Special Issue: Inflammatory Bowel Diseases and Intestinal Epithelial Stem Cells

Mini Review

Regenerative medicine for inflammatory bowel disease

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Inflammatory bowel disease (IBD) is potentially curable by "immune rest" and correction of the genetic predisposition inherent in allogeneic hematopoietic stem cell transplantation. However, balancing risks against benefits remains challenging. Recently, application of mesenchymal stem cells (MSCs) serving as a site-regulated "drugstore" is a new concept which creates the possibility of an alternative treatment for many intractable diseases, such as IBD. Depending on the required function of MSC as a cell provider, immune moderator, and/or trophic resource, MSC therapy should be optimized; surprisingly, therapeutic effects do not always require full engraftment of MSCs, but rely on the capacity of MSCs to inhibit pathogenic immune responses and release trophic factors favoring tissue repair. Therefore, optimization of pleiotropic gut trophic factors produced by MSCs must directly enhance new drug discovery for IBD.

Stem cell biology holds great promise for a new era of cell-based therapy, sparking considerable interest among scientists, clinicians, and their patients. However, the translational arm of stem cell science is in a relatively primitive state. Although several clinical studies using MSCs have been initiated, the early results suggest several inherent problems. In all of them, optimization of MSC therapy appears to be the most urgent problem, to be resolved only by scientifically unveiling the mechanisms of therapeutic action. The authors believe that such information would facilitate the critical steps in the paradigm shift from stem cell biology to regenerative medicine for conquering IBD in near future.

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Introduction

Although intestinal wound healing comprises each step of epithelial restitution, proliferation, and maturation, the process is classically divided into four phases: namely, hemostasis, inflammation, proliferation, and remodeling[1]. The pathogenesis of inflammatory bowel disease (IBD) involves multiple complex inter-relationships among genetic predisposition, environmental factors and immunological abnormalities. Intriguingly, it notes, in addition to environmental factors, the extreme differences in IBD susceptible genes identified by recent genome-wide association studies[2] among the population examined. It is strongly suggested whether true culprit disease susceptibility to IBD beyond racial difference leaves undiscovered or Japanese IBD could be quite different from western IBD to the genetic predisposition. In any case, such new findings must provide important clues for further novel approaches against IBD.

Unfortunately, established therapy for IBD has been dominated by a focus on inflammation in the wound healing process and on immunological abnormalities in its pathogenesis. The optimal IBD therapy aiming for complete cure should not only block inflammation but also enhance proliferation and remodeling during healing. Furthermore, intestinal homeostasis should be regulated by extra-intestinal as well as local machinery in the larger picture of health and disease in the intestine. Consequently, therapeutic target cells should transform inflammatory cells to intestinal (stem) cell, stromal cells, or bone marrow (stem) cells, and therapeutics should advance to include stem cell-based or gene-based therapy. Stem cell biology and regenerative medicine must provide a backbone to such a paradigm shift in future IBD therapeutics.

HSCT and IBD

The concept and practice of hematopoietic stem cell transplantation (HSCT) as a primary treatment for immune-mediated inflammatory diseases (IMIDs), such as IBD, began in the late 1990s. Allogenic HSCT should theoretically be preferable for cure than autologous HSCT, as there is a graft-versus-host reaction that removes the presumed disease-causing T cells that might survive conditioning[3]. Since allogenic transplantation is associated with higher transplant-related morbidity and mortality owing to graft-versus-host disease (GvHD), more autologous transplants have been performed for IMIDs. The rationale of autologous HSCT for autoimmune diseases is to reset the immune system, that is, to generate new self-tolerant lymphocytes after chemotherapy-induced elimination of self- or auto-reactive lymphocytes (i.e., lymphoablation). Allogeneic HSCT is based on the rationale of both immune reset and on correcting the genetic predisposition to disease by replacing with non-disease-prone HSCs from a healthy donor.

