Role of reduced intensity conditioning in T-cell and B-cell immune reconstitution after HLA-identical bone marrow transplantation in ADA-SCID

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ABSTRACT

The treatment of choice for severe combined immunodeficiency is bone marrow transplantation from an HLA-identical donor sibling without conditioning. However, this may result in low donor stem cell chimerism, leading to reduced long-term immune reconstitution. We compared engraftment, metabolic, and T-cell and B-cell immune reconstitution of HLA-identical sibling bone marrow transplantation performed in 2 severe combined immunodeficiency infants with adenosine deaminase deficiency from the same family treated with or without a reduced intensity conditioning regimen (busulfan/ fludarabine). Only the patient who received conditioning showed a stable mixed chimerism in all lineages, including bone marrow myeloid and B cells. The use of conditioning resulted in higher thymus-derived naïve T cells and T-cell receptor excision circles, normalization of the T-cell repertoire, and faster and complete B-cell and metabolic reconstitution. These results suggest the utility of exploring the use of reduced intensity conditioning in bone marrow transplantation from HLA-identical donor in severe combined immunodeficiency to improve long-term immune reconstitution.

Key words: primary immunodeficiency, bone marrow transplantation, reduced intensity conditioning.

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Introduction

Adenosine deaminase severe combined immunodeficiency (ADA-SCID) is a complex immune and metabolic disorder due to the lack of adenosine deaminase (ADA), a crucial enzyme of the purine metabolism. Patients suffer from recurrent, severe infections, growth arrest and organ alterations due to metabolic toxicity.[1-3] As for other SCID, the treatment of choice, when available, is bone marrow transplantation (BMT) from an HLA-identical sibling donor without preparative conditioning regimen.[4] Second-line treatment options for patients without an HLA-identical donor include enzyme replacement therapy,[2] unrelated BMT,[4] and gene therapy with autologous hematopoietic stem cells (HSC).[5,6]

For ADA-SCID patients undergoing HLA-identical BMT, excellent survival (95%) and adequate immune reconstitution is usually reported,[4] although there is concern about potential impairment of T-cell functions in long-term follow up.[7] The progressive loss of thymopoiesis has been attributed to a defective thymic microenvironment due to pre-existing alterations or damage induced by graft-*versus*-host-disease (GVHD) as well as to the absence of an adequate reservoir of healthy donor stem cells associated with the lack of conditioning, insufficient HSC dose, or the age of the recipient.[7-9] It has been reported that engraftment of donor-derived myeloid cells, favored by pre-conditioning, correlates with higher total $CD4^+$ and naïve $CD4^+$ T-cell counts many years after BMT in SCID patients.[7,8] In the absence of conditioning, HSC, B cells, myeloid cells and erythroid cells generally remain of recipient origin[9] and poor B-cell reconstitution has been shown to be associated to a significantly higher number of clinical events during follow up.[7,10]

In the case of ADA-SCID, the engraftment of donor-derived cells in all lineages, even as mixed chimerism, could also improve systemic alterations linked to the accumulation of toxic metabolites, such as skeletal defects[11] or neuro-psychological defects.[12,13]

On the other hand, the use of a pre-transplant conditioning regimen in ADA-SCID patients who are at risk of severe toxicity is still debated. A more satisfactory approach could be

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represented by the use of reduced or minimal intensity conditioning regimens in BMT and gene therapy.[14-17]

Here, we compared the immunological reconstitution in 2 ADA-SCID infants belonging to the same family who were treated with an HLA-identical healthy sibling BMT with or without a reduced intensity conditioning.

