Analysis of Mesenchymal Stromal Cell Engraftment After Allogeneic HSCT in Pediatric Patients: A Large Multicenter Study

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Summary: The mesenchymal stem cell (MSC) role after allogeneic hematopoietic stem cell transplantation (HSCT) is still a matter of debate; in particular, MSC engraftment in recipient bone marrow (BM) is unclear. A total of 46 patients were analyzed for MSC and hemopoietic stem cell engraftment after HSCT. The majority of patients had the BM as the stem cell source, and acute leukemia was the main indication for HSCT. Mesenchymal and hematopoietic stem cell chimerism analysis was carried out through specific polymorphic tandemly repeated regions. All patients reached complete donor engraftment; no evidence of donor-derived MSC engraftment was noted. Our data indicate that MSCs after HSCT remain of recipient origin despite the following: (i) myeloablative conditioning; (ii) the stem cell source; (iii) the interval from HSCT to BM analysis; (iv) the underlying disease before HSCT; and (v) the patients' or the donors' age at HSCT.

Key Words: mesenchymal stem cells, chimerism, hematopoietic stem cell transplantation

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Bone marrow (BM) hematopoiesis takes place in defined niches, wherein several cell types can be identified. Among these cells, the mesenchymal stem cells (MSCs) are able to support hematopoiesis through the production of several growth factors. BM-derived MSCs retain the potential to differentiate readily into adipocytes and chondrocytes and, with variable efficiency, into a variety of further cell types.[1] Furthermore, they can grow readily in culture, in which they proliferate extensively with an adherent, fibroblastic-like morphology. Because MSCs support hematopoiesis, and some studies have highlighted a beneficial role for graft-versus-host disease (GvHD) treatment, it is of interest to determine whether these cells are susceptible to conditioning therapy, engraft with donor hematopoietic stem cells, or depend on the graft source. This information will be necessary to understand in more detail the process of engraftment and, possibly, also the immunologic events after allogeneic HCT. The aim of this study was to determine whether donor-derived MSCs could be identified after hematopoietic stem cell transplantation (HSCT) in a heterogenous group of patients and whether there are any correlations with conditioning regimen (myeloablative vs. nonmyeloablative), the source of the graft (BM, peripheral blood stem cells [PBSC], or cord blood [CB]), the interval from HSCT, and the patient's or the donor's age at HSCT.

Despite it being well known that the literature data have shown how mesenchymal stromal cells remain of recipient origin in adult patients, we studied whether this was also the case in a large heterogenous pediatric population in more recent years.

PATIENTS AND METHODS

Patients' Characteristics

From March 2013 to 2017, a total of 46 recipients of HSCT were analyzed for HSC and MSC chimerism (Table 1). The majority of our patients had undergone HSCT for acute leukemia (acute lymphoblastic leukemia, 45%; acute myeloid leukemia, 37%); 27 received BM grafts (59%), 18 patients had PBSC as the stem cell rescue (39%), and 1 patient had a CB rescue (2%). A total of 37 patients received myeloablative conditioning (80%), and 9 patients had nonmyeloablative conditioning (19%). Written consent was obtained from all the patients or their legal guardians.

MSC Isolation

BM cells were plated and expanded in α-minimum essential medium (Cambrex Bioscience) with 10% of fetal bovine serum in 75 cm^2 T-flasks, 2 mmol/L L-glutamine, and penicillin/streptomycin (1×).

MSC Differentiation

The culture was maintained at 37°C in a 5% CO_2 atmosphere. After 5 to 7 days, nonadherent cells were removed, whereas the adherent cells were expanded until they reached confluence (14 to 20 d of culture). The MSCs were then harvested by treatment with trypsin/EDTA (Cambrex Bioscience) for 5 minutes at 37°C and used for further analysis.

MSC Flow Cytometry

The cells were then characterized according to the International Society for Cellular Therapy.[2,3] Phenotypic characterization was performed using the following antibodies combination: anti-CD90 FITC, CD73 PE, CD34 FITC, CD14

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TABLE 1. Details of Patients' Demographics

Demographics	n (%)
Patients	46 (100)
Sex	
Male	29 (63)
Female	17 (37)
Patient's age (y)	13.5 (1-67)
Donor's age (y)	25 (0-56)
Diagnosis	
AML	17 (37)
ALL	21 (45)
Myelodysplastic syndrome	2 (4)
NHL	1 (2)
Neuroblastoma	1 (2)
Multiple myeloma	1 (2)
Nonmalignant disease	3 (6)
Days after transplant	138 (29-1715)
Stem cell source	
Bone marrow	27 (59)
Peripheral blood	18 (39)
Cord blood	1 (2)
Conditioning	
Myeloablative	37 (80)
Nonmyeloablative	9 (19)
Hematopoetic cell chimerism	Full donor 100%
Mesenchymal cell chimerism	Full recipients 100%

ALL indicates acute lymphoblastic leukemia; AML, acute myeloid leukemia; NHL, non-Hodgkin Lymphoma.