Since the first report was published in 1993[4], a total of 25 IBD consisting of 20 CD and 5 UC patients who underwent HSCT (7 autologous and 18 allogeneic) for cancer have been reported (Table 1)[5-12]. Overall, 22 of 25 (88%) patients have achieved clinical remission over a median follow-up of 20 months. HSCT may result in long-lasting remission without any need for IBD medications in the most patients. There have been two transplant-related infectious deaths. Interestingly, the patient who relapsed 18 months after transplantation in the Seattle series had mixed chimerism, whereas the four patients who achieved sustained remission were assumed to be complete bone marrow chimeras[13]. These studies support the notion that "lymphoablation" and "immune reset" can induce long-lasting remissions in IBD patients. In contrast, a patient without IBD undergoing allogeneic HSCT developed severe CD soon after transplantation[14]. Investigations showed that the transplanted stem cell harbored a pathogenetic *NOD2* mutation.

The first Phase I study[15] included 12 patients with active CD refractory to conventional therapies including anti-TNFα treatment. There was an early and sustained clinical remission in 11 of the 12 (91.7%) patients after a median follow-up of 18.5 months. One patient experienced CD relapse at 15 months post-transplantation. A second Phase I/II study included four refractory patients[16]. Three of the four (75%) patients had sustained remission after a median follow-up of 16.5 months. All patients tolerated HSCT well and no mortality was observed in the above two series of patients. Observations of three additional case reports published so far also suggest that sustained clinical remission with HSCT is initially likely to result from lymphoablation by drugs used in the conditioning regimen; later may be an effect of altered immune reconstitution[17-19]. Although these observations underscore a curative trend of allogenic HSCT, a balance between risks and benefits remains ambiguous in the setting[20].

Table 1 Hematopoietic stem cell transplantation for the treatment of cancer in patients with inflammatory bowel disease

Author (year) [Ref]	Primary Disease	Type	IBD	Duration (yr)	Age Sex (F/M)	Mortality (n)	AEs	Outcome
Drakos (1993) [4]	NHL	Auto	1 CD	22	41 F	0	0	R (6 mo)
Castro (1996) [7]	Breast cancer	Auto	1 CD	11	NA F	0	NA	R (> 2yr)
Kashyap (1998) [8]	NHL	Auto	1 CD	8	21 M	0	0	R (7 yr)
Talbot (1998) [6]	Leukemia	Allo	1 CD	7	35 M	0	GvHD	R (8 yr)
Lopez-Cubero (1998) [13]	Leukemia	Allo	6 CD	3-29	27-46 M	3	6 GvHD	R (4-15yr) 1r (1.5yr)
Musso (2000) [9]	HL	Auto	1 CD	10	30 M	0	0	R (3 yr)
Marti (2001) [12]	Breast cancer	Auto	1 UC	7	57 F	0	0	r (20 mo)
Soderholm (2002) [10]	Leukemia	Auto	1 CD	3	57 F	0	Sepsis	R (5 yr)
Ditschkowski (2003) [5]	1 MDS/ 10 leukemia	Allo	4 UC 7 CD	Median 10	27-55 6/5	1	8 GvHD 1 inf	R (median 34 mo)
Anumakonda (2007) [11]	NHL	Auto	1 CD	16	32 F	0	NA	r (8 yr)

Abbreviation: AEs; adverse effects, NHL; non-Hodgkin lymphoma, HL; Hodgkin lymphoma, MDS; myelodysplastic syndrome, Auto; autologous, Allo; allogeneic, CD; Crohn disease, UC; ulcerative colitis, NA; not available, R; remission, r; relapse, inf; infection.

