Human Mesenchymal Stem Cell Therapy for Acute Graft Versus Host Disease

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Abstract

Acute graft versus host disease (GVHD) is the most critical complication after allogeneic hematopoietic stem cell transplantation (allo-HSCT). The understanding of major histocompatibility complex (MHC) and the development of acute GVHD prophylaxis have contributed to decreasing the incidence of severe acute GVHD. However, these progresses expand the chance of receiving allo-HSCT from human leukocyte antigen (HLA) mismatched donor. In addition, the expanding of indication for allo-HSCT to elderly patients or patients with organ dysfunction by the pervasiveness of reduced-intensity conditioning is associated with increased risk of acute GVHD. Unexpectedly, severe refractory acute GVHD can occur even in allo-HSCT from HLA matched sibling donor. The first line therapy for severe acute GVHD is corticosteroid, but the second line therapy has not been established and the prognosis of steroid-resistant acute GVHD remains poor. Recently, the efficacy of human bone marrow mesenchymal stem cell (MSC) therapy for steroid-resistant acute GVHD has been consistently reported. MSCs are approved as a cell therapy drug for steroid-resistant acute GVHD in some countries. We here review the history and the future of MSC therapy for acute GVHD.

Keywords: Mesenchymal stem cell; Immunomodulation; Graft versus host disease; Hematopoietic stem cell transplantation

Introduction

Although the development of molecular-targeted therapies has improved prognosis of hematopoietic malignancies for the last couple of decades, allogeneic hematopoietic stem cell transplantation (allo-HSCT) remains the only therapeutic approach to relapsed/refractory diseases. The development of supportive cares for many complications like infectious diseases and graft versus host disease (GVHD) has remarkably improved the outcomes of allo-HSCT [1]. However, severe acute GVHD that occurs within the first 100 days after allo-HSCT is one of the most critical problems. The frequency and severity of acute GVHD differ depending on HSC source, human leukocyte antigen (HLA) disparity, conditioning regimen, acute GVHD prevention, and comorbidities [2]. The development of GVHD prophylaxis and reduced-intensity conditioning expanded the chance of receiving allo-HSCT, but the frequency of refractory severe acute GVHD definitely increased. A lot of novel drugs, such as anti-thymocyte immunoglobulin, mycophenolate mofetil, infliximab, have been used for steroid-resistant acute GVHD. Response rate of these drugs has been reported to be about 50% (complete response; about 10-30%) and median overall survival after treatment less than 1 year [3].

Mesenchymal stem cells (MSCs) were discovered as fibroblastic cells to differentiate to osteo-progenitor cells by Freidenstein et al. [4]. Pittenger et al. indicated that MSCs existed in human bone marrow [5]. Definition of human MSC includes adherence to plastic dish, expression of CD105, CD73 and CD90, lack of expression of CD45, CD34, CD14 or CD11b, CD79a or CD19 and HLA-DR and differentiation to osteoblast, adipocyte, and chondroblast in vitro [6]. MSCs are isolated from several tissues including bone marrow, fatty tissue, and umbilical cord with standard adherent method, and these cells include a heterogeneous cell population. MSCs have immunomodulatory potential as well as multi-differentiation potential. MSCs and their progenies support hematopoiesis as niche components in bone marrow [7,8]. MSCs are most currently investigated as a cell therapy source because of their accessibility and multipotency [9]. MSC therapy for steroid-resistant acute GVHD has achieved the most attractive results. In this review we describe the potential and problems of MSC therapy.

Immunomodulatory function of MSCs

MSC therapy for acute GVHD exploits the immunomodulatory properties of MSCs. The immunomodulatory effect of MSCs was first indicated by long term engraftment in a xenogeneic environment in 2000 [10]. Although its mechanisms have not been fully understood, previous studies have revealed that MSCs inhibited the function of immune cells via cell to cell contact dependent mechanism and several soluble immune modulators [11-14].