FITC, CD45 FITC, HLA-DR PE, CD105 PC7, and CD19 APC (Miltenyi Biotec, Bologna, Italy). Data acquisition was performed using Navios (Beckman Coulter). Only cells negative for hematopoietic antigens (CD34, CD14, CD45) and for HLA-DR, and expressing > 90% of CD90, CD73, and CD105, could be considered as MSCs and used for chimerism analysis (Fig. 1).

Chimerism Analysis

Specific polymorphic tandemly repeated regions (STRs) were amplified by means of the polymerase chain reaction (PCR) following the specific manufacturers' instructions. After cell detachment, nuclear DNA was extracted and amplified (AmpFISTR Identifiler kit; Applied Biosystems). This assay allows the amplification of 15 STR loci plus the sex-determining locus amelogenin (CSF1PO, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D21S11, FGA, TH01, TPOX, and vWA, D2S1338 and D19S433) (X) that were resolved by capillary electrophoresis (3500 Ruo Genetic Analyzer, Applied Biosystems) and analyzed by comparing the genotype electropherograms of the BM recipient following HSCT (BM and mesenchymal cells) with those of the donor and the BMT recipient before HSCT.[4] The same technique was used to test the engraftment of donor hematopoietic cells.

RESULTS

Although all patients had complete donor hematopoietic engraftment (100%), full recipient chimerism (100%) was observed in all patients when we analyzed the mesenchymal cell cultures despite the following factors: (i) myeloablative conditioning; (ii) the stem cell source (BM vs. PBSC vs. CB); (iii) the interval from HSCT to BM analysis (from day +29 to 1715); (iv) the underlying disease before HSCT (malignant vs. nonmalignant); and (v) the patients' or the donors' age at HSCT. In particular, nested-PCR was not carried out because the clinical role of engraftment under 1% was considered clinically negligible.

DISCUSSION

The significance of the host nature of MSCs is still not clear. Although there is experimental evidence suggesting the presence of a common mesoderm cell, it is still controversial whether transient or durable engraftment of native donor-derived MSCs without ex vivo treatment can occur in allogeneic HSCT recipients and whether other sources of hematopoietic progenitors could contain MSCs. Villaron and Almeida[5] and Poloni and Leoni[6] reported some cases, in which MSCs from BM of patients who received mobilized peripheral blood hematopoietic transplantation were from donor origin, suggesting the presence of MSCs in apheresis products and their capacity for homing to the BM.

In particular, Poloni and Leoni[6] demonstrated that following allogenic transplantation from HLA-identical siblings with reduced-intensity conditioning, stromal progenitors reinfused with hemopoietic stem cells induced a mixed chimerism (7% to 86% donor) in 8 of 22 patients (36%) 5 of whom have been transplanted with mobilized peripheral blood.

Furthermore, Villaron and Almeida[5] showed that in 2 of 19 patients with multiple myeloma, who had received a reduced-intensity transplantation using PBSCs, MSCs were partially from donor origin (60.17% and 26.13%).

Actually, several other studies indicated that, irrespective of the use of MSC coadministration, the post-transplant chimerism of BM-derived MSCs after allogeneic HSCT has been reported to remain of host origin, suggesting that the infused donor MSCs are either immunologically rejected or not capable of long-term engraftment in the host BM microenvironment.[7,8]

Furthermore, Javier-Garcia et al[9] demonstrated that, after an allogenic HSCT, the presence of donor MSCs within the inoculums, the type of underlying disease, the intensity of pre-transplant therapies, or the conditioning regimen, do not seem to influence the chimerism status of MSCs, resulting in 100% of recipient origin in each of the 13 BM samples analyzed. To explain this phenomenon, the authors hypothesized that circulating MSCs may come also from non-BM tissues, and that chimerism status evaluation of MSCs isolated from tissues other than marrow would be helpful.