Design and Methods

The patients are first-degree cousins carrying the same ADA mutation (homozygous R211H on exon 7). Patient 1 was diagnosed at two months of age during hospitalization for bronchiolitis, while Patient 2 was diagnosed prenatally. A sibling of Patient 2 was previously diagnosed with ADA-SCID and treated with enzyme replacement therapy followed by HSC gene therapy.[5] Patients' toxic metabolite (dAXP) levels in red blood cells (RBC) were comparable at diagnosis (Pt 1: 61%; Pt 2: 51%). Both patients received a short course of enzyme replacement therapy before BMT. At five months of age, Patient 1 received an unconditioned bone marrow without graft-*versus*-host-disease (GVHD) prophylaxis, according to the EBMT guidelines of the Inborn Error Working Party. Patient 2 first received an unconditioned umbilical cord blood (UCB) transplant at the age of one month (1.55×10^8 nucleated cells/kg, with 0.79×10^6 $CD34^+$ cells/kg) from an HLA-identical sibling. Due to the lack of engraftment, Patient 2 was then considered for an HLA-identical BMT from the same donor preceded by a reduced intensity conditioning regimen. At the age of four months, the patient received reduced dose busulfan IV (6.7 mg/kg total), targeted according to plasma busulfan levels, and fludarabine (total dose: 100 mg/m^2) with standard cyclosporine A (CsA) prophylaxis. Total nucleated cell and $CD34^+$ cell doses were respectively: 9.23×10^8 cells/kg and 23.7×10^6 cells/kg for Patient 1; 7.2×10^8 cells/kg and 32×10^6 cells/kg for Patient 2. Cytomegalovirus (CMV) status was the same (positive donor/negative recipient). Patients were maintained on standard anti-infectious prophylaxis until immune reconstitution. Patient 2 tolerated the conditioning well except for a transient hypertension.

References for controls were obtained from the literature[18,19] or internal laboratory standards. Chimerism studies were performed on bone marrow and peripheral blood (PB) by PCR analyses of tandem repeat polymorphisms on cells purified by immunomagnetic beads and cell sorting. ADA metabolism, immune phenotyping, proliferation assays, measurement of thymic activity (TREC), and spectratyping were performed as previously described.[5,20] Patients' parents gave written informed consent to immunological research studies according to research protocols approved by the ethical committees of San Raffaele Hospital and Bambino Gesù Children's Hospital.

Results and Discussion

We describe here the immunological and metabolic reconstitution in 2 ADA-SCID infants treated with HLA-identical BMT, performed either without conditioning (Pt 1), or with a reduced intensity regimen (busulfan 6.7 mg/kg, fludarabine 100 mg/m^2) (Pt 2), following failure of a previous unconditioned transplant. These patients represent a unique setting since they carry the same ADA mutation and were treated at a similar age with a comparable cell dose. Comparison of the 2 patients allowed evaluation of the role in HLA-identical BMT of the conditioning regimen influencing the degree of chimerism and the kinetic of immune reconstitution, with special regard to thymic function and clonal composition. Posttransplant clinical course was without major complications in both patients and no signs of GVHD were observed. As expected, only Patient 2 displayed a transient neutropenia (ten days, with nadir at day +15, absolute neutrophil count (ANC) 70/μL), with no severe thrombocytopenia (nadir of platelets 49,000/μL at day +15). At current follow up (three years after BMT), the patients are currently clinically well, with normal behavioral development and growth, and have not suffered from any major infectious episodes.

Both patients displayed a high donor engraftment in the T and NK-cell compartment, in agreement with the strong selective advantage in these lineages (Table 1). However, only Patient 2 showed persistent, stable donor engraftment in myeloid PB cells (Pt 1: <5%; Pt 2: 75%) and in B cells (Pt 1: 5%; Pt 2: 60%) at a substantial level (Table 1). Interestingly, the initial engraftment in B cells derived from the infused bone marrow was lost with time in Patient 1. The use of conditioning in Patient 2 could have contributed to the better donor B-cell engraftment, as suggested in other studies based on larger cohort of patients; although in those studies other factors, such as the type of donor and SCID disease (B+ *vs.* B-) might have influenced the outcome.[9,10,21]