MSCs can suppress the proliferation of naïve T cells as well as activated CD4+ helper T (Th) and CD8+ cytotoxic T cells regardless of major histocompatibility complex (MHC) restriction [15, 16]. MSCs decrease interferon (IFN)-γ production from Th1 cells and interleukin (IL)-4 production from Th2 cells. The immunosuppressive effect is considered to keep T cells in the G0/G1 cell cycle phase via the downregulation of cyclin D2 expression [17]. MSCs do not express MHC class II and co-stimulatory molecules such as CD40, CD80, and CD86 [5, 16], which may relate with T cell anergy. MSCs inhibit Th17 cells proliferation and IL-17 production [18]. MSCs can expand regulatory T cells, which are thought to inhibit allogeneic lymphocyte...
proliferation [19]. MSCs can also suppress the proliferation and IFN-γ production of natural killer cells [20]. MSCs have been shown to inhibit the proliferation, immunoglobulin production and chemokine secretion of activated B cell proliferation [21]. The differentiation from monocyes or hematopoietic cells to dendritic cells (DCs) and the maturation of DCs can be impaired by MSCs [22]. In contrast, MSCs can promote the differentiation to M2 macrophages which abundantly produce IL-10 [13]. These modulations of immune cells are associated with many soluble factors released from MSCs, including transforming growth factor-β1, hepatocyte growth factor, IL-10, indoleamine 2, 3-dioxygenase, prostaglandin E2, nitric oxide, matrix metalloproteinases, HLA-G (Figure 1) [11-14]. The secretion of these factors from MSCs has been shown to be induced by IFN-γ alone, or in combination with tumor necrosis factor (TNF)-α, IL-1α, or IL-1β [23]. Toll-like receptors (TLR) also have been reported to be expressed on MSCs and to trigger the induction of cytokines and chemokines [24]. Thus, various and complicated mechanisms are involved with immunomodulatory properties of MSCs.

**Clinical studies of MSC therapy for refractory acute GVHD using individual donors**

The first success of MSC therapy for acute GVHD was reported by Le Blanc et al. in 2004 [28]. A 9-year-old boy with acute lymphoblastic leukemia who received allo-HSCT from an unrelated donor suffered from grade IV acute GVHD, though he was treated with steroid pulse therapy, extracorporeal PUV A, infliximab, decidum, mycophenolate mofetil, and methotrexate. MSCs at a dose of 2 × 106 cells/kg isolated from bone marrow of a haploidentical donor, his mother, were administered to him. His acute GVHD was rapidly improved within a week after MSC therapy. Following the amazing result, Le Blanc et al. conducted a multi-institutional joint phase II experimental study of MSC therapy for steroid-resistant severe acute GVHD [29]. Thirty of 55 patients with steroid-resistant acute GVHD (grade II, n = 5; grade III, n = 25; grade IV, n = 25) achieved CR complete response (CR) just 3-63 days (median, 18 days) after MSC therapy. Surprisingly, 90% of them achieved CR with only a single MSC administration. Both donor type and cell dose were not associated with overall response. Severity of acute GVHD also had no significant effect on response. Two-year overall survival in the patients with CR (52%, 95% CI: 34-70%) was better than that in the patients with PR or no response (16%, 95% CI: 0-32%, p = 0.018). Subsequently, many studies of MSC therapy for refractory acute GVHD have been conducted until now (summarized in Table 1) [29-37]. MSCs were mostly derived from third party donors in all the studies. Median administered dose of MSCs were 1-2 × 106 cells/kg and median number of doses was 1 to 3. Response was rapidly observed in responders. CR rate (8-65%) and overall response rate (16-86%) were varied, which were far superior to conventional salvage treatment for steroid resistant acute GVHD. Overall survival in the responders was significantly better compared to non-responders as reported by Le Blanc. In the three reports containing both pediatric and adult patients, response in pediatric patients was significantly superior to that in adult patients [29,35,36]. However, a study comprising only pediatric patients reported lower complete response rate (24%) [34]. In that study MSCs were cultured in platelet lysate-containing medium, which may be associated with the inferior result. A study for adult patients using MSCs cultured in platelet lysate-containing medium also showed poor response (CR rate: 8%) [31]. Further studies are needed to clarify the superiority of MSC therapy in children. Acute toxicity related with MSC infusion was extremely rare in all the study. In contrast, infectious episodes were reported during the long term observation. Because the existence of severe acute GVHD itself contributes to compromised condition, the direct contribution of MSC therapy to frequency of infectious diseases needs to be evaluated in the future.

