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Clinical and laboratory features of mesenchymal stromal cells in pediatric stem cell transplantation

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Chapter 5

Multiple infusions of haploidentical mesenchymal stromal cells are not immunogenic in children undergoing myeloablative stem cell transplantation.

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Both umbilical cord blood transplantation (UCBT) and T-cell depleted haploidentical peripheral blood stem cell transplantation (PBSCT) may be associated with an increased risk of graft failure. Mesenchymal stromal cell (MSCs) infusion has been used in children undergoing these procedures to overcome the problems of delayed engraftment and rejection.^{1,2}

MSCs do not constitutively express MHC class II or co-stimulatory molecules and were once considered non-immunogenic.³ However, Nauta *et al.* reported that MSCs may elicit an immune-mediated rejection in a murine model of allogeneic hematopoietic stem cell transplantation (HSCT).⁴ This observation has raised clinical concerns as to whether MSCs may trigger an immune mediated rejection especially if repeatedly administered in the setting of HSCT.

In our institution, with ethical committee approval, children undergoing UCBT are co-transplanted with haploidentical, bone marrow-derived, *ex vivo* expanded MSCs with the aim of improving hematological engraftment. Also children requiring haploidentical PBSCT receive same donor, bone marrow-derived, *ex vivo* expanded MSCs designed to overcome rejection.

We report data suggesting that exposure to multiple infusions of haploidentical MSCs can be administered in children undergoing HSCT after a myeloablative regimen without inducing rejection of human allogeneic haematopoietic stem cells from the same donor.

A 12-year old boy received a 5/6 HLA matched unrelated UCBT in combination with MSCs obtained from the father for homozygous beta-thalassemia, after having received a myeloablative conditioning (see Table 1 for details). Graft-versus-host disease (GvHD) prophylaxis consisted of Cyclosporine A (CSA) and steroids; dose of CSA was adjusted according to blood sampling to maintain trough blood levels between 150-200 g/L.

Leukocyte recovery ($0.7 \times 10^9/l$ mainly consisting of CD8+ T-cells)) was observed on day +15. As VNTR polymorphic chimerism analysis showed 0% donor chimerism, the patient received an autologous bone marrow rescue.

Ten months later, with full autologous haematological and immune recovery, he underwent a haploidentical PBSCT from his father also in combination with MSCs obtained from the same donor. CD34+ cells were positively selected using the CliniMacs one-step procedure (Miltenyi Biotech, Bergisch Gladbach, Germany).

Conditioning was identical to the first transplant with the addition of Fludarabine. No post-transplantation pharmacological immune suppressive therapy was used. Characteristics of both UCBT and PBSCT and co-transplanted MSCs are summarized in Table 1.

Full hematological recovery occurred on day +12 with 100% donor chimerism. His post-transplant period was complicated by reactivation of Cytomegalovirus infection, successfully treated with gancyclovir and one year later he is transfusion-independent with 100 % donor engraftment and normal immune reconstitution.

Similarly, a 2-year old boy underwent a 6/6 HLA matched unrelated UCBT in combination with MSCs from his father for haemophagocytic lymphohistiocytosis (HLH). He also received myeloablative conditioning. GvHD prophylaxis was identical to that employed in the first patient (CSA trough blood levels between 100-150 g/L). Lymphocyte recovery of 100% (mainly consisting of CD4+ and CD8+ T-cells) of recipient origin was observed at day +22 and he later received autologous stem cell rescue.

Six months following UCBT, he too underwent a haploidentical PBSCT from his father with CD34+ cell selection as previously described for the first patient. Paternal MSCs were also infused. Characteristics of both HSCT and co-transplanted MSCs are also summarized in Table 1.

Full hematological recovery occurred on day +19 with 100% donor chimerism. At +4 months after the allograft, he died from disseminated adenovirus infection, despite therapy with cidofovir and donor-derived, adenovirus-specific, T-cell infusions, with 100% donor chimerism.

