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Mini Review

Immunomodulation of mesenchymal stem/stromal cells for the onset of cGVHD

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Mesenchymal stem/stromal cells (MSC) present in many tissues that are multipotent which can differentiate into several different cell types. Mesenchymal stem cells are also believed to act on the inflammatory-immune reactions in the local to release humoral factors by integrating the tissue injury site. MSCs migrate to the damaged tissue site to involve in tissue repair when they administered exogenously. Communicating with the inflammatory microenvironment is an important part of this process. In recent years, it has been studied that the cellular and molecular mechanisms of interaction between MSC and the various participants in inflammation. Depending on their type and strength of the inflammatory stimulus, the MSC changes its role either for suppressive or inducible for the immune response. Here, we review the current paradigm about the immunomodulatory functions of MSCs in according with our recent finding of cGVHD animal model study.

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Graft-versus-host disease (GVHD)

GVHD is a common complication following an allogeneic bone marrow transplant. Immune cells in the graft recognize the recipient (the host) as "foreign". The transplanted immune cells then attack the host's body cells. In the clinical setting, GVHD is divided into two forms. The acute form of the disease (aGVHD) is normally observed within the first 100 days post-transplant, and is a major challenge to transplants owing to associated morbidity and mortality. The chronic form (cGVHD) normally occurs after 100 days. And it shows distinct clinical findings, which resemble features of autoimmune disease such as systemic sclerosis or Sjögren's syndrome involving exocrine glands[1, 2], and is believed to be distinct from the potentially lethal acute form of GVHD. This autoimmune phenotype has been attributed to donor-derived T cells that escape negative selection by the host thymus damaged by acute GVHD[3, 4].

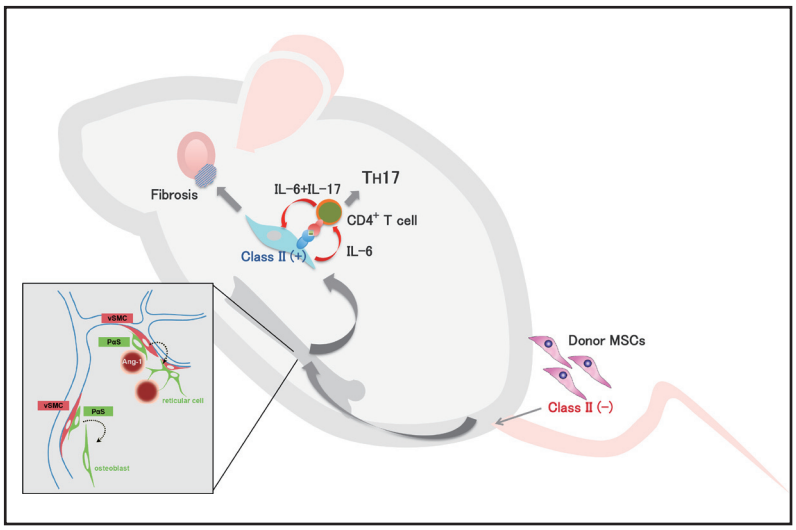

Fig. 1 Immunomodulation of MSC derived cells in periphery

Anti-inflammatory effect of MSCs

The therapeutic potential of MSCs was first reported in a case of steroid resistance acute GVHD. The lack of lymphocyte activation by MSC co-cultures as evidence for the immuno-suppressive properties of MSCs[5]. A trans-fusion of haploidentical MSCs dramatically improves the symptoms[6]. This was followed by a phase II trial of MSC infusion for severe acute GVHD anticipating that allogeneic MSCs play an immunosuppressive role following BMT[7]. Several factors and molecules secreted by MSCs have been linked to the immunoregulatory function of these cells. It is reported that MSCs act on the anti-inflammatory effects by promoting the secretion of IL-10 from macrophages by producing PGE2[8]. Indoleamine2,3-dioxygenese (IDO) production of MSCs induce differentiation of monocytes into M2 macrophages[9]. In a myocardial infarction animal model, trapped MSCs produce anti-inflammatory protein TNF-α stimulated gene / protein 6 (TSG-6) to the lungs, is to suppress the inflammatory response of the ischemic myocardium by diffusion act[10], and the TSG-6 acting on anti-inflammatory effects as well in corneal injury model[11]. In addition, MSCs secrete soluble receptor 1 (sTNFR1) to exert anti-inflammatory effects in peritonitis model[12]. Together these secreted factors may inhibit inflammatory responses, promote endothelial and fibroblast activities, and facilitate the proliferation and differentiation of progenitor cells in tissues *in situ*.

