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T-cell suppression mediated by mesenchymal stem cells is deficient in patients with severe aplastic anemia

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***Objective*. To compare the suppressive effect of mesenchymal stem cells (MSC), derived from normal individuals or severe aplastic anemia patients (SAA), on T-cell activation.**

***Patients and Methods*. We studied bone marrow MSC from 19 healthy donors and 23 SAA patients in different phases of the disease: at diagnosis (n = 3), following immunosuppressive therapy (IS) (n = 16), or after a bone marrow transplant (BMT) (n = 4). MSC were tested for T-cell suppression in the following assays: mixed lymphocyte reaction (MLR), phytohemaglutinin (PHA)-primed cultures, activation surface markers, γ-IFN production, hematopoietic colony formation (CFC), production of cyclic ADP–ribose (cADPR).**

***Results*. The abnormalities of SAA MSC included: 1) significantly lower suppression of T-cell proliferation induced by alloantigens ($p = 0.009$) or PHA ($p = 0.006$); 2) impaired capacity to suppress CD38 expression on PHA-primed T cells ($p = 0.001$); 3) impaired ability to suppress γ-IFN production in PHA cultures, resulting in an 11-fold higher γ-IFN concentration; 4) no preventive effect on T cell–mediated inhibition of CFC; and 5) significantly reduced ($p = 0.009$) production of cADPR, a universal calcium mobilizer. MSC-mediated suppression of PHA-induced T-cell proliferation was restored to control levels in 3 of 4 patients post-BMT.**

***Conclusion*. The ability of MSC to downregulate T-cell priming, proliferation, and cytokine release is deficient in patients with SAA, persists indefinitely after immunosuppressive therapy, but seems to be restored after BMT. Whether these abnormalities are relevant to the pathogenesis of aplastic anemia remains to be determined.**

Introduction

Bone marrow (BM) mesenchymal stem cells (MSC) have been shown to possess immunosuppressive activity, both in vitro and in vivo, in the experimental transplant setting [1–4]. The terms MSC identifies cells which are harvested from adult bone marrow and are then purified through several passages by plastic adherence in appropriate mediun [5]. These cells have been shown to be capable of differentiating to osteoblasts, myoblasts, chondroblasts, tenoblasts, adipocytes and marrow stromal cells [5]. In a major MHC-mismatched model, mice given lethal radiation and a BM transplant fail to engraft or die of graft-vs-host disease (GVHD) [6]; however, when donor marrow is co-infused with an equal number of donor osteoblasts, 100% of the animals engraft, with full hematologic and immunologic reconstitution, and are free of GVHD [6]. Similar in vivo effects have been shown in primates [7], suggesting that MSC may be able to set up an immunoprotective site within the BM microenvironment capable of facilitating allogeneic engraftment [7]. Indeed, MSC promote engraftment of human $CD34^+$ cord blood cells in preimmune fetal sheep [8] and in NOD/SCID mice [9], and facilitate platelet recovery in the autologous setting in humans [10].

MSC are also immunosuppressive in vitro, as shown by Di Nicola and coworkers [3]: T-cell responses could be abrogated by addition of human MSC in experiments with and without cell contact. Antibodies against hepatocyte growth factor (HGF) and TGF-β impaired the suppressive effect of MSC [3]. In keeping with a role of HGF on T-cell function, it has recently been shown that HGF reduces GVHD in a mouse model [11]. More recently, prostaglandin (PGE2) has

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been shown to be released after interaction of MSC with dendritic cells, NK cells, and T cells [12]; this would result in skewing the immune system towards an anti-inflammatory phenotype, with upregulation of interleukin (IL)-4 and IL-10 and downregulation of IFN and TNF production [12].

Now why should marrow MSC, which are crucial for hemopoietic stem cell (HSC) survival and pluripotency [13,14], inhibit T-cell function: is BM stroma preventing T-cell activation in the proximity of hemopoietic stem cells, as suggested by Bartholomew and coworkers [7]? If this is the case, would the lack of MSC immunoprotection be important in patients with marrow failure? To answer this question we compared the inhibitory effect of normal MSC on T-cell function in vitro, with the effect of MSC obtained from patients with aplastic anemia.

Materials and methods

Healthy donors and aplastic anemia patients

Bone marrow was collected from healthy donors (n = 19) undergoing a marrow harvest for marrow donation, and from aplastic anemia patients either at diagnosis (n = 3) or after immunosuppressive therapy (IS) (n = 16). Of the 16 IS-treated patients, 13 were responders and transfusion independent, 2 were studied at the time of relapse and were transfusion dependent, and one was refractory to IS. Four patients had received a bone marrow transplant (BMT). Clinical data of patients are outlined in Table 1.

