Brief report

Serial transplantation of mismatched donor hematopoietic cells between HLA-identical sibling pairs with congenital immunodeficiency: in vivo tolerance permits rapid immune reconstitution following T-replete transplantation without GVHD in the secondary recipient

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We report serial transplantation procedures in 2 sets of brothers with X-linked primary immunodeficiency. The first boy in each family received a T-cell–depleted transplant from a mismatched donor. The recipients then acted as donors for T-replete transplantation of the "tolerized" graft into their HLA-identical brothers with the same disorder. Immune reconstitution was noted to occur at a significantly faster rate in the secondary recipients, and without the occurrence of graft-versus-host disease (GVHD), despite the presence of donor cells mismatched for 1 to 3 HLA antigens. This serial transplantation technique allows the primary recipient of HLA-mismatched donor cells to act as a functionally "HLA-matched" donor for subsequent affected siblings, and should be considered as a therapeutic option in families with congenital disorders. (Blood. 2006;108:2124-2126)

Introduction

We report 2 sets of brothers with X-linked primary immunodeficiency, severe combined immune deficiency (SCID) due to absence of the common gamma chain, and Wiskott-Aldrich syndrome (WAS). In patients with primary immunodeficiencies, a stem cell transplant (SCT) from an HLA-identical donor achieves a superior 3-year survival compared with an HLA-mismatched SCT (77% vs 54% for SCID patients, 71% vs 42% for non-SCID).[1] Similarly in WAS patients, improved survival is seen following genotypically identical stem cell transplantation compared with related HLA-mismatched stem cell transplantation (81% vs 43%). These differences reflect the higher incidence of graft-versus-host disease (GVHD) and slower immune reconstitution following HLA-mismatched stem cell transplantation.[2] No matched related or unrelated donors were available for either of the sibling pairs in this study, so the first boy in each family underwent a T-cell–depleted mismatched stem cell transplantation procedure. Following recovery, the recipients then acted as donors for non–T-cell–depleted SCT of the presumed "tolerized" graft into their HLA-identical brothers.

Study design

Case descriptions

Family X. Patient X1 was diagnosed at the age of 6 months with T-B+NK-X–linked SCID due to a nonfunctional mutation in the common gamma-chain gene. He presented with pneumonia following recurrent chest infections, chronic diarrhea, and failure to thrive. At the age of 13 months, he underwent T-cell–depleted bone marrow transplantation from a 9/10 HLA-mismatched, CTLP-reactive unrelated donor (mMUD) with busulphan/cyclophosphamide conditioning, in vivo T-cell depletion with Campath 1G, and cyclosporin/methotrexate as graft-versus-host disease prophylaxis (Table 1). Neutrophil recovery occurred on day 14, and 100% donor engraftment was documented on day 43. On day 6, he developed a severe croup like illness. No causative organism was identified, but he responded to nebulized adrenaline and budesonide. He developed grade 2 skin GVHD on day 10 that responded to oral prednisolone and was discharged home on day 41. While recovery of T-cell numbers was reasonably prompt (by day 26 his $CD3^+$ cells $> 0.5 \times 10^9$/mL and $CD3^+4^+$ cells $> 0.3 \times 10^9$/mL), $CD8^+$ T-cell and B-cell recovery and normalization of functional proliferative responses to PHA were slow ($CD8^+$ cells $> 0.2 \times 10^9$/mL day 91, $CD19^+$ cells $> 0.5 \times 10^9$/mL 6 months, normal PHA SI 6 months). Furthermore, despite penicillin prophylaxis he developed penicillin-resistant pneumococcal sepsis 18 months following transplantation, having responded poorly to pneumococcal vaccination. His immune reconstitution is shown in Table 2. At last review he was 9 years old, was growing well, and was in good health.

His brother, X2, was diagnosed in utero with the same mutation by molecular genetic analysis of chorionic villous tissue and found to be HLA identical to patient X1. Pregnancy was uneventful and the child was delivered by caesarian section at 37 weeks' gestation. SCID was demonstrated at birth by flow cytometric analysis of umbilical cord blood, which showed absence of $CD3^+45^+$ and $CD16^+56^+45^+$ cells. Low-level maternal engraftment was present with 1:350 female cells detected by fluorescent in situ hybridization. No functional assay of graft tolerance was performed. On day 2 of life, patient X2 received T-replete bone marrow harvested from

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Table 1. Details of serial hematopoietic stem cell transplantations within 2 families