**Industrial MSC therapy for acute GVHD**

Osiris therapeutics, Inc. developed industrial mesenchymal cells derived from third-party bone marrow donors, Prochymal, as the world's first cell therapy drug. MSCs that are culture-expanded for 5 passages are cryopreserved as product lots. The cells are thawed and diluted before administration. Kebreria et al. reported the first randomized, multicenter phase II trial of Prochymal for acute GVHD in 2009 [38]. In the study, Prochymal was administered at two different dose (2 or 8 × 106 cells/kg) with corticosteroid as the first line therapy to the patients with grade II-IV acute GVHD and the overall response rate was 93% (CR, 77%; PR, 16%). Subsequently, several studies of manufactured MSC therapy for steroid-resistant refractory acute GVHD have been reported (summarized in Table 2) [39-44]. CR and
overall response rate were 24-58% and 60-93%, which were similar to the results of MSC therapy from individual donors. However, the median number doses for a treatment of manufactured MSC therapy was more than 8, which was so many compared to individual MSC therapy. In addition, there was no significant difference of CR rate between Prochymal group and placebo group in a double-blind placebo-controlled randomized phase III trial (40% and 28%, p = 0.08) [39]. On the other hand, in a small prospective, open-label, nonrandomized, multicenter study, CR rate in patients using MSCs in combination with conventional therapies for refractory acute GVHD was significantly higher than that in patients treated with only conventional therapies (61% and 26%, p = 0.02) [43]. Prochymal has been approved in Canada and New Zealand as a treatment for steroid-resistant refractory acute GVHD only in children. Another MSC drug, Temcell HS, which is manufactured according to Prochymal [41,44], was recently approved for refractory acute GVHD in both adults and children in Japan. However, further experience is required to make a definitive assessment of these MSC drugs.

### Table 1: Summary of MSC therapy for steroid-resistant acute GVHD using individual MSC donors. MSD: HLA-matched sibling donor, haplo: HLA-haploidentical donor, NA: data not available.

<table>
<thead>
<tr>
<th>Reference</th>
<th>No. of patients</th>
<th>Median age of patients (years)</th>
<th>MSC donor</th>
<th>Median MSC dose ((\times 10^6/\text{kg}))</th>
<th>Number of infusion (median)</th>
<th>CR (%)</th>
<th>CR+PR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ringden et al. (30)</td>
<td>8</td>
<td>56</td>
<td>MSD (2), haplo (3), third party (3)</td>
<td>1</td>
<td>1 (1)</td>
<td>63</td>
<td>63</td>
</tr>
<tr>
<td>Le Blanc et al. (29)</td>
<td>55</td>
<td>22</td>
<td>MSD (5), haplo (18), third party (69)</td>
<td>1.4</td>
<td>1-5 (2)</td>
<td>55</td>
<td>71</td>
</tr>
<tr>
<td>von Bonin et al. (31)</td>
<td>13</td>
<td>58</td>
<td>Third party</td>
<td>0.9</td>
<td>1-5 (2)</td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td>Lucchini et al. (32)</td>
<td>11</td>
<td>10</td>
<td>Third party</td>
<td>1.2</td>
<td>1-5 (NA)</td>
<td>24</td>
<td>73</td>
</tr>
<tr>
<td>Herrmann et al. (33)</td>
<td>12</td>
<td>48.5</td>
<td>MSD (1), mismatched sibling (1), third party (10)</td>
<td>1.7-2.3</td>
<td>2-19 (2)</td>
<td>58</td>
<td>33</td>
</tr>
<tr>
<td>Resnick et al. (35)</td>
<td>50</td>
<td>19</td>
<td>The same donor as HSC donor (7), third party (67)</td>
<td>1.1</td>
<td>1-4 (NA)</td>
<td>34</td>
<td>66</td>
</tr>
<tr>
<td>Ball et al. (34)</td>
<td>37</td>
<td>7</td>
<td>Haplo (3), third party (34)</td>
<td>2</td>
<td>1-13 (2)</td>
<td>65</td>
<td>86</td>
</tr>
<tr>
<td>Introna et al. (36)</td>
<td>31</td>
<td>NA</td>
<td>Third party</td>
<td>1.5</td>
<td>2-11 (3)</td>
<td>29</td>
<td>74</td>
</tr>
<tr>
<td>Sanchez-Guijo et al. (37)</td>
<td>25</td>
<td>NA</td>
<td>Third party</td>
<td>1.1</td>
<td>2-4 (4)</td>
<td>46</td>
<td>71</td>
</tr>
</tbody>
</table>

