

## LETTER TO THE EDITOR

# Expansion of B cell precursors after unrelated cord blood transplantation for an adult patient

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In April 2006, a 26-year-old woman with a history of minimal residual disease (MRD)-positive T cell acute lymphoblastic leukemia was referred for allogeneic stem cell transplantation. A few months before, during leukemia treatment, a small population of atypical lymphocytes, TdT + CD10 + CD19 + CD34 +, accounting for 5% of the bone marrow (BM) cells, was observed. These cells did not exhibit immunoglobulin light-chain restriction or aberrant T cell antigen expression and were classified as B cell precursors. No suitable candidate among related or unrelated BM donors was identified. We searched for a cord blood (CB) unit; 1 month later, an unrelated CB unit, collected 8 years before, was provided by a CB bank within the national network. Characteristics of CB unit and recipient are shown in Table 1. In June 2006, she underwent unrelated single CB transplant after myeloablative conditioning regimen with total body irradiation, fludarabine and cyclophosphamide. Cyclosporine A, rabbit antithymocyte globulin and methylprednisolone were used for graft-versus-host-disease (GVHD) prophylaxis. CB unit was thawed at bedside and infused without manipulation. The number of infused nucleated cells and CD34 + cells were  $3.3 \times 10^7$  and  $0.49 \times 10^5/\text{kg}$ , respectively. Neutropenia-related infections observed during the first 100 days included *Staphylococcus epidermidis* sepsis and probable invasive pulmonary aspergillosis (IPA). Itraconazole antifungal prophylaxis was discontinued and voriconazole treatment was administered, followed by prolonged secondary prophylaxis. No grade II–IV acute GVHD was observed. Time from transplantation to absolute neutrophil count  $>0.5 \times 10^9/\text{l}$  was 45 days and time to sustained platelets recovery  $>20 \times 10^9/\text{l}$  was 191 days.

On day 45, BM aspirate revealed 8% of heterogeneous atypical lymphocytes with round or indented nucleus, condensed nuclear chromatin, absent or indistinct nucleoli and high nuclear/cytoplasmic ratio. The same day, chimerism was evaluated in BM and peripheral blood (PB) polymorfonuclear (PMNs) and mononuclear cells (MNCs), by quantitative molecular genotype analysis using 15 markers of DNA short tandem repeats (STR) tagged with four fluorochromosomes and analyzed with PCR assay. Full CB chimerism was observed.

Further BM samples were obtained on days 97 and 139 for morphological evaluation, flow cytometry immunophenotyping, chimerism and MRD analysis. Atypical lymphocytes increased up to 30%. Flow cytometry analysis showed lymphoid cells TdT + CD10 + CD19 + CD20 + CD38 +; no immunoglobulin light-chain restriction or aberrant

T cell antigen expression were observed. Chimerism was full in CB. MRD was assessed by PCR/RQ-PCR with two case-specific molecular markers with a sensitivity of  $10^{-4}$  targeting clonal T cell receptor gene rearrangement. Patient was MRD-negative. Cyclosporine A treatment was then discontinued on day 102 when the patient exhibited no GVHD symptoms with the aim to prevent acute lymphoblastic leukemia (ALL) relapse.

PMN and MNC clonality was assessed; after purification from heparinized PB collected on day 142, the highly polymorphic STR within the X-linked human androgen receptor (HUMARA) gene were analyzed. The patient was heterozygous for HUMARA locus and was therefore considered informative for X-chromosome inactivation analysis performed according to Karasawa *et al.*<sup>1</sup> A polyclonal pattern in both PMNs and MNCs with an allele expression of 66:34% in MNCs and 69:31% in PMNs was observed. A maculopapular rash was observed on day 162; patient was given combined cyclosporine A and methylprednisolone treatment.

Cyclosporine A administration was discontinued and tacrolimus treatment was started on day 206 because of neurologic toxicity. A single infliximab infusion was administered on day 220 because of intractable diarrhea and ileus. Two weeks later, patient died of IPA refractory

**Table 1** Cord blood and recipient characteristics

	Donor cord blood	Recipient
HLA-A	A250101, A3201	A250101, A3201
HLA-B	B350101, B44020101	B350101, B44020101
HLA-C	CW050101, CW04010101	CW050101, CW04010101
HLA-DRB1	130501, 140101	040101, 130501
ABO type	0, RhD (–)	0, RhD (+)
Sex	Female	Female
<i>Characteristics of infused cord blood</i>		
Unit volume	134.9 ml	Recipient body weight 65 kg
Total nucleated cells	$217.2 \times 10^7$	$3.3 \times 10^7/\text{kg}$
Total mononucleated cells	$64.8 \times 10^7$	$1 \times 10^7/\text{kg}$
CFU-GM	$22.5 \times 10^4$	$0.35 \times 10^4/\text{kg}$
BFU-E	$49.5 \times 10^4$	$0.76 \times 10^4/\text{kg}$
CD34+ (at thawing)	$3.2 \times 10^6$	$0.49 \times 10^5/\text{kg}$
Cell viability	82%	

Maternal serum collected at delivery and at 6 months was negative for HBsAg, HCV, HIV1/2, HTLV I/II and syphilis.

**Table 2** Flow cytometric T/B cell marker expression on lymphoblasts and hematogones

	<i>Lymphoblasts (%)</i>	<i>B cell precursors (day 139) (%)</i>
<i>Single fluorescence</i>		
CytoplasmicCD1a	47	NA
CD1a	36	NA
CytoplasmicCD3	100	0.3
CD5	99	2
CD7	99	8
TdT	10	22
CD34	10	1.4
CD10	98	78
CD19	0.4	87
CD20	0.1	27
SurfaceCD22	0.4	31
CytoplasmicCD22	NA	21
CD38	100	99
<i>Double fluorescence</i>		
CD19/K	NA	19
CD19/λ	NA	13
CD20/K	0.07	15
CD20/λ	0.03	11
CD19/CD34	NA	1
CD19/TdT	NA	NA
CD10/CD19	NA	76
CD19/CD38	NA	83

Abbreviation: NA = not available.

B-cell precursors exhibited a spectrum of antigen expression typical of stage 2 hematogones which have downregulated CD34 completely before progressive upregulation of CD20 and CD22. No aberrant expression was observed; in contrast, lymphoblasts at diagnosis exhibited aberrant CD10 expression.

to combined liposomal amphotericin B and posaconazole treatment.

Hematogones are morphologically distinct B cell precursors appearing as lymphoid cells with homogeneous, condensed chromatin and scant cytoplasm which can be observed in large numbers in BM of children with a variety of hematologic and non-hematologic diseases, sometimes accounting for greater than 50% of BM cells and creating a picture that can be confused with ALL.<sup>2</sup>

Hematogones exhibit a typical complex spectrum of lymphoid antigen differentiation extending