Our results, obtained mainly from BM of pediatric patients (35 patients, 76%), are consistent with the relative resistance of stromal cells to myeloablative conditioning therapy (including total body irradiation, 20 patients, 43%), so that regenerative demand can be satisfied by the surviving intrinsic MSCs. However, while the absence of donor-derived MSCs might also reflect an insufficient number of MSCs in the graft or a poor capability of transplanted MSCs to engraft, the transplant ability of BM stem cells has been shown in patients with osteogenesis imperfecta.[10] In contrast with results that we and others observed, a further aspect of interest is the clinical evidence that MSCs, also third parties, were shown to be able to (i) treat acute GvHD[11] and (ii) to prevent GvHD even after haploidentical or cord stem cell transplantation.[12,13] Therefore, the role of MSCs after allogeneic stem cell transplantation remains to be defined.

If it is true that MSCs are able to control GvHD and if it is equally true that donor MSCs do not replace the patient's marrow stroma, perhaps the analysis of MSCs should be targeted at the lymph node germinal centers, where the autoallo-reactive T-cell clones might be responsible for triggering and maintaining the GvHD cascade.

Furthermore, the conventional method of HSC chimerism (PCR amplification of STRs followed by fragment

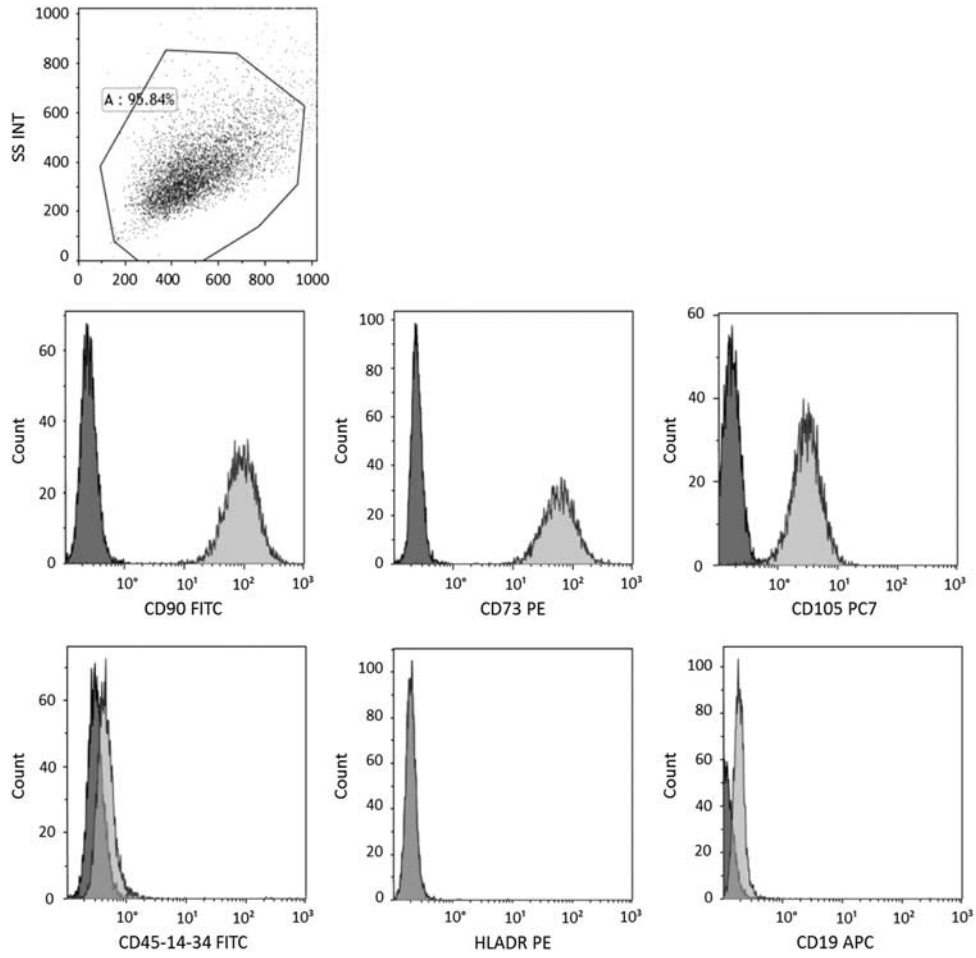

FIGURE 1. Representative immunophenotype of in vitro expanded mesenchymal cells. Light gray. histogram: positive reactivity for the specific antibodies against each CD marker. Dark gray histogram: isotype controls.

analysis), has a 1% to 5% sensitivity limit, which is probably insufficient for expected levels of MSC engraftment.[14] However, BM cells were not analyzed by nested-PCR, as the sensitivity limit was considered clinically negligible.

To get a better understanding of the fate of donor-derived MSCs after allogeneic HSCT or some other MSC-based therapy, the development of more sensitive methods to track infused MSCs is required.

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