Table 2 Hematopoietic stem cell transplantation as a primary treatment for Crohn's disease

Author (year) [Ref]	Type	n	Cell Selection	Duration (yr)	Age Sex (F/M)	AEs	Outcome
Kreisel (2003) [17]	Auto	1	Yes	14	36 M	0	R (9 mo)
Scime (2004) [18]	Auto	1	Yes	2.5	55 M	0	R (5 mo)
Oyama (2005) [15]	Auto	12	Yes	1.5-20	15-38 6/6	1 Stenosis	11 R (12 mo) 1 r (18 mo)
Cassinotti (2008)[16]	Auto	4	No	NA	26-45 1/3	0	3 R (3-12 mo) 1 r (4 mo)
Glocker (2009) [19]	Allo	1	NA	9	9 M	0	R (2 yr)

Abbreviation: AEs; adverse effects, Auto; autologous, Allo; allogeneic, NA; not available, R; remission, r; relapse.

MSC and IBD

Mesenchymal stem cells (MSCs) are noted for active proliferation, plastic differentiation, strong immunomodulation, low immunogenicity, and abundant trophic factor production. Collectively, the wide range of advantageous *in vivo* effects of MSCs, from cell replacement and immunosuppression to trophic effects, drives their increasing use in regenerative medicine and immune intervention. Detailed reviews of MSCs as a cell provider are found elsewhere[21]. Successful preclinical studies using MSCs in models of autoimmunity, inflammation and tissue damage have paved the way for clinical trials. Le Blanc K, et al reported a milestone GvHD case who was successfully treated with an immunosuppressive effect of MSCs *in vivo*, rather than an induction of tolerance[22]. Since then, in humans, *ex vivo*-expanded allogeneic MSCs have been infused in several Phase I studies[23-25]. No adverse events during or after MSC infusion have been observed so far. In Crohn's disease (CD), encouraging results from studies using locally administered adipose stem cells to treat complex perianal

fistulae support the use of this approach for patients unresponsive to infliximab[26].

However, possible long-term adverse effects and the mechanisms of the therapeutic action remain unproven. Since the transplantation efficacy of MSC is extremely poor and the duration of detectable MSC-derived cells in the impaired intestinal tissues is short, maximum benefit from their therapeutic action will require more efficient engraftment, proliferation, and/or differentiation of repopulating MSCs in recipient tissues. The likelihood of achieving this remains uncertain. In our experiments, we found a wide array of cues in the micromilieu for MSC engraftment in experimental colitis; MSC engrafted into the intestinal epithelial region in the condition[27] while into stromal tissues in the other condition[28]. In either case, engrafted MSCs are so scarce that we cannot observe their dynamics in the recipient intestine. There also seem to be conflicting reports on the role of MSCs on carcinogenesis in the previous reports[29, 30], MSCs can promote or inhibit carcinogenesis depending on the micromilieu cue in our study (data not shown).

Stem cell-based therapy with curative intent is growing in importance as the next generation therapy for IBD. However, before broader clinical application of MSCs as a cell-based therapy, it is essential to set clear therapeutic targets and to understand the precise mechanism of repair in each clinical setting to ensure optimal MSC therapy. This includes the source and type of MSC (autologous or allogeneic), the quality control of prepared MSCs, procedure of administration (route, schedule, dose, pretreatment with cytokines or chemokines, etc), which factors raise transplantation efficacy and control appropriate differentiation in the desired location. Surprisingly, the therapeutic effects of MSCs do not depend on their full engraftment, but rely on the capacity of MSCs to inhibit pathogenic immune responses and release trophic factors favoring tissue repair[31, 32]. We found that conditioned medium of MSCs including pleiotropic gut trophic factors promoted intestinal epithelial repair. Consequently, we believe that strategies to optimize MSC preconditioning and the contents of MSC conditioned medium would open new avenues for drug discovery and establish a basis for cell-based therapy for IBD.

Intestinal stem cell and IBD

Recently, gastrointestinal stem cell culture has been established in humans as well as rodents[33, 34]; transplantation of these stem cells opens real possibilities of novel approaches to intractable gastrointestinal disease, such as IBD. However, transplantation of intestinal stem cells remains in its infancy, readers should refer elsewhere for more on this subject.

