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Case report

Successful unrelated cord blood transplantation in two children with severe combined immunodeficiency syndrome

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Summary:

We describe the successful unrelated cord blood transplantation in two patients affected by a Zap-70 deficiency and an Omenn-like syndrome, respectively. The patients were hospitalised for recurrent infections at the age of 13 and 2 months, respectively. An unrelated cord blood unit was found for each. The conditioning regimen was cyclophosphamide, busulfan and antithymocyte globulin. The total number of infused cells was 15.1×10^7/kg and 17×10^7/kg, respectively. Neutrophil engraftment was achieved on days +15 and +23, and platelet count $>50 \times 10^9$/l was achieved on days +21 and +52, respectively. One patient presented acute Graft-versus-host disease (GVHD) grade I and the other grade III. Chimerism was mixed and full donor. Normal lymphoproliferative response to mitogens and alloantigens was detectable at 6 months for both. No chronic GVHD was observed in either. The patients are alive and well at 53 and 15 months after transplantation. In conclusion, umbilical cord blood represents a valid alternative source of haemopoietic stem cells.

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Severe combined immunodeficiencies (SCID) are a group of rare congenital disorders characterised by the impairment of both humoral and cell-mediated immunity, leukopenia, and low or absent antibody levels. They usually manifest in the first months of life with severe and recurring infections leading to death.[1] Since 1968, these diseases have been successfully treated by BMT[2–5] and, in recent years, by umbilical cord blood transplantation (UCBT).[6–9]

In this report, we describe the successful immunological reconstitution, following myeloablative UCBT, in two patients affected by a Zap-70 deficiency and by an Omenn-like syndrome. Zap-70 deficiency is a rare autosomal recessive form of SCID because of mutations within the kinase domain of ZAP-70, which is critical for CD8+ T-cell development.[10] Omenn-like syndrome is the engraftment of maternal T-cells in a child with a T-SCID, which is clinically reminiscent of Omenn syndrome.[11–12]

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Case report

Patient 1

A 13-month-old child was referred to our hospital after a 2-month long Salmonella gastroenteritis and several recurring respiratory infections. The child presented a low height–weight growth. Laboratory evaluation revealed normal B-cell values (2830/μl – 45%) with normal immunoglobulin levels, a near absence of CD8+ cells (251/μl – 0.04%) with an increased CD4/CD8 cell ratio and a decreased proliferative response to allogeneic stimulus, mitogens and anti-CD3 stimulus (stimulus involving TCR). When the response was evaluated with anti-CD3, together with phorbol ester, the proliferative response was maintained. A mutation of the tyrosine kinase ZAP-70 protein was identified by molecular biology analysis (Roncarolo *et al*, personal communication).

As no matched related donor was found, the patient underwent haemopoietic stem cell transplantation with a two-loci-mismatched cord blood unit, from the Milan Cord Blood Bank, identified by low-resolution typing for loci A and B and high-resolution typing for locus DRβ1 (Table 1). The time from the start of the search to the identification of a suitable cord blood unit was 43 days and then 12 days to transplantation.

The conditioning regimen was busulfan 2 mg/kg in divided doses daily on days −10, −9, −8, −7 (total dose 8 mg/kg), cyclophosphamide 50 mg/kg daily on days −5, −4, −3, −2 (total dose 200 mg/kg) and antithymocyte globulin (ATG) 2 mg/kg daily on days −2, −1 and +1 (total dose 6 mg/kg). Graft-versus-host disease (GVHD) prophylaxis consisted of cyclosporin A (CSA) 3 mg/kg daily. The cord blood unit was thawed according to Rubinstein's technique.[13] The total number of cells infused was 15.1×10^7/kg. CD34+ cells/kg were 8×10^5, CFU-GM were 2.9×10^4/kg. PMN count $>0.5 \times 10^9$/l was achieved on day +15 and PLT count $>50 \times 10^9$/l on day +21. The patient presented acute GVHD grade I. No chronic GVHD was observed. CSA administration was stopped at 10 months from transplantation.

