2005;7:393-395.
13. Copelan EA. Hematopoietic stem-cell transplantation. N Engl J Med 2006;354:1813-1826.
14. Ikehara S. Treatment of autoimmune diseases by hematopoietic stem cell transplantation. Exp Hematol 2001;29:661-669.
15. Van Bekkum DW. Stem cell transplantation in experimental models of autoimmune disease. J Clin Immunol 2000;20:10-16.
16. Marmont AM. Stem cell transplantation for autoimmune disorders. Coincidental autoimmune disease in patients transplanted for conventional indications. Best Pract Res Clin Haematol 2004;17:223-232.
17. Van Laar JM, Tyndall A. Adult stem cells in the treatment of autoimmune diseases. Rheumatology (Oxford) 2006;45:1187-1193.
18. Tyndall A, Gratwohl A. Blood and marrow stem cell transplants in autoimmune disease. A consensus report written on behalf of the European League Against Rheumatism (EULAR) and the European Group for Blood and Marrow Transplantation (EBMT). Br J Rheumatol 1997;36:390-392.
19. Castro J, Bentch H.L., Smith L, Kalter S, Bachier C, Meneghatti C, Moore A, Oliversen S, LeMaistre CF. Prolonged clinical remission in patients with inflammatory bowel disease (IBD) after high dose chemotherapy (HDC) and autologous blood stem cell transplantation. ASH Annual Meeting Abstracts 1996;88:133a.
20. Hawkey CJ, Snowden JA, Lobo A, Beglinger C, Tyndall A. Stem cell transplantation for inflammatory bowel disease: practical and ethical issues. Gut 2000;46:869-872.
21. Kashyap A, Forman SJ. Autologous bone marrow transplantation for non-Hodgkin's lymphoma resulting in long-term remission of coincidental Crohn's disease. Br J Haematol 1998;103:651-652.
22. Musso M, Porretto F, Crescimanno A, Bondi F, Polizzi V, Scalone R. Crohn's disease complicated by relapsed extranodal Hodgkin's lymphoma: prolonged complete remission after unmanipulated PBPC autotransplant. Bone Marrow Transplant 2000;26:921-923.
23. Soderholm JD, Malm C, Juliusson G, Sjodahl R. Long-term endoscopic remission of crohn disease after autologous stem cell transplantation for acute myeloid leukaemia. Scand J Gastroenterol 2002;37:613-616.
24. Musso M, Porretto F, Crescimanno A, Bondi F, Polizzi V, Scalone R. Crohn's disease complicated by relapsed extranodal Hodgkin's lymphoma: prolonged complete remission after unmanipulated PBPC autotransplant. Bone Marrow Transplant 2000;26:921-923.
25. Talbot DC, Montes A, Teh WL, Nandi A, Powles RL. Remission of Crohn's disease following allogeneic bone marrow transplant for acute leukaemia. Hosp Med 1998;59:580-581.
26. Lopez-Cubero SO, Sullivan KM, McDonald GB. Course of Crohn's disease after allogeneic marrow transplantation. Gastroenterology 1998;114:433-440.
27. Ditschkowski M, Einsele H, Schwerdtfeger R, Bunjes D, Trenschel R, Beelen DW, Elmaagacli AH. Improvement of inflammatory bowel disease after allogeneic stem-cell transplantation. Transplantation 2003;75:1745-1747.
28. Hawkey CJ. Stem cell transplantation for Crohn's disease. Best Pract Res Clin Haematol 2004;17:317-325.