Future perspectives

Notwithstanding the enticing perspective of immune reset, it is unrealistic to believe that autologous HSCT can eradicate immune disease because a genetic predisposition may remain unmodified in the transplanted stem cells and disease recurrence remains a potential risk. The last decade has seen a remarkable shift in our appreciation of the potential uses for MSCs instead of HSCs. The challenge now is the scientific evaluation of MSC therapy against conventional treatments for IBD in randomized controlled trials. The possibility of using allogeneic MSCs and the immunomodulatory effects of MSCs in preventing GvHD is a promising and safer perspective for testing this treatment in IBD. Furthermore, optimization of pleiotropic gut trophic factor produced by MSCs should potentiate new drug discoveries for IBD. Significant issues remain respecting the design and interpretation of studies on MSCs, including patient selection, disease stage, disease activity, and long-term safety.

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References

1) Stadelmann WK, Digenis AG, Tobin GR: Physiology and healing dynamics of chronic cutaneous wounds. Am J Surg. 1998; 176: 26S-38S.
2) Van Limbergen J, Wilson DC, Satsangi J: The genetics of Crohn's disease. Annu Rev Genomics Hum Genet. 2009; 10: 89-116.
3) Marmont AM: Immunoablation followed or not by hematopoietic stem cells as an intense therapy for severe autoimmune diseases. New perspectives, new problems. Haematologica. 2001; 86: 337-345.
4) Drakos PE, Nagler A, Or R: Case of Crohn's disease in bone marrow transplantation. Am J Hematol. 1993; 43: 157-158.
5) Ditschkowski M, Einsele H, Schwerdtfeger R, Bunjes D, Trenschel R, Beelen DW, Elmaagacli AH: Improve-

ment of inflammatory bowel disease after allogeneic stem-cell transplantation. Transplantation. 2003; 75: 1745-1747.
6) 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.
7) Castro J, Bentch HI, Smith HL: Prolonged clinical remission in patients with inflammatory bowel disease after high dose chemotherapy and autologous blood stem cell transplantation. Blood. 1996; 88: 133A.
8) 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.
9) 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.
10) 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.
11) Anumakonda V, Hayee B, Chung-Faye G: Remission and relapse of Crohn's disease following autologous haematopoietic stem cell transplantation for non-Hodgkin's lymphoma. Gut. 2007; 56: 1325.
12) Marti JL, Mayordomo JI, Isla MD, Saenz A, Escudero P, Tres A: PBSC autotransplant for inflammatory bowel disease (IBD): a case of ulcerative colitis. Bone Marrow Transplant. 2001; 28: 109-110.
13) Lopez-Cubero SO, Sullivan KM, McDonald GB: Course of Crohn's disease after allogeneic marrow transplantation. Gastroenterology. 1998; 114: 433-440.
14) Sonwalkar SA, James RM, Ahmad T, Zhang L, Verbeke CS, Barnard DL, et al: Fulminant Crohn's colitis after allogeneic stem cell transplantation. Gut. 2003; 52: 1518-1521.
15) Oyama Y, Craig RM, Traynor AE, Quigley K, Statkute L, Halverson A, et al: Autologous hematopoietic stem cell transplantation in patients with refractory Crohn's disease. Gastroenterology. 2005; 128: 552-563.
16) Cassinotti A, Annaloro C, Ardizzone S, Onida F, Volpe AD, Clerici1 M, et al: Autologous hematopoietic stem cell transplantation without CD34+ cell selection in refractory Crohn's disease. Gut. 2008; 57: 211-217.
17) Scimè R, Cavallaro AM, Tringali S, Santoro A, Rizzo A, Montalbano L, Cottone M: Complete clinical remission after high-dose immune suppression and autologous hematopoietic stem cell transplantation in severe Crohn's disease refractory to immunosuppressive and immunomodulator therapy. Inflamm Bowel Dis. 2004; 10: 892-894.
18) Kreisel W, Potthoff K, Bertz H, Schmitt-Graeff A, Ruf G, Rasenack J, FinkeJ: Complete remission of Crohn's disease after high-dose cyclophosphamide and autologous stem cell transplantation. Bone Marrow Transplant. 2003; 32: 337-340.
19) Glocker EO, Kotlarz D, Boztug K, Gertz EM, Schäffer AA, Noyan F, et al: Inflammatory bowel disease and mutations affecting the interleukin-10 receptor. N Engl J Med. 2009; 361: 2033-2045.
20) Bohgaki T, Atsumi T, Koike T: Multiple autoimmune diseases after autologous stem-cell transplantation. N Engl J Med.2007; 357: 2734-2736.
21) Richardson SM, Hoyland JA, Mobasheri R, Csaki C, Shakibaei M, Mobasheri A: Mesenchymal stem cells in regenerative medicine: opportunities and challenges for articular cartilage and intervertebral disc tissue engineering. J Cell Physiol. 2010; 222: 23-32.
22) Le Blanc K, Rasmusson I, Sundberg B, Götherström C, Hassan M, Uzunel M, Ringdén O: Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. Lancet. 2004; 363; 1439-1441.
23) Koc ON, Day J, Nieder M, Gerson SL, Lazarus HM, Krivit W: Allogeneic mesenchymal stem cell infusion for treatment of metachromatic leukodystrophy (MLD) and Hurler syndrome (MPS-IH). Bone Marrow Transplant. 2002; 30: 215-222.
24) Horwitz EM, Gordon PL, Koo WK, Marx JC, Neel MD, McNall RY, et al: Isolated allogeneic bone marrow-derived mesenchymal cells engraft and stimulate growth in children with osteogenesis imperfecta: implications for cell therapy of bone. Proc Natl Acad Sci USA. 2002; 99: 8932-8937.
25) Le Blanc K, Frassoni F, Ball L, Locatelli F, Roelofs H, Lewis I, et al: Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: a Phase II study. Lancet. 2008; 371: 1579-1586.