### Table 2: Summary of MSC therapy for steroid-resistant acute GVHD using manufactured MSCs. NA: data not available.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study type</th>
<th>No. of patients</th>
<th>Median age of patients (years)</th>
<th>Median MSC dose ((\times 10^6/\text{kg}))</th>
<th>Number of infusions (median)</th>
<th>CR (%)</th>
<th>CR+PR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prasad et al. (40)</td>
<td>Phase I/II</td>
<td>12</td>
<td>5</td>
<td>2 or 8</td>
<td>2-21 (8.5)</td>
<td>58</td>
<td>75</td>
</tr>
<tr>
<td>Muroi et al. (41)</td>
<td>Phase I/II</td>
<td>14</td>
<td>52</td>
<td>2</td>
<td>3-12 (8)</td>
<td>57</td>
<td>93</td>
</tr>
<tr>
<td>P. J. Martin et al. (39)</td>
<td>Phase III</td>
<td>163 (Prochymal®)</td>
<td>NA</td>
<td>2</td>
<td>8</td>
<td>40</td>
<td>82</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>81 (placebo)</td>
<td>NA</td>
<td></td>
<td>28</td>
<td>73</td>
</tr>
<tr>
<td>Kurtzberg et al. (42)</td>
<td>Phase III</td>
<td>75</td>
<td>7.8</td>
<td>2</td>
<td>1-20 (10)</td>
<td>NA</td>
<td>61.3</td>
</tr>
<tr>
<td>Muroi et al. (44)</td>
<td>Phase III/IV</td>
<td>25</td>
<td>33</td>
<td>2</td>
<td>4-8 (8)</td>
<td>24</td>
<td>60</td>
</tr>
<tr>
<td>Zao et al. (43)</td>
<td>Open-label non-randomized</td>
<td>28 (MSC+)</td>
<td>26</td>
<td>1</td>
<td>2-8 (4)</td>
<td>61</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td></td>
<td>19 (MSC-)</td>
<td>29</td>
<td></td>
<td></td>
<td>26</td>
<td>42</td>
</tr>
</tbody>
</table>

### MSC for acute GVHD prophylaxis

Recently, some researchers attempt to utilize MSCs derived from the same donors as hematopoietic stem cell (HSC) donors for prophylaxis of acute GVHD. Shinpounova et al. conducted a randomized trial to examine the efficacy of MSC therapy on acute GVHD prophylaxis [45]. Authors compared 39 patients received MSC therapy for acute GHVD prophylaxis with 38 patients received standard GVHD prophylaxis. In the study, MSCs were administered the day when peripheral leukocyte count reached 109/L at a median dose of 1.2 × 106 cells/kg (range, 0.9-1.65 × 106 cells/kg). The incidence of acute GVHD with grade II-IV were significantly decreased in the MSC prophylaxis group compared to the standard prophylaxis group (9.4% versus 29.4%, p =
There was no significant difference in donor characteristics and MSCs' properties between two groups. Gene expression profile analysis in MSCs suggested that increased gene expression of fibroblast growth factor receptor 1 (FGFR1) and platelet-derived growth factor receptor beta (PDGFRB) in MSCs might be related with decrease of the incidence of acute GVHD. Mazziarz et al. conducted phase I dose escalation study to evaluate the availability and the safety of an industrial MSC, "allogeneic multipotent adult progenitor cell (MAPC) (MultiStem®, Athersys, Inc., Cleveland, OH)" for acute GVHD prophylaxis [46]. Authors compared the efficacy of a single dose injection of MAPC (1, 5, or 10 × 106 cells/kg, n = 18) with that of once a week multiple dose injection of MAPC (1 or 5 × 106 cells/kg, n = 18).

No infusion-related toxicities were observed, but 3 patients had a grade 2–3 infection. Of these, 2 had CMV (100%) and Epstein-Barr virus (EBV) infections between MSC-treated group and the single-dose arm and the multiple-dose arm in this study. The usage of MSCs for acute GVHD prophylaxis should be carefully considered.

**Problems and future directions of MSC therapy for acute GVHD**

There is room for improvement on MSC therapy for refractory acute GVHD. The quality assurance of MSCs is the most important problem. As described above, the requirement of repeated administration in industrial MSC therapy probably could arise from their dysfunction induced by bulk production. Von Bahr et al. reported that the patients who received MSCs from passage 1–2 have better survival (75% at 1 year) than those who receive MSCs from passage 3–4 (21%) (p < 0.01) [47]. In addition, the immune plasticity of MSCs may vary depending on donors. Many donors are required to secure the quality and quantity of MSCs. The usage of alternative source, like cord blood (CB) [48, 49], Wharton's jelly cells (WJCs) [50], adipose tissue [51], teeth pluripotent stem cells [52,53], could be available for MSC source. Especially, CB and WJCs are alternative sources of MSCs.

The requirement of repeated administration in industrial MSC therapy probably could arise from their dysfunction induced by bulk production. The authors indicated that virus-specific T cell activation and proliferation were impaired by MSC therapy. We need to devote enough attention to viral infection.

MSC therapy for refractory acute GVHD has been conducted in many countries because of its attractive results, but there are many issues to be investigated: cell dose, cell source, culture condition, quality, long term effect, and late complications. MSC therapy is still on the way to be established and should be used appropriately in clinical setting now. Future progress would contribute to establish MSC therapy as is the case with HSCT.

**References**