29. Lin Yin JA, Jowitt SN. Resolution of immune-mediated diseases following allogeneic bone marrow transplantation for leukaemia. Bone Marrow Transplant 1992;9:31-33.
30. Lin Yin JA. Transplantation for new indications: autoimmune diseases. In: Atkinson K, ed. Clinical Bone Marrow Transplantation. Cambridge: Cambridge University Press, 1994:699-703.
31. Hinterberger W, Hinterberger-Fischer M, Marmont A. Clinically demonstrable anti-autoimmunity mediated by allogeneic immune cells favorably affects outcome after stem cell transplantation in human autoimmune diseases. Bone Marrow Transplant 2002;30:753-759.
32. Burt RK, Traynor A, Oyama Y, Craig R. High-dose immune suppression and autologous hematopoietic stem cell transplantation in refractory Crohn disease. Blood 2003;101:2064-2066.
33. Craig RM, Traynor A, Oyama Y, Burt RK. Hematopoietic stem cell transplantation for severe Crohn's disease. Bone Marrow Transplant 2003;32 Suppl 1:S57-S59.
34. Kreisel W, Potthoff K, Bertz H, Schmitt-Graeff A, Ruf G, Rasenack J, Finke J. Complete remission of Crohn's disease after high-dose cyclophosphamide and autologous stem cell transplantation. Bone Marrow Transplant 2003;32:337-340.
35. Oyama Y, Craig RM, Traynor AE, Quigley K, Statkute L, Halverson A, Brush M, Verda L, Kowalska B, Krosnjar N, Kletzel M, Whitington PF, Burt RK. Autologous hematopoietic stem cell transplantation in patients with refractory Crohn's disease. Gastroenterology 2005;128:552-563.
36. Armitage JO. Bone marrow transplantation. N Engl J Med 1994;330:827-838.
37. Tyndall A, Passweg J, Gratwohl A. Haemopoietic stem cell transplantation in the treatment of severe autoimmune diseases 2000. Ann Rheum Dis 2001;60:702-707.
38. Tabbara IA, Zimmerman K, Morgan C, Nahleh Z. Allogeneic hematopoietic stem cell transplantation: complications and results. Arch Intern Med 2002;162:1558-1566.
39. Friedenstein AJ, Deriglasova UF, Kulagina NN, Panasuk AF, Rudakowa SF, Luria EA, Ruadkow IA. Precursors for fibroblasts in different populations of hematopoietic cells as detected by the in vitro colony assay method. Exp Hematol 1974;2:83-92.
40. Dominici M, Le BK, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop D, Horwitz E. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 2006;8:315-317.
41. Bartholomew A, Sturgeon C, Siatskas M, Ferrer K, McIntosh K, Patil S, Hardy W, Devine S, Ucker D, Deans R, Moseley A, Hoffman R. Mesenchymal stem cells suppress lymphocyte proliferation in vitro and prolong skin graft survival in vivo. Exp Hematol 2002;30:42-48.
42. Glennie S, Soeiro I, Dyson PJ, Lam EW, Dazzi F. Bone marrow mesenchymal stem cells induce division arrest anergy of activated T-cells. Blood 2005;105:2821-2827.
43. Krampera M, Glennie S, Dyson J, Scott D, Laylor R, Simpson E, Dazzi F. Bone marrow mesenchymal stem cells inhibit the response of naive and memory antigen-specific T-cells to their cognate peptide. Blood 2003;101:3722-3729.
44. Corcione A, Benvenuto F, Ferretti E, Giunti D, Cappiello V, Cazzanti F, Risso M, Gualandi F, Mancardi GL, Pistoia V, Uccelli A. Human mesenchymal stem cells modulate B-cell functions. Blood 2006;107:367-372.
45. Sotiropoulou PA, Perez SA, Gritzapis AD, Baxevanis CN, Papamichail M. Interactions between human mesenchymal stem cells and natural killer cells. Stem Cells 2006;24:74-85.
46. Spaggiari GM, Capobianco A, Becchetti S, Mingari MC, Moretta L. Mesenchymal stem cell-natural killer cell interactions: evidence that activated NK cells are capable of killing MSCs, whereas MSCs can inhibit IL-2-induced NK-cell proliferation. Blood 2006;107:1484-1490.
47. Nauta AJ, Kruisselbrink AB, Lurvink E, Willemze R, Fibbe WE. Mesenchymal stem cells inhibit generation and function of both CD34+-derived and monocyte-derived dendritic cells. J Immunol 2006;177:2080-2087.
48. Aggarwal S, Pittenger MF. Human mesenchymal stem cells modulate allogeneic immune cell responses. Blood 2005;105:1815-1822.
49. Ren G, Zhang L, Zhao X, Xu G, Zhang Y, Roberts AI, Zhao RC, Shi Y. Mesenchymal stem cell-mediated immunosuppression occurs via concerted action of chemokines and nitric oxide. Cell Stem Cell 2008;2:141-150.