Human bone marrow mesenchymal stem cells

Light-density human bone marrow cells were cultured in MesenCult Medium (Stem Cell Technologies, Vancouver, BC, Canada) in 35-mm tissue culture dishes and incubated in humified atmosphere at 37°C and 5% CO_2 for 24 to 48 hours. The whole medium and nonadherent cells were then removed and replaced with fresh medium; cultures were fed twice weekly, by changing half of the medium. At the end of the culture period (approximatively 14 days, when the cells become confluent), residual nonadherent cells and medium were removed by washing with phosphate-buffered saline. The adherent cells were trypsinized, washed, and expanded to the third generation. MSC were then harvested and frozen until a week before coculture with lymphoid cells. MSC were used both after exposure to 3 Gy radiation or un-irradiated (^{137}Cs source, Gammacell, Atomic Energy of Canada Limited [AECL], Mississauga, Ontario, Canada); no differences were observed in the effect of irradiated and un-irradiated MSC.

We tested normal MSC for their differentiation capacity. Briefly, chondrogenic induction was performed at each culture passage with aliquots of 250,000 cells pelleted in standard medium. Twenty-four hours later, pelleted micromass detached from the wall of tubes were mantained in culture with chondrogenic medium (IMDM with 10% FCS, Euroclone, Milan, Italy; 50 ug/mL ascorbic acid, Sigma; 1 ng/mL human recombinant TGF-β, Sigma Aldrich, St. Louis, MO) for 14 days, changing the medium every 2 or 3 days. Histochemical analysis of pellet sections was performed with alcian blue (Bioptical Tec., Sigma Aldrich) pH 2.5 for 30 minutes and then washed in water. Immunohistochemical stains of the slides were performed with anti-type II collagen antibody (Southern Biotech [SBA],

Table 1. Clinical data of aplastic anemia patients

					At time of MSC analysis			
N.	Init	Sex	Date Dx	Date IS or BMT	Date	Age	Disease status	Transfusion dependence
1	RM	M	01.04.95	29.05.95	05.11.03	25	relapse	Yes
2	GP	M	10.04.82	30.05.82	20.05.02	40	responder	No
3	ZG	M	09.11.95	23.11.95	13.02.03	35	responder	No
4	PE	F	15.04.98	20.05.98	15.07.02	37	refractory	Yes
5	BI	F	24.07.85	20.08.85	15.05.02	70	responder	No
6	GP	F	01.02.85	18.02.85	15.05.02	43	responder	No
7	ML	M	15.06.75	23.12.97	05.06.02	27	responder	No
8	CA	M	15.06.99	16.08.99	12.06.02	62	responder	No
9	DA	F	15.09.94	17.10.94	02.07.02	22	relapse	Yes
10	BF	F	15.06.88	08.04.92	02.07.02	32	responder	No
11	PG	M	12.02.97	13.02.97	03.07.02	23	responder	No
12	MP	M	17.11.94	19.11.94	03.07.02	20	responder	No
13	LP	M	01.07.88	01.12.88	15.11.02	35	responder	No
14	RR	F	05.07.02	29.08.02	13.01.03	51	responder	No
15	MC	M	07.02.03	-	13.02.03	28	diagnosis	Yes
16	TP	M	05.12.81	15.01.82	20.03.03	33	responder	No
17	MA	M	15.03.92	06.04.92	20.11.03	16	responder	No
18	Gv	M	15.12.03	-	28.01.04	30	diagnosis	Yes
19	RP	M	15.12.03	-	02.01.04	45	diagnosis	Yes
20	RG	M	04.04.03	30.09.03	12.05.04	18	post-BMT	No
21	AM	M	15.08.00	09.03.04	19.05.04	42	post-BMT	Yes
22	SPA	M	01.09.94	31.10.94	19.05.04	40	post-BMT	No
23	MA	F	15.03.92	12.01.99	04.06.04	28	post-BMT	No

Abbreviations: Dx = diagnosis; IS = immunosuppressive therapy; BMT = bone marrow transplantation; Hb = hemoglobin; PMN = neutrophils; Plt = platelets; Pt = patient; M = male; F = female; PR = partial remission; CR = complete remission; resp = response; Date = day/month/yr.