26) 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.
27) Yabana T, Arimura Y, Tanaka H, Goto A, Hosokawa M, Nagaishi K, et al: Enhancing epithelial engraftment of rat mesenchymal stem cells restores epithelial barrier integrity. J Pathol. 2009; 218: 350-359.
28) Tanaka H, Arimura Y, Yabana T, Goto A, Hosokawa M, Nagaishi K, et al: Myogenic lineage differentiated mesenchymal stem cells enhance recovery from dextran sulfate sodium-induced colitis in the rat. J Gastroenterol. 2011; 46: 143-152.
29) Khakoo AY, Pati S, Anderson SA, Reid W, Elshal MF, Rovira II, et al: Human mesenchymal stem cells exert potent antitumorigenic effects in a model of Kaposi's sarcoma. J Exp Med. 2006; 203: 1235-1247.
30) Karnoub AE, Dash AB, Vo AP, Sullivan A, Brooks MW, Bell GW, et al: Mesenchymal stem cells within tumour stroma promote breast cancer metastasis. Nature. 2007; 449: 557-565.
31) Uccelli A, Laroni A, Freedman MS: Mesenchymal stem cells for the treatment of multiple sclerosis and other neurological diseases. Lancet Neurol. 2011; 10: 649-656.
32) Hansson EM,Lindsay ME, Chien KR: Regeneration next: Toward heart stem cell therapeutics. Cell Stem Cell. 2009; 5: 364-377.
33) Jung P, Sato T, Merlos-Suárez A, Barriga FM, Iglesias M, Rossell D, et al: Isolation and in vitro expansion of human colonic stem cells. Nat Med. 2011; 17: 1225-1227.
34) Sato T, Stange DE, Ferrante M, Vries RGJ, Vanes JH, Van Den Brink S, et al: Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium. Gastroenterology. 2011; 141: 1762-1772.