While the lethal nature of acute GVHD may necessitate such and other therapeutic trials[13], the mechanisms involved are unknown other than reports suggesting the release of immunomodulatory cytokines by MSCs trapped in the pulmonary capillary and the cells disappear within 2-3 days[14-17]. This type of “Hit and Run” treatment does not require HLA compatibility. Third-party (haploidentical) MSCs transfusion is sufficient for the transient immunomodulatory effect.

Chronic GVHD animal model

In clinical setting, GVHD can occur even when HLA-identical siblings are the donors. HLA-identical siblings or unrelated donors often genetically different, therefore minor histocompatibility antigens (miHA) can be presented by Major histocompatibility complex (MHC) molecules to the donor's T-cells. These antigens act as “foreign” and mount an immune response.

Bone marrow transplantation with 8 week-old donor B10. D2 (H-2d) mice and recipient BALB/c mice (H-2d) has been reported as a MHC-compatible, miHA-incompatible model of cGVHD (Fig. 1)[18]. The phenotype of the mice closely resembles clinical samples of patients suffering from cGVHD. Signs of cGVHD appear by 3 weeks after BMT, and progresses to full-blown disease by 8 weeks characterized by low tear volume and excessive fibrosis of the lacrimal gland, conjunctiva, salivary gland, skin, lung, liver and intestine. Accumulation of donor-derived fibroblasts in fibrotic lesions surrounding exocrine ducts was observed, which was similar to human patients as shown in our previous report[19]. These results suggested that donor-derived fibroblasts were part of the pathological process leading to cGVHD.

Prospective isolation of mouse mesenchymal stem/stromal cells (MSCs)

Multipotent mesenchymal stem/stromal cells (MSCs) in the bone marrow differentiate into several mesenchymal lineages such as fibroblasts, adipocytes, osteocytes and chondrocytes[20, 21]. However, a crucial step involving *in vitro* expansion was required to isolate these cells, which may modify their phenotype and function[22]. Most current information on MSCs comes from such *in vitro* studies of adherent cells referred to as fibroblast CFUs (CFU-Fs) [20, 21, 23, 24], which are a heterogeneous population of cells at best. Many preclinical and clinical studies have provided growing evidence of the efficacy of MSC-based treatments. However, in most cases, the rate of MSC engraftment is poor, and engrafted MSCs tend to be short-lived, which indicates that there should be other mechanisms by which MSCs exert their therapeutic effects.

Therefore, little is known about *in vivo* dynamics of MSCs after whole bone marrow transplantation (WBMT). Tracing the cell fate of MSCs following transplantation is required. Our group have recently succeeded in prospectively isolating murine MSCs based on their expression of PDGF receptor α and Sca-1 (PDGFRα+/ Sca-1+ (PαS) cells)[17,25]. Isolated PαS-MSCs without *in vitro* expansion can differentiate into hematopoietic niche cells, osteoblasts and adipocytes after systemic *in vivo* transplantation[17].