Multilineage donor engraftment was confirmed in the bone marrow of Patient 2, reaching levels of 65% in $CD34^+$ progenitors, 80-85% in erythroid, megakaryocytic, and granulocytic precursors, and 55% in B cells (*data not shown*). The higher chimerism level in Patient 2 led to higher ADA activity in PB lymphocytes (Pt 1: 344; Pt 2: 1346 nmol/h/mg protein) and red blood cells (Pt 1: 0.06; Pt 2: 2.64 μmol/h/ml packed cells). This resulted in a faster metabolic detoxification in PB of Patient 2, whereas Patient 1 reached appropriate purine toxic metabolite levels (dAXP <50 nmoles/mL) only 1.5 years after transplant (*data not shown*). A faster systemic detoxification could prevent the occurrence of systemic metabolic effects including neurological complications that are usually not corrected by standard BMT.[12]

Immunological studies showed a more rapid reconstitution of T-cell compartment in Patient 1, reaching normal T-cell counts for age at day +48 (Figure 1A). However, this

Table 1. Engraftment of donor cells in different cell lineages and at different time points posttransplant.

		Donor cell engraftment (%)			
		T cells	B cells	NK cells	Myeloid cells
Pt1	8 mo	>95%	95%	n.a.	<5%
	1.5 y	>95%	5%	>95%	<5%
	2.5 y	>95%	5%	>95%	<5%
Pt2	8 mo	80%	65%	80%	85%
	1 y	85%	65%	90%	80%
	2 y	85%	60%	90-95%	70%
	3 y	90-95%	60%	>95%	75%

Analyses of donor cell engraftment by chimerism analysis (PCR co-amplification of 15 tandem repeats on recipient and donor DNA samples) in peripheral blood of Pt1 and Pt2 at different time points after BMT. The multilineage engraftment was confirmed at multiple time points in purified cell subsets of Pt2 from the BM.

increase was mainly sustained by homeostatic proliferation of PB CD8$^+$ cells and CD4$^+$CD45RO$^+$ memory T cells (Figure 1A-C), associated with reduced TREC levels (Figure 2A), which persisted over time. Immune reconstitution in Patient 2 was initially slower but, after discontinuation of GVHD prophylaxis, a progressive normalization of lymphocyte counts was observed, with CD3$^+$ and CD4$^+$ cells reaching normal values for age at day +282 (Figure 1B-D). In contrast to Patient 1, T-cell reconstitution was sustained mainly by the generation of CD4$^+$CD45RA$^+$ naïve cells (Figure 1B-D). Indeed, thymic activity measured by TREC on PB CD4$^+$ and CD8$^+$ T cells was one log higher in Patient 2 as compared to Patient 1 (Figure 2A), indicating a better thymic output in the patient who received pre-conditioning. This was consistent with a wide polyclonal TCR distribution in CD4$^+$ cells in Patient 2, whereas Patient 1 showed an altered repertoire one year after BMT, which improved over time (Figure 2B). The repertoire was more skewed in CD8$^+$ T cells in both patients as reported in previous studies[22,23] but also consistently more polyclonal in Patient 2. Moreover, Patient 2 showed normalization of proliferative responses to polyclonal mitogens (PHA, anti-CD3), Candida, and allogeneic stimuli, while proliferative responses were reduced in Patient 1 (*data not shown*).

Collectively, these results suggest the presence of a continuous regeneration of T-cell repertoire of a wide antigenic-specificity, fundamental for the competence of the immune system against a broad spectrum of infectious agents and prevention of autoimmunity.

In agreement with the prevalent B-cell donor chimerism, B-cell counts were restored earlier in Patient 2 (Figure 1E-F), while Patient 1 showed persistently low levels of circulating B cells, including memory B cells. In addition, IVIg were discontinued later in Patient 1 (2.5 years) than in Patient 2 (one year). Nevertheless, both patients achieved normal immunoglobulin levels and protective antibody responses post-vaccination (*data not shown*). One could also hypothesize that the metabolic detoxification and cross correction from ADA-expressing donor T cells might have contributed to the partial B-cell reconstitution in Patient 1, despite low levels of donor B cells. It is possible that the previous UCB infusion in Patient 2 might have favored the subsequent engraftment of bone marrow donor cells, but this is unlikely since no GVHD was observed and donor cells were found three weeks after umbilical cord blood (UCB) infusion only in T cells, and were subsequently lost.