The chimerism study performed by FISH showed persistent mixed chimerism from day +30 after UCBT. The analysis of lymphocyte subpopulations performed from month +7 revealed a persistent mixed chimerism of NK and B cells and a full donor chimerism of T cells.

Immunological reconstitution is summarised in Table 2. Normal lymphoproliferative response to mitogens and alloantigens was detectable from 6 months after UCBT. Immunoglobulin administration was stopped on month +15 and, from then on, the patient's immunoglobulin values were normal. The child was vaccinated against poliomyelitis, hepatitis B, tetanus and diphtheria 18 months after UCBT. A normal specific antibody production was evaluated after the third vaccine dose.

The patient is alive and well 53 months after transplantation with full haematological and immunological reconstitution.

Patient 2

A 2-month-old child presented with diarrhoea, generalised oedema, generalised exudative erythrodermia, infiltrate liftable skin, inguinal and axillary adenopathy and moderate hepatosplenomegaly. Laboratory evaluation revealed moderate anaemia, leukocytosis (66 200/μl) with marked eosinophilia (11 900/μl), low serum immunoglobulin values (IgG: 260 mg/dl, IgA: 8 mg/dl, IgM: 38 mg/dl) and hypoalbuminemia (3.14 g/dl). Liver function tests were mildly deranged. Immunophenotype analysis showed the following percentages and absolute number of lymphocyte subpopulations: CD3+ 86.1% – 15575/μl, CD3+/CD4+ 76.6% – 13 864/μl, CD3+/CD8+ 5.2% – 941/μl, CD19+ 4.9% – 887/μl, CD3-/CD56+ 3.8% – 688/μl and the presence of a large population of activated T cells. The proliferative response to mitogens was markedly reduced. FISH analysis on peripheral blood showed that 75% of total cells were of maternal origin. Maternal lymphocytes were found by HLA typing of peripheral blood cells and lymphonode biopsy.[14] The diagnosis was Omenn-like syndrome.

As no matched related donor was found, the child underwent haemopoietic stem cell transplantation with a one locus-mismatched cord blood unit, from the Milan Cord Blood Bank, identified by low-resolution typing for loci A and B and high-resolution typing for locus DRβ1 (Table 1). The time from the start of the search to the identification of a suitable cord blood unit was 33 days and then 25 days to transplantation.

The conditioning regimen was busulfan 4 mg/kg in divided doses daily on days −10, −9, −8, −7 (total dose 16 mg/kg), cyclophosphamide 50 mg/kg daily on days −5, −4, −3, −2 (total dose 200 mg/kg) and ATG 3.5 mg/kg daily on days −5, −4, −3 (total dose 10.5 mg/kg). GVHD prophylaxis consisted of CSA 3 mg/kg/day from day –1 and prednisone 1 mg/kg/day. The cord blood unit was thawed according to Rubinstein's technique.[13] The total number of cells infused was 17×10^7/kg. CD34+ cells/kg were 14×10^5, CFU-GM were 1.7×10^4/kg. CD3+ lymphocytes in the graft were 43.43×10^6/kg. PMN count $>0.5 \times 10^9$/l was achieved on day +23 and platelet count $>50 \times 10^9$/l

Table 1 HLA typing and ABO group of donors and recipients

	Patient 1		*Patient 2*	
	Donor	*Recipient*	*Donor*	*Recipient*
Sex	*Female*	*Male*	*Male*	*Male*
ABO group	*A*	*A*	*O*	*A*
	Rh negative	*Rh positive*	*Rh negative*	*Rh positive*
HLA typing:				
Locus A[a]	23/32	23/29	2/3	30/3
Locus B[a]	44/51	44/51	7/13	7/13
Locus DRβ1[b]	0701/1101	0701/1104	04011/07011	0401/0701

[a]Serological typing.
[b]Molecular typing.