50. Krampera M, Cosmi L, Angeli R, Pasini A, Liotta F, Andreini A, Santarlasci V, Mazzinghi B, Pizzolo G, Vinante F, Romagnani P, Maggi E, Romagnani S, Annunziato F. Role for interferon-gamma in the immunomodulatory activity of human bone marrow mesenchymal stem cells. Stem Cells 2006;24:386-398.
51. Gieseke F, Schutt B, Viebahn S, Koscielniak E, Friedrich W, Handgretinger R, Muller I. Human multipotent mesenchymal stromal cells inhibit proliferation of PBMCs independently of IFNgammaR1 signaling and IDO expression. Blood 2007;110:2197-2200.
52. Ren G, Zhang L, Zhao X, Xu G, Zhang Y, Roberts AI, Zhao RC, Shi Y. Mesenchymal stem cell-mediated immunosuppression occurs via concerted action of chemokines and nitric oxide. Cell Stem Cell 2008;2:141-150.
53. Le BK, Ringden O. Immunomodulation by mesenchymal stem cells and clinical experience. J Intern Med 2007;262:509-525.
54. Rasmusson I, Le BK, Sundberg B, Ringden O. Mesenchymal stem cells stimulate antibody secretion in human B cells. Scand J Immunol 2007;65:336-343.
55. Zappia E, Casazza S, Pedemonte E, Benvenuto F, Bonanni I, Gerdoni E, Giunti D, Ceravolo A, Cazzanti F, Frassoni F, Mancardi G, Uccelli A. Mesenchymal stem cells ameliorate experimental autoimmune encephalomyelitis inducing T-cell anergy. Blood 2005;106:1755-1761.
56. Wu GD, Nolta JA, Jin YS, Barr ML, Yu H, Starnes VA, Cramer DV. Migration of mesenchymal stem cells to heart allografts during chronic rejection. Transplantation 2003;75:679-685.
57. Chapel A, Bertho JM, Bensidhoum M, Fouillard L, Young RG, Frick J, Demarquay C, Cuvelier F, Mathieu E, Trompier F, Dudoignon N, Germain C, Mazurier C, Aigueperse J, Borneman J, Gorin NC, Gourmelon P, Thierry D. Mesenchymal stem cells home to injured tissues when co-infused with hematopoietic cells to treat a radiation-induced multi-organ failure syndrome. J Gene Med 2003;5:1028-1038.
58. Lee RH. Intravenous hMSCs improve myocardial infarction in mice because cells embolized in lung are activated to secrete the anti-inflammatory protein TSG-6. Cell Stem Cell. 2009 Jul 2;5(1):54-63.
59. Parekkadan B, Upadhyay R, Dunham J, Iwamoto Y, Mizoguchi E, Mizoguchi A, Weissleder R, Yarmush ML. Bone Marrow Stromal Cell Transplants Prevent Experimental Enterocolitis and Require Host CD11b(+) Splenocytes. Gastroenterology 2010.
60. Stolzing A, Jones E, McGonagle D, Scutt A. Age-related changes in human bone marrow-derived mesenchymal stem cells: consequences for cell therapies. Mech Ageing Dev 2008;129:163-173.
61. Caplan AI. Adult mesenchymal stem cells for tissue engineering versus regenerative medicine. J Cell Physiol 2007;213:341-347.
62. Sun LY, Zhang HY, Feng XB, Hou YY, Lu LW, Fan LM. Abnormality of bone marrow-derived mesenchymal stem cells in patients with systemic lupus erythematosus. Lupus 2007;16:121-128.
63. Corre J, Mahtouk K, Attal M, Gadelorge M, Huynh A, Fleury-Cappellesso S, Danho C, Laharrague P, Klein B, Reme T, Bourin P. Bone marrow mesenchymal stem cells are abnormal in multiple myeloma. Leukemia 2007;21:1079-1088.
64. Arnulf B, Lecourt S, Soulier J, Ternaux B, Lacassagne MN, Crinquette A, Dessoly J, Sciaini AK, Benbunan M, Chomienne C, Fermand JP, Marolleau JP, Larghero J. Phenotypic and functional characterization of bone marrow mesenchymal stem cells derived from patients with multiple myeloma. Leukemia 2007;21:158-163.
65. Nauta AJ, Westerhuis G, Kruisselbrink AB, Lurvink EG, Willemze R, Fibbe WE. Donor-derived mesenchymal stem cells are immunogenic in an allogeneic host and stimulate donor graft rejection in a nonmyeloablative setting. Blood 2006;108:2114-2120.
66. Eliopoulos N, Stagg J, Lejeune L, Pommey S, Galipeau J. Allogeneic marrow stromal cells are immune rejected by MHC class I- and class II-mismatched recipient mice. Blood 2005;106:4057-4065.
67. Horwitz EM, Prockop DJ, Fitzpatrick LA, Koo WW, Gordon PL, Neel M, Sussman M, Orchard P, Marx JC, Pyeritz RE, Brenner MK. Transplantability and therapeutic effects of bone marrow-derived mesenchymal cells in children with osteogenesis imperfecta. Nat Med 1999;5:309-313.