Our results suggest that the use of a conditioning regimen prior HLA-identical bone marrow transplantation in SCID, even at reduced intensity, might be sufficient to obtain a stable donor chimerism in all lineages, including B and myeloid cells. This results in higher thymus-derived naïve T cells, TREC levels, and normalization of the T-cell repertoire, which are strong predictors of long-term sustained immune reconstitution.[7,8] On the contrary, the low thymic output and the alterations in T-cell repertoire observed in Patient 1 without conditioning may be

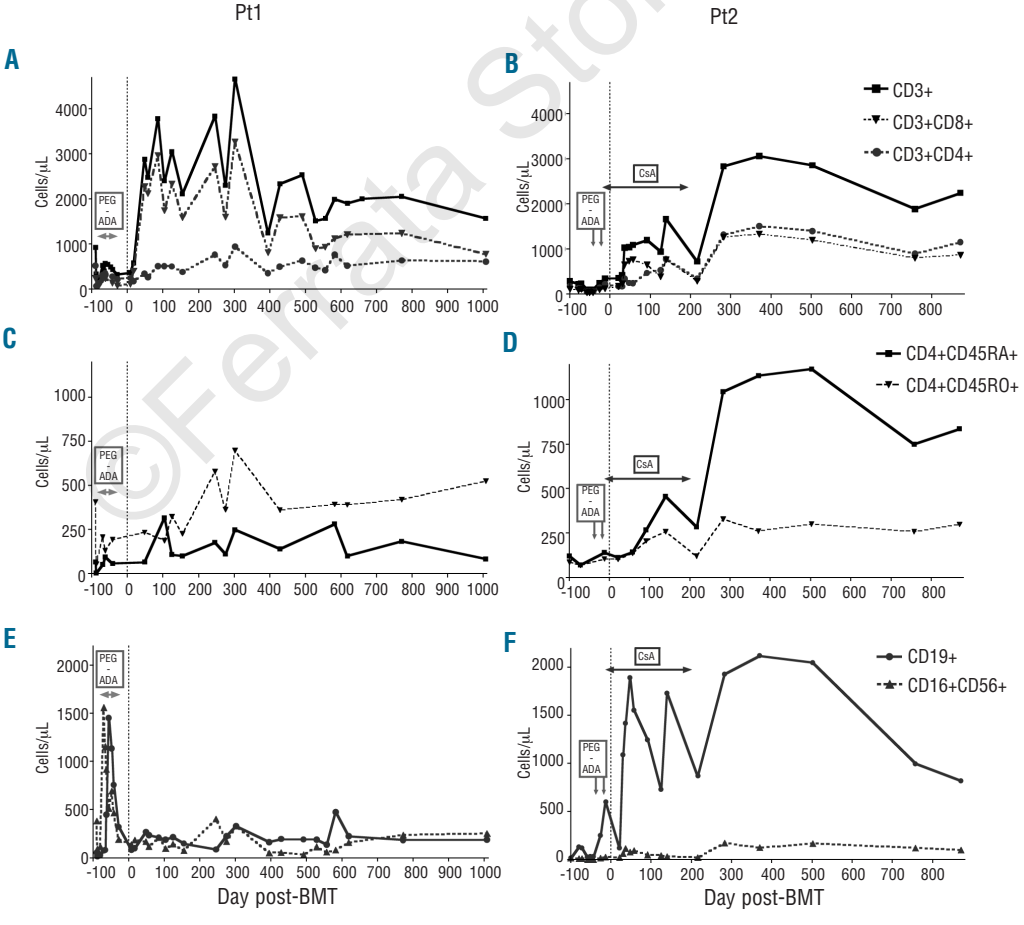

Figure 1. Immune reconstitution after HLA-identical BMT with or without conditioning in ADA-SCID. Pt1 received no conditioning, Pt2 reduced intensity conditioning with busulfan and fludarabine. Kinetics of reconstitution of lymphocyte subsets in Pt1 (A, C, E) and Pt2 (B, D, F), analyzed by flow cytometry. Values indicate absolute numbers of CD3$^+$, CD3$^+$CD4$^+$, CD3$^+$CD8$^+$ T cells (A, B), CD4$^+$CD45RA$^+$ naïve and CD4$^+$CD45RO$^+$ memory T cells (C, D), CD19$^+$ B cells and CD16$^+$CD56$^+$ NK cells (E, F). Normal values for age for T cells are >2400 cells/μL (5-9 months); >1600 cells/μL (9-15 months), >1400 cells/μL (15-24 months), >900 cells/μL (2-5 years).[18] Other reference values for age were obtained from literature.[18,19] The period of PEG-ADA supplementation and GVHD prophylaxis (CsA) are also indicated.

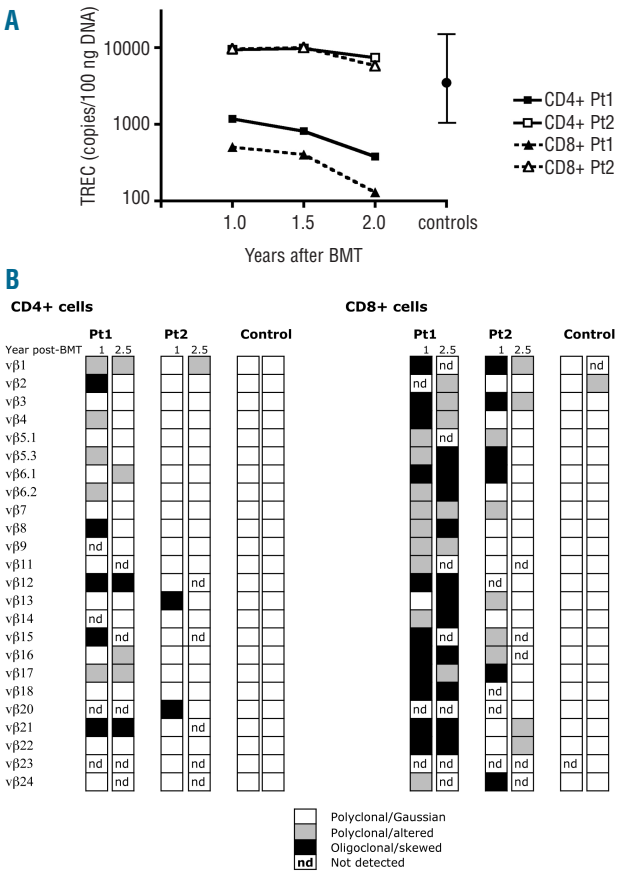