Table 2 Patient 1 immune reconstitution after UCBT

Months after UCBT	*CD3+/μl*	*CD3+4+/μl*	*CD3+8+/μl*	*CD19+/μl*	*CD56+3−/μl*
1	400 (1400–8000)[a]	300 (900–5500)	100 (400–2300)	4 (600–3100)	80 (100–1400)
3	320 (1400–8000)	270 (900–5500)	50 (400–2300)	60 (600–3100)	130 (100–1400)
6	650 (1400–8000)	500 (900–5500)	130 (400–2300)	1050 (600–3100)	100 (100–1400)
9	620 (900–4500)	440 (500–2400)	150 (300–1600)	720 (200–2100)	190 (100–1000)
12	1900 (900–4500)	1170 (500–2400)	570 (300–1600)	320 (200–2100)	530 (100–1000)
24	4130 (900–4500)	1830 (500–2400)	1770 (300–1600)	880 (200–2100)	1150 (100–1000)
30[b]	1800 (900–4500)	980 (500–2400)	550 (300–1600)	850 (200–2100)	140 (100–1000)
39	2426 (900–4500)	1333 (500–2400)	698 (300–1600)	1069 (200–2100)	141 (100–1000)
53	2123 (700–4200)	1065 (300–2000)	629 (300–1800)	744 (200–1600)	212 (90–900)

[a]In parentheses: age-matched reference values for blood lymphocyte immunophenotype.[17]
[b]Month +31: acute virosis.

Table 3 Patient 2 immunological reconstitution after UCBT

Months after UCBT	*CD3+/μl*	*CD3+4+/μl*	*CD3+8+/μl*	*CD19+/μl*	*CD56+3−/μl*
1	308 (2400–6900)[a]	62.3 (1400–5100)	242.5 (600–2200)	1.05 (700–2500)	25.3 (100–1000)
3	2779 (2400–6900)	233 (1400–5100)	2521 (600–2200)	89 (700–2500)	240.8 (100–1000)
6	1882 (1600–6700)	181 (100–4600)	1679 (400–2100)	951 (600–2700)	137 (200–1200)
9	1116 (1600–6700)	163 (100–4600)	955 (400–2100)	1251 (600–2700)	197 (200–1200)
12	1869 (1400–8000)	205 (900–5500)	1640 (400–2300)	1598 (600–3100)	402 (100–1400)
15	1497 (1400–8000)	195 (900–5500)	1296 (400–2300)	1319 (600–3100)	510 (100–1400)

[a]In parentheses: age-matched reference values for blood lymphocyte immunophenotype.[17]

on day +52. The patient presented acute GVHD grade III (skin and liver). No chronic GVHD was observed. CSA was administered for 10 months after transplantation.

Persistent full donor chimerism was demonstrated in blood cells by the variable number tandem repeat (VNTR) technique from day +47.

Immunological reconstitution is summarised in Table 3. Normal lymphoproliferative response to mitogens and alloantigens was detectable from 6 months after UCBT.

The patient is alive and well at 15 months after transplantation with full haematological and immunological reconstitution.

Discussion

Haemopoietic stem cell transplantation needs to be considered as soon as possible after a diagnosis of SCID because these disorders usually run an unpredictable course and may rapidly prove fatal. Successful UCBT has now been performed in several SCID patients.[6–9]

No data on UCBT for either type of disease presented in this report are available. A ZAP-70 deficiency patient successfully treated by bone marrow transplantation has recently been reported.[15] Data presented in this report suggest that umbilical cord blood represents an alternative source of hemopoietic stem cells in patients affected by SCID,[6–9] as both patients had a full and rapid B and T lymphocyte reconstitution after UCBT with normal values and response to mitogens 6 months after transplantation. Furthermore, in spite of the HLA disparity, one of our patients had acute GVHD grade I and the other grade III, but at transplant he presented in an overall poor condition, which may have influenced the onset of GVHD. In both patients no chronic GvHD was observed. Data on UCBT for other kinds of disease indicate that the lower incidence and severity of acute and, in particular, chronic GVHD favours a prompt immune reconstitution, because of the lower doses and the earlier discontinuation of immunosuppressive treatment.[9]

In conclusion, the rapid availability of the donor and the lower risk of acute and chronic GVHD,[16] irrespective of HLA matching, suggest UCBT as a procedure of choice in these kinds of patients.

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