These two cases suggest that following rejection of UCBT, no immune reactivity apparently developed against either paternal donor antigens, as in both patients a full engraftment of paternal hematopoietic stem cell was achieved. Support to this interpretation is provided by the observation that subsequent cross-match between patient serum and paternal donor lymphocytes and between paternal donor serum and patient lymphocytes was negative. HLA antibody screening including ELISA antibody detection for HLA class I and II antigens was also negative. It cannot be excluded that the use of two consecutive myeloablative conditioning regimens in these patients may have contributed to the lack of sensitization against paternal

antigens expressed on MSCs.

While able to suppress T, B and NK function and inhibit dendritic cell maturation, the precise mechanisms underlying the immunomodulatory effects of MSCs remain largely unknown.⁵ MSCs have also been shown *in vitro* to function as weak antigen presenting cells.⁶ They can take up antigens and, after stimulation with IFN γ , induce T-cell responses to recall antigens.⁷ Infusion of allogeneic MSCs can prime *naïve* T cells in immunocompetent mice⁴ and subcutaneously implanted allogeneic MSC can be rejected in non-immunosuppressed recipient mice.⁸ Although more research is required to determine the exact nature of MSCs initiated immune responses, clinical trials need to consider this potential immunogenicity of multiple MSCs infusions, which under specific circumstances could lead to donor hematopoietic stem cell rejection.

We were able to demonstrate that, despite previous exposure to the hematopoietic stem cell donor derived MSCs, further exposure to same donor MSCs and hematopoietic stem cells did not result into rejection of the donor graft. In contrast to the experimental model of Nauta *et al.*⁴, our observations were made following a myeloablative conditioning regimen, which may have abrogated any immunogenicity subsequent to the MSCs exposure.

More studies are undoubtedly needed to assess the most effective way to administer MSCs, but our case report demonstrates that in immunosuppressed patients MSCs can be used to promote hematopoietic engraftment, without running the risk of graft rejection. Of course, caution should be exercised in patients who are transplanted following reduced intensity conditioning and in immune competent patients treated with allogeneic MSCs (i.e. for autoimmune disorders).

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Table 1. Patient and graft characteristics

		Patient 1	Patient 2
	Recipient gender	Male	Male
	Age at 1 st HSCT	10 years	2 years
	Indication for HSCT	Homozygous β thalassemia	EBV induced HLH
Cord blood stem cells	HLA match(low resolution)	5/6	6/6
	MNC harvested $\times 10^7/\text{kg}$	4.0	3.6
	CD 34+ infused $\times 10^5/\text{kg}$	4.1	1.5
	MSC donor	Father	Father
	MSC infused $\times 10^6/\text{kg}$	3.3	1.5
	Conditioning	Treo. 42gr/ m^2 Cyclo. 120mg/kg Melfalan 140mg/ m^2 ATG- Sangstat lmtix [®] 10mg/kg	Bu. 480mg / m^2 Cyclo. VP16 30mg/kg ATG-Sangstat lmtix [®] 10mg/kg
	GvHD prophylaxis	CSA 2mg /kg/d and pred.1mg/kg/d	CSA 2mg /kg/d and pred. 1mg/kg/d
Haplo PBSCs	Stem cell donor	Father	Father
	MSC donor	Father	Father
	CD34+ cells infused $\times 10^6/\text{kg}$	16.2	20
	CD3 + cells infused $\times 10^7/\text{kg}$	<0.2	<0.1
	MSC infused $\times 10^6/\text{kg}$	3.0	1.5
	Conditioning	Treo. 42gr/ m^2 Cyclo. 120mg/kg Melfalan 140mg/ m^2 Flu. 80mg/ m^2 ATG-Sangstat lmtix [®] 10mg/kg	Treo. 42gr/ m^2 Melfalan 140mg/ m^2 Flu. 150mg/ m^2 ATG-Sangstat lmtix [®] 10mg/kg
	GvHD prophylaxis	None	None

KEY: Bu: Busulfan[®] targeted drug dosing aimed at an AUC of 17.5 mg*h/l; Treo: Treosulfan; Cyclo: Cyclophosphamide with Mesna; Flu;

Fludarabine: ATG: Anti-thymocyte globulin. CSA: Cyclosporine (from day -2); Pred: prednisolone (from day +5)