Patient no. (conditioning/ serotherapy/ GVHD prophylaxis) and donor	HLA-match (mismatched locus)	Source	In vitro T-cell depletion	Total cells/kg	$CD3^+$ cells/kg	$CD34^+$ cells/kg
X1 (Bu16, Cy200/Campath 1G, d −13 to −9/CSA, MTX)						
UD	9/10 (1C)	BM	Campath 1M	9.0×10^7	1.0×10^5	—
X2 (none/none/none)						
Brother	10/10	BM	None	3.5×10^8	1.0×10^8	5.0×10^6
Y1						
First transplant (Bu8, Flu120, Mel125/ATG, d −15 to −11/CSA)						
Father	7/10 (1B, 1C, 1DR)	PBSCs	Miltenyi	4.6×10^7	2.0×10^5	3.6×10^7
Second transplant (Cy150/Campath 1G, d −4 to −1/MP, CSA)						
Father	7/10 (1B, 1C, 1DR)	BM	Campath 1M	6.0×10^8	—	1.9×10^7
Father	7/10 (1B, 1C, 1DR)	PBSCs	Miltenyi	2.6×10^7	1.1×10^5	2.0×10^7
Y2						
First transplant (Flu150, Mel140/Campath 1H, d −8 to −4/CSA)						
Brother	10/10 (before BMT)	BM	None	2.4×10^8	2.8×10^7	4.8×10^6
Second transplant (Bu16, Cy200/ATG, d −15 to −11/CSA)						
Brother	10/10 (before BMT)	BM	None	2.3×10^8	2.5×10^7	2.8×10^6
Father	7/10 (1B, 1C, 1DR)	PBSCs	Miltenyi	2.7×10^7	0.8×10^4	2.3×10^7

Miltenyi is located in Auburn, CA.

Bu16 indicates busulphan 16 mg/kg; Cy200, cyclophosphamide 200 mg/kg; Campath 1G, Campath 1G 1 mg/kg; CSA, cyclosporin A; MTX, methotrexate; UD, unrelated donor; BM, bone marrow; Bu8, 8 mg/kg busulphan; Flu120, 120 mg/m^2 fludarabine; Mel125, 125 mg/m^2 melphalan; ATG, 25 mg/kg rabbit antithymocyte globulin; PBSCs, peripheral blood stem cells; Cy150, 150 mg/kg cyclophosphamide; MP, methylprednisolone; Flu150, 150 mg/m^2 fludarabine; Mel140, 140 mg/m^2 melphalan; and Campath 1H, 1 mg/kg Campath 1H.

his brother X1, who had undergone SCT 3 years ago, without conditioning or GVHD prophylaxis, as described in Table 1. He remained well and was discharged home at the age of 7 days. Immune reconstitution was much more rapid than in his brother, as shown in Table 2. By 6 days of age, his ALC was more than 1.0×10^9/mL, with $CD3^+4^+$ cells more than 0.3×10^9/mL and $CD3^+8^+$ cells more than 0.2×10^9/mL, and he had a normal PHA response by 30 days. His B cells were never less than 0.5×10^9/mL. Three months after transplantation, he had stable mixed chimerism (20% mMUD mononuclear cells, and 30% mMUD granulocytes), with no evidence of engraftment of X1′s own cells into X2. X2 did not develop GVHD at any stage. He was last reviewed at 7 years of age, when he was well, and had not suffered any significant infections. Analysis at this latest time point shows a normal T-cell receptor (TCR) spectratype with Gaussian profiles in both $CD4^+$ and $CD8^+$ T cells (data not shown) and normal TREC (TCR excision circles) levels indicating engraftment of prethymic progenitors.[3]

Family Y. Monochorionic twin boys both developed recurrent episodes of cellulitis, purulent ear infections, and eczema in infancy. Investigation revealed thrombocytopenia and leucopenia. A diagnosis of WAS was made in both boys at 7 months of age based on absent expression of WAS protein. At 11 months, patient Y1 received a peripheral blood stem cell transplant, using purified $CD34^+$ cells from his 7/10 HLA-mismatched father, with busulphan/fludarabine/melphalan/ATG conditioning, as detailed in Table 1. At 20 days after transplantation, bone marrow examination revealed no evidence of engraftment. A repeat procedure was performed using both T-cell–depleted bone marrow and peripheral blood stem cells from his father, with cyclophosphamide/Campath 1G conditioning, and methylprednisolone/cyclosporin as GVHD prophylaxis. Neutrophil recovery occurred by day 16, and engraftment studies showed full donor chimerism in both mononuclear and granulocyte cell lines by 3 weeks after transplantation. It took more than 4 months for his ALC to reach 1.0×10^9/mL, T cells to reach 0.5×10^9/mL, $CD3^+4^+$ cells to reach 0.3×10^9/mL, and $CD3^+8^+$ cells to reach 0.2×10^9/mL, and nearly a year to develop B cells more than 0.5×10^9/mL. He had recovered a normal PHA response by 210 days after transplantation. At last review at 5 years of age he was free from infections and other WAS-related problems.