Figure 2. Thymic function and TCR repertoire after HLA-identical BMT with or without conditioning in ADA-SCID. (A) TREC levels measured by quantitative PCR in purified CD4$^+$ and CD8$^+$ T cells at different time points of follow up compared to normal values in T cells obtained from 16 pediatric healthy controls (median and range). (B) TCR repertoire analysis through Vβ spectratyping in purified CD4$^+$ and CD8$^+$ cells analyzed at one and 2.5 years after BMT. The pattern of clonality was defined as reported.[20] A representative age-matched control is included.

explained by an insufficient engraftment of donor stem cells and by the peripheral expansion of mature donor T cells.[24] These advantages should be balanced with a higher risk of toxicity and infectious complications in SCID, and particularly in ADA deficiency, due to increased sensitivity to chemotherapy in these patients. In addition, the genotoxic effects on host cells exposed to chemotherapy should be considered in patients who received reduced intensity conditioning and which resulted in mixed chimerism in the hematopoietic stem cell compartment.

In conclusion, our study, although obtained only by the comparison of 2 patients, introduces the option of exploring the use of a reduced-intensity conditioning regimen in the setting of HLA-identical bone marrow transplantation for ADA deficiency and other severe combined immunodeficiencies to achieve a better long-term immune reconstitution. Initially, this approach could be considered in early-diagnosed infants without organ damage and potentially expanded later to patients at greater risk.

Authorship and disclosures

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