Chronic GVHD caused by mismatched bone marrow MSCs

In our resent studies, a massive fibrosis in lacrimal and salivary gland, skin, lung, liver and intestine in a cGVHD mouse model was attributed to donor derived MSCs, and not mismatched HSCs (manuscript in revise). Our results also suggested that radio-resistant residual recipient T cells, but not donor-HSC derived *de novo* T cells, were activated following mismatched MSC transplantation. The development of cGVHD in our model started as early as 3 weeks, although at least 4 weeks are required for maturation of T cells to appear in the peripheral blood following purified HSC transplantation[26]. Furthermore, we found a statistically higher ratio of recipient-derived T cells remaining in patients suffering from cGVHD compared to non-cGVHD patients following WBMT. In view of all the supporting evidence, we conclude that residual host T cells are responsible for the pathogenesis of cGVHD in both this cGVHD animal model, as well as in human cGVHD patients. These results are compatible with other reports showing how residual recipient CD4+ T cells regulate cGVHD[27,28]. If the hypothesis is correct, the cGVHD is not "graft vs host" disease but "host vs graft" disease, since immune reaction by host cells against graft cells is playing a major role in this animal model. Our point of view may affect current paradigm of the pathogenesis of cGVHD.

Inflammatory functions of MSCs

It has been shown that MSCs inhibit differentiation into the T_H1 and T_H17 subsets of helper T cells and promote the generation of Treg[29]. In our results, however, the onset of cGVHD is associated with progressive loss of Tregs and increase of T_H17. These opposite results suggested that MSCs acquire distinct immunophenotypes and activate different signaling pathways that may regulate immune responses differently. Such findings bring new insight to understanding of the crosstalk between MSCs and the inflammatory niche. Recently, the role of host non-hematopoietic antigen presenting cells (APCs), and not hematopoietic professional APCs, including DCs, was shown to be responsible for the progression of lethal acute GVHD[30]. These non-professional APCs were suggested to be mesenchymal lineage cells due to the expression of αSMA. We also had a similar result that donor-derived MSCs were responsible for the T_H17 transition of naïve T cells via TCR signaling and IL-6 secretion. These findings together strongly challenge conventional paradigms for an enhancive immunomodulatory functions of MSCs. We hope that these finding will develop in the new treatment such as administering anti-IL-17, anti-IL-6 receptor antibody for cGVHD.

Finally, the pros and cons of MSC therapy should be carefully weighed before further clinical use of these cells since they seem to exert different results in acute and chronic forms of GVHD. Furthermore, since it is now possible to prospectively isolate MSCs, a thorough re-evaluation of previous works that have relied on *in vitro* expansion for the collection of MSCs is required. The lack of specific markers with which to monitor MSCs *in vivo* has slowed elucidation of how MSCs respond to different inflammatory conditions. Nevertheless, learning how to control the plasticity of immunomodulation by MSCs, both endogenous and exogenous, may provide an important new modality for better therapeutic application of MSCs, as well as improved understanding of the role of MSCs in different stages of disease.

