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UNRELATED BONE MARROW TRANSPLANTATION IN A WISKOTT-ALDRICH SYNDROME PATIENT SHARING TWO HLA-EXTENDED HAPLOTYPES WITH THE DONOR

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ABSTRACT

We report a case of BMT from an unrelated donor (MUD) in a patient affected by Wiskott-Aldrich syndrome (WAS). The donor-recipient pair was completely identical for two HLA-extended haplotypes. The conditioning regimen consisted of Bu 14 mg/kg followed by CY 200 mg/kg. GVHD prophylaxis was carried out with CsA plus short-term MTX. Allogeneic engraftment was obtained without any signs of acute or chronic GVHD. At fifteen months from the transplant the patient was in an excellent clinical condition. This case confirms the role of BMT from MUD in WAS, and suggests that complete donor-recipient identity for two entire HLA-extended haplotypes is a particularly favorable immunogenetical condition in this type of transplant.

Keywords: unrelated BMT, Wiskott-Aldrich syndrome, extended HLA-haplotypes

The Wiskott-Aldrich syndrome (WAS) is an X-linked recessive disorder characterized by severe thrombocytopenia, eczema, combined B- and T-cell immunodeficiency, impaired humoral immune response to polysaccharide antigens and increased susceptibility to infections and lymphoreticular malignancies.[1]

Allogeneic BMT from an HLA-matched sibling donor is the treatment of choice in WAS patients.[2,3] Unfortunately, the probability of finding an HLA-identical donor among family members ranges from 25-30%. HLA haploidentical T-cell-depleted bone marrow has been used but with contrasting results.[4-6] Recently, favorable results were reported in WAS patients transplanted with phenotypically-matched unrelated donors (MUD),[7,8] suggesting that BMT from MUD may play a significant role in the treatment of WAS. Some clinical[9] and *in vitro*[10] findings seem to indicate that better donor-recipient matching is obtained when two HLA-extended haplotypes are shared.

Accordingly, BMT was carried out in a patient affected by WAS, using an unrelated donor identical for two HLA-extended haplotypes.

Case Report

The patient, a 4-year-old Sardinian boy, was admitted to our Institute in January 1994. From the first few months of life the patient revealed thrombocytopenia (platelets $\leq 20\times10^9$/L), recurrent episodes of gastrointestinal bleeding and eczema. Hospitalization had been necessary on several occasions for bacterial and viral infections due to severe immune deficiency.

On admission to our Institute the total lymphocyte count was 2.28×10^9/L with 1.54×10^9/L $CD3^+$ T cells (1.28×10^9/L $CD4^+$ and 0.26×10^9/L $CD8^+$), 0.36×10^9/L B cells and 0.28×10^9/L NK cells. Lymphocyte analysis by scanning electron microscopy revealed surface abnormalities characteristic of WAS. Serum immunoglobulin assays yielded low values of IgG (427 mg/dL,

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normal range 650-1700) and IgM (19 mg/dL, normal range 55-300) and high values of IgA (494 mg/dL, normal range 70-380) and IgE (109 kU/L, normal range < 70). Serological investigation detected IgG antibodies for CMV.

The patient, who lacked an HLA-identical family donor, carried two HLA-extended haplotypes which are in strong linkage disequilibrium in the Sardinian population and differ for only one antigen in the HLA-A locus:

1. A*3002, Cw5, B18, C2C, BfF1, C4A3, C4BQ0, DRB1*0301, DRB3*0202, DQA1*0501, DQB1*0201, DPA1*01, DPB1*0301;
2. A2, Cw5, B18, C2C, BfF1, C4A3, C4BQ0, DRB1*0301, DRB3*0202, DQA1*0501, DQB1*0201, DPA1*01, DPB1*0301.

A search for a donor in the Italian Bone Marrow Donor Registry (IBMDR) made it possibile to select 7 HLA-A, Cw, B and DR phenotypically-identical donors. A family study including class III specificities showed that 4 of the donors (of Sardinian origin) shared both HLA-A-DQ extended haplotypes with the patient. Molecular typing demonstrated that two of these donors were DPB1*0301/0401 heterozygous, while the other two (both females) were completely identical with the patient. Of the two HLA-A-DP identical donors, we chose the one who was completely negative in MLC (see Table 1, donor 1). This donor, a 32-year-old woman and mother of one child, had serum IgG antibodies for CMV like the patient. On the whole, the time required for the search was 30 days.