68. Lazarus HM, Koc ON, Devine SM, Curtin P, Maziarz RT, Holland HK, Shpall EJ, McCarthy P, Atkinson K, Cooper BW, Gerson SL, Laughlin MJ, Loberiza FR, Jr., Moseley AB, Bacigalupo A. Cotransplantation of HLA-identical sibling culture-expanded mesenchymal stem cells and hematopoietic stem cells in hematologic malignancy patients. Biol Blood Marrow Transplant 2005;11:389-398.

69. Le Blanc K., Rasmusson I, Sundberg B, Gotherstrom C, Hassan M, Uzunel M, Ringden O. Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. Lancet 2004;363:1439-1441.

70. Rubio D, Garcia-Castro J, Martin MC, de la FR, Cigudosa JC, Lloyd AC, Bernad A. Spontaneous human adult stem cell transformation. Cancer Res 2005;65:3035-3039.

71. Tolar J, Nauta AJ, Osborn MJ, Panoskaltsis MA, McElmurry RT, Bell S, Xia L, Zhou N, Riddle M, Schroeder TM, Westendorf JJ, McIvor RS, Hogendoorn PC, Szuhai K, Oseth L, Hirsch B, Yant SR, Kay MA, Peister A, Prockop DJ, Fibbe WE, Blazar BR. Sarcoma derived from cultured mesenchymal stem cells. Stem Cells 2007;25:371-379.

72. Djouad F, Plence P, Bony C, Tropel P, Apparailly F, Sany J, Noel D, Jorgensen C. Immunosuppressive effect of mesenchymal stem cells favors tumor growth in allogeneic animals. Blood 2003;102:3837-3844.

73. Karnoub AE, Dash AB, Vo AP, Sullivan A, Brooks MW, Bell GW, Richardson AL, Polyak K, Tubo R, Weinberg RA. Mesenchymal stem cells within tumour stroma promote breast cancer metastasis. Nature 2007;449:557-563.

74. Centeno CJ, Schultz JR, Cheever M, Robinson B, Freeman M, Marasco W. Safety and complications reporting on the re-implantation of culture-expanded mesenchymal stem cells using autologous platelet lysate technique. Curr Stem Cell Res Ther 2010;5:81-93.

75. Vogel G. Cell biology. To scientists' dismay, mixed-up cell lines strike again. Science 2010;329:1004.

76. Schrepfer S, Deuse T, Reichenspurner H, Fischbein MP, Robbins RC, Pelletier MP. Stem cell transplantation: the lung barrier. Transplant Proc 2007;39:573-576.

77. Hayashi Y, Tsuji S, Tsujii M, Nishida T, Ishii S, Iijima H, Nakamura T, Eguchi H, Miyoshi E, Hayashi N, Kawano S. Topical implantation of mesenchymal stem cells has beneficial effects on healing of experimental colitis in rats. J Pharmacol Exp Ther 2008;326:523-531.