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Conflict of interests

None

Reference

1) Ferrara JL, Levine JE, Reddy P, Holler E: Graft-versus-host disease. Lancet. 2009; 373: 1550-1561.
2) Filipovich AH, et al: National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report. Biol Blood Marrow Transplant. 2005; 11: 945-956.
3) Fukushi N, et al: Thymus: a direct target tissue in graft-versus-host reaction after allogeneic bone marrow transplantation that results in abrogation of induction ofself-tolerance. Proc Natl Acad Sci U S A. 1990; 87: 6301-6305.
4) Hollander GA, Widmer B, Burakoff SJ: Loss of normal thymic repertoire selection and persistence of autoreactive T cells in graft vs host disease. J Immunol. 1994; 152: 1609-1617.
5) Le Blanc K, Tammik L, Sundberg B, Haynesworth SE, Ringden O: Mesenchymal stem cells inhibit and stimulate mixed lymphocyte cultures and mitogenic responses independently of the major histocompatibility complex. Scand J Immunol. 2003; 57: 11-20.
6) Le Blanc K, et al: Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. Lancet. 2004; 363: 1439-1441.
7) Le Blanc K, 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.
8) Nemeth K, Leelahavanichkul A, Yuen PS, et al: Bone marrow stromal cells attenuate sepsis via prostaglandin E(2)-dependent reprogramming of host macrophages to increase their interleukin-10 production. Nature Med. 2009; 15: 42-49.
9) François M, Romieu-Mourez R, Li M, et al: Human MSC suppression correlates with cytokine induction of indoleamine 2,3-dioxygenase and bystander M2 macrophage differentiation. Mol Ther. 2010; 20: 187-195.
10) Lee RH, Pulin AA, Seo MJ, et al: 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; 5: 54-63.
11) Oh JY, Roddy GW, Choi H, et al: Anti-inflammatory protein TSG-6 reduces inflammatory damage to the cornea following chemical and mechanical injury. Proc Natl Acad Sci U S A. 2010; 107: 16875-16880.
12) Yagi H, Soto-Gutierrez A, Navarro-Alvarez N, et al: Reactive bone marrow stromal cells attenuate systemic inflammation via sTNFR1. Mol Ther. 2010; 18: 1857-1864.
13) Ringden O, et al: Mesenchymal stem cells for treatment of therapy-resistant graft-versus-host disease. Transplantation. 2006; 81: 1390-1397.
14) Zangi L, et al: Direct imaging of immune rejection and memory induction by allogeneic mesenchymal stromal cells. Stem Cells. 2009; 27: 2865-2874.
15) English K, French A, Wood KJ: Mesenchymal stromal cells: facilitators of successful transplantation? Cell Stem Cell. 2010; 7: 431-442.
16) Uccelli A, Moretta L, Pistoia V: Mesenchymal stem cells in health and disease. Nat Rev Immunol. 2008; 8: 726-736.
17) Morikawa S, et al: Prospective identification, isolation, and systemic transplantation of multipotent mesenchymal stem cells in murine bone marrow. J Exp Med. 2009; 206: 2483-2496.
18) Zhang Y, McCormick LL, Desai SR, Wu C, Gilliam AC: Murine sclerodermatous graft-versus-host disease, a model for human scleroderma: cutaneous cytokines, chemokines, and immune cell activation. J Immunol. 2002; 168: 3088-3098.
19) Ogawa Y, et al: Donor fibroblast chimerism in the pathogenic fibrotic lesion of human chronic graft-versus-host disease. Invest Ophthalmol Vis Sci. 2005; 46: 4519-4527.
20) Pittenger MF, et al: Multilineage potential of adult human mesenchymal stem cells. Science. 1999; 284: 143-147.
21) Prockop DJ: Marrow stromal cells as stem cells for nonhematopoietic tissues. Science. 1997; 276: 71-74.
22) Banfi A, et al: Proliferation kinetics and differentiation potential of ex vivo expanded human bone marrow stromal cells: Implications for their use in cell therapy. Exp Hematol. 2000; 28: 707-715.
23) Conget PA, Minguell JJ: Phenotypical and functional properties of human bone marrow mesenchymal

progenitor cells. J Cell Physiol. 1999; 181: 67-73.
24) Friedenstein AJ, et al: Precursors for fibroblasts in different populations of hematopoietic cells as detected by the in vitro colony assay method. Exp Hematol. 1974; 2: 83-92.
25) Morikawa S, et al: Development of mesenchymal stem cells partially originate from the neural crest. Biochem Biophys Res Commun. 2009; 379: 1114-1119.
26) Okada S, et al: Sequential analysis of hematopoietic reconstitution achieved by transplantation of hematopoietic stem cells. Blood. 1993; 81: 1720-1725.
27) Anderson BE, et al: Recipient CD4+ T cells that survive irradiation regulate chronic graft-versus-host disease. Blood. 2004; 104: 1565-1573.
28) Bettelli E, et al: Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. Nature. 2006; 441: 235-238.
29) Luz-Crawford P, et al: Mesenchymal stem cells generate a CD4+CD25+Foxp3+ regulatory T cell population during the differentiation process of Th1 and Th17 cells. Stem Cell Res Ther. 2013; 4: 65.
30) Koyama M, et al: Recipient nonhematopoietic antigen-presenting cells are sufficient to induce lethal acute graft-versus-host disease. Nat Med. 2011; 18: 135-142.