After obtaining informed consent for BMT, a conditioning regimen was started with Bu 3.5 mg/kg/day orally for 4 days followed by CY 50 mg/kg/day iv for 4 days. Busulphan was administered at doses lower than those generally recommended (14 mg/kg instead of 16 mg/kg) to reduce toxicity and in consideration of the patient's T-cell immunodeficiency and the high degree of matching between donor and recipient. On day 0 (May 30, 1994) the patient was housed in a sterile laminar airflow room and 1.9×10^8/kg donor nucleated cells were infused. GVHD prophylaxis was carried out with CsA 3 mg/kg/day iv from day –2, combined with MTX 10 mg/m^2 iv on days +2, +4 and +7.

Infection prophylaxis consisted of acyclovir, fluconazole, oral non-absorbable antibiotics and polyspecific human immunoglobulins. From day +8 to day +21, 5 mg/kg/day recombinant G-CSF were administered. Engraftment was demonstrated on day +13 by PCR, using highly polymorphic microsatellite markers of 4 different chromosomes (D1S104, D2S72, D4S174 and D8S88).

The post-BMT course was regular, without any signs of infection. Hematological reconstitution was quick with granulocytic neutrophils exceeding 0.5×10^9/L from day +14 and a total leukocyte count of $> 3.5\times10^9$/L from day +21;

		HLA molecular typing					MLC (IRR%)	
		DRB1*	DRB3*	DQA1*	DQB1*	DPB1*	D-Rx	R-Dx
Recipient		0301/0301	0202/0202	0501/0501	0201/0201	0301/0301		
Donor	1	0301/0301	0202/0202	0501/0501	0201/0201	0301/0301	0	0
"	2	0301/0301	0202/0202	0501/0501	0201/0201	0301/0301	0	7.9
"	3	0301/0301	0202/0202	0501/0501	0201/0201	0301/0401	4.1	12.6
"	4	0301/0301	0202/0202	0501/0501	0201/0201	0301/0401	3.0	51.4

MLC: mixed lymphocyte culture; IRR: relative response index; D-Rx: relative response index of donor cells stimulated by recipient cells; R-Dx: relative response index of recipient cells stimulated by donor cells;

Table 1. Molecular HLA class II alleles and MLC data in the recipient and 4 unrelated donors sharing two HLA-extended haplotypes.

the latter remained stable after suspension of rG-CSF. The platelet count rose steadily above 35×10^9/L from day +14 and Hb concentration spontaneously stayed > 10 g/dL from day +29. All other standard laboratory parameters remained within normal values during the post-BMT course.

The patient was discharged from the hospital on day +48 without any sign of acute GVHD.

At 15 months post-BMT, the patient was in excellent clinical condition without any signs of infection or acute/chronic GVHD, and with platelet counts of $>200\times10^9$/L.

The lymphocyte subsets were normal: lymphocytes 2.76×10^9/L with 1.47×10^9/L $CD3^+$ T cells (1.25×10^9/L $CD4^+$ and 0.53×10^9/L $CD8^+$), 0.32×10^9/L B cells and 0.26×10^9/L NK cells. Serum immunoglobulins were also within the normal range: IgG 765 mg/dL, IgA 54 mg/dL, IgM 115 mg/dL and IgE 0 kU/L.

The lymphoproliferative response to allogeneic and mitogen stimulation (PHA, Pokeweed) was normal. Scanning electron microscopy did not show any alterations in lymphocyte morphology and flow cytofluorometry revealed normal surface expression of CD43 sialoglycoprotein.

Discussion

Recently, several cases of unrelated BMT in WAS have been reported.[7,8] Out of a total of 7 cases, 6 were successful. GVHD was controlled by therapy in all cases but its incidence was high, with 5 out of 6 cases of acute GVHD (83%) and 4 out of 5 evaluable cases of chronic GVHD (80%). In 2 cases the donor-recipient pair differed for an HLA class I antigen, while the remaining 5 were completely matched, although only at the serological level.

Our case differs from the ones described above for the immunogenetical criteria in donor selection and the clinical outcome. In fact, our donor-recipient pair shared two HLA-extended haplotypes, which were determined by a family study that included class III investigation and molecular typing of the DR, DQ and DP alleles.

Furthermore, in another case of unrelated BMT carried out in a 17-year-old thalassemic patient according to the same immunogenetical criteria,[11] the clinical course was characterized by a total absence of infections, GVHD and other serious hematological complications. Although it is not yet possible to draw definitive conclusions, these two cases suggest that a complete donor-recipient matching of two HLA-extended haplotypes is a particularly favorable condition for the outcome of BMT from unrelated donors, at least in patients with genetic diseases. A similar situation has also been reported in the Japanese program of unrelated bone marrow transplantations;[12] however, this situation is relatively frequent only in populations that are extremely homogeneous genetically. In most cases of BMT in which donor/recipient identity concerns non-extended HLA haplotypes, donor selection must also be based on other criteria predictive of GVHD, such as sex, donor parity and functional tests such as CTLp and HTLp, which are probably not crucial when two extended haplotypes are shared by the donor and the recipient.

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