Table 2. Time to immune reconstitution by cell type in primary (X1, Y1) and secondary (X2, Y2) recipients of serial hematopoietic stem cell transplantation

	Time to reconstitute, d			
	X1	X2	Y1	Y2
Neutrophil count above 0.5×10^9/L	14	0	22	18
ALC above 1.0×10^9/L	26	6	150	30
CD3 count above 0.5×10^9/L	26	10	150	30
CD3/4 count above 0.3×10^9/L	26	6	140	55
CD3/8 count above 0.2×10^9/L	91	6	130	30
CD19 count above 0.5×10^9/L	182	0	353	125
Normal PHA response	180	30	210	150

At the time of his brother's transplantation, patient Y2 had experienced recurrent bacterial infections and autoimmune vasculitis. Bone marrow transplantation (BMT) was proposed using marrow from his brother, who had previously undergone transplantation. Mixed lymphocyte culture showed some reactivity in the host-versus-graft direction (stimulation index [SI] 7.8 versus control 28.4), but tolerance in the graft-versus-host direction (SI 1.3 versus control 32.0); further evidence of tolerance was obtained, showing a low cytotoxic T-lymphocyte precursor frequency of 1 in 9724. At 19 months of age, patient Y2 received a T-replete bone marrow transplant from his twin brother, following nonmyeloablative conditioning with fludarabine/melphalan/Campath 1H conditioning and cyclosporin alone as GVHD prophylaxis, as shown in Table 1. This achieved partial chimerism, with a maximum of only 10% donor cells in whole blood and in the mononuclear cells. He was subsequently treated with rituximab and a splenectomy for autoimmune hemolytic anemia and thrombocytopenia. At 3 years of age, he was clinically deteriorating. Following myeloablative conditioning with busulphan/cyclophosphamide/ATG, he received a second transplant with T-cell–replete bone marrow from his brother Y1 and CD34-purified peripheral blood stem cells from his father, with cyclosporin as GHVD prophylaxis as shown in Table 1. A month later, he had 100% engraftment of paternal cells in both lineages. His immune reconstitution was more rapid than that of his brother, with ALC more than 1.0×10^9/mL, T cells more than 0.5×10^9/mL, and $CD3^+8^+$ cells more than 0.2×10^9/mL by 30 days. His $CD3^+4^+$ cells were more than 0.3×10^9/mL by 55 days after transplantation, and his B-cell count was more than 0.2×10^9/mL by 125 days. PHA response was normal by 150 days. Despite receiving a T-replete haploidentical graft, he did not develop GVHD. He was last reviewed at 5 years of age. He has had no significant infections or other WAS-related problems.

Results and discussion

This serial transplantation technique shows how HLA-mismatched grafts may be tolerized into "HLA-matched" grafts through sibling donors who, by virtue of previous transplantation, have been converted from being disease-affected recipients into healthy matched donors. Potential mechanisms of tolerance include deletion of alloreactive T cells in the thymus of the primary recipient and/or the transfer of peripheral T-regulatory cells with the secondary graft.

Successful serial BMT has been described previously,[4] where the recipient of a haploidentical transplant for SCID acted as a donor for his/her unconditioned affected sibling, who received cyclosporin as GVHD prophylaxis. No comparison of immune reconstitution was made in that paper. To our knowledge, patient X2 is the first report of a successful serial transplant from an unrelated donor. He successfully underwent transplantation without the development of GVHD even in the absence of GVHD prophylaxis. The transplantation was performed as soon as possible after birth to reduce the risk of maternal engraftment with the consequent need for pretransplantation conditioning. This precluded a functional assay of tolerance and donor/recipient compatibility. However, the infusion of a CD3 cell dose 1000 times larger than the dose patient X1 received, and lack of GVHD in the absence of GVHD prophylaxis, demonstrated this tolerance in vivo. Furthermore, B-cell and $CD4^+$ and $CD8^+$ T-cell reconstitution occurred faster in patient X2 than in X1. Of interest, despite the absence of conditioning, TREC analysis suggests early progenitor engraftment, which may reflect the early time of transplantation and large cell dose or may be related to the tolerized nature of the graft.

For patient Y2, there was sufficient time to ensure that there was immunologic tolerance of potential donor cells toward the second recipient's HLA antigens by means of both MLC and CTLP. Twin Y2 was initially conditioned with a reduced-intensity nonmyeloablative protocol. This has been shown to be successful in the transplantation of both SCID and non-SCID congenital immunodeficiencies,[5] but low-level donor chimerism has become increasingly recognized following the use of matched sibling donors with reduced-intensity conditioning protocols,[6] presumably due to a reduced graft-versus-marrow effect. In this case, successful engraftment was achieved with myeloablative conditioning and the addition of tolerized bone marrow from Y1. Patient Y2 "tolerated" 2.5×10^7/kg $CD3^+$ cells originating from a haploidentical donor with only cyclosporin as GVHD prophylaxis. Again, immune reconstitution occurred faster for all subsets in the secondary recipient (Table 2).

These cases demonstrate how serial BMT can lead to successful treatment of sibling pairs with congenital immunodeficiencies, with rapid immune reconstitution and cure in the subsequent sibling. Such an approach should be considered for similar families when no HLA-matched donor is available.

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