78. Tanaka F, Tominaga K, Ochi M, Tanigawa T, Watanabe T, Fujiwara Y, Ohta K, Oshitani N, Higuchi K, Arakawa T. Exogenous administration of mesenchymal stem cells ameliorates dextran sulfate sodium-induced colitis via anti-inflammatory action in damaged tissue in rats. Life Sci 2008;83:771-779.

79. Gonzalez MA, Gonzalez-Rey E, Rico L, Buscher D, Delgado M. Adipose-derived mesenchymal stem cells alleviate experimental colitis by inhibiting inflammatory and autoimmune responses. Gastroenterology 2009;136:978-989.

80. Gonzalez-Rey E, Anderson P, Gonzalez MA, Rico L, Buscher D, Delgado M. Human adult stem cells derived from adipose tissue protect against experimental colitis and sepsis. Gut 2009;58:929-939.

81. Zhang Q, Shi S, Liu Y, Uyanne J, Shi Y, Shi S, Le AD. Mesenchymal stem cells derived from human gingiva are capable of immunomodulatory functions and ameliorate inflammation-related tissue destruction in experimental colitis. J Immunol 2009;183:7787-7798.

82. Garcia-Olmo D, Garcia-Arranz M, Herreros D, Pascual I, Peiro C, Rodriguez-Montes JA. A phase I clinical trial of the treatment of Crohn's fistula by adipose mesenchymal stem cell transplantation. Dis Colon Rectum 2005;48:1416-1423.

83. Garcia-Olmo D, Herreros D, Pascual I, Pascual JA, Del-Valle E, Zorrilla J, De-La-Quintana P, Garcia-Arranz M, Pascual M. Expanded adipose-derived stem cells for the treatment of complex perianal fistula: a phase II clinical trial. Dis Colon Rectum 2009;52:79-86.

84. Newman RE, Yoo D, LeRoux MA, nilkovitch-Miagkova A. Treatment of inflammatory diseases with mesenchymal stem cells. Inflamm Allergy Drug Targets 2009;8:110-123.

85. Duijvestein M, Vos AC, Roelofs H, Wildenberg ME, Wendrich BB, Verspaget HW, Kooy-Winkelaar EM, Koning F, Zwaginga JJ, Fidder HH, Verhaar AP, Fibbe WE, van den Brink GR, Hommes DW. Autologous bone marrow-derived mesenchymal stromal cell treatment for refractory luminal Crohn's disease: results of a phase I study. Gut 2010;59:1662-1669.

86. Arnulf B, Lecourt S, Soulier J, Ternaux B, Lacassagne MN, Crinquette A, Dessoly J, Sciaini AK, Benbunan M, Chomienne C, Fermand JP, Marolleau JP, Larghero J. Phenotypic and functional characterization of bone marrow mesenchymal stem cells derived from patients with multiple myeloma. Leukemia 2007;21:158-163.

87. Buhler L, Kurilla-Mahon B, Chang Q, Abraham S, Alwayn IP, Appel JZ, III, Newman D, Awwad M, White-Scharf ME, Sackstein R, Sachs DH, Cooper DK, Down JD. Cryopreservation and mycophenolate therapy are detrimental to hematopoietic progenitor cells. Transplantation 2002;74:1159-1166.

88. Chen TG, Chen JZ, Wang XX. Effects of rapamycin on number activity and eNOS of endothelial progenitor cells from peripheral blood. Cell Prolif 2006;39:117-125.

89. Guo J, Zeng Y, Liang Y, Wang L, Su H, Wu W. Cyclosporine affects the proliferation and differentiation of neural stem cells in culture. Neuroreport 2007;18:863-868.

90. Miriuka SG, Rao V, Peterson M, Tumiati L, Delgado DH, Mohan R, Ramzy D, Stewart D, Ross HJ, Waddell TK. mTOR inhibition induces endothelial progenitor cell death. Am J Transplant 2006;6:2069-2079.

91. Yabana T, Arimura Y, Tanaka H, Goto A, Hosokawa M, Nagaishi K, Yamashita K, Yamamoto H, Adachi Y, Sasaki Y, Isobe M, Fujimiya M, Imai K, Shinomura Y. Enhancing epithelial engraftment of rat mesenchymal stem cells restores epithelial barrier integrity. J Pathol. 2009 Jul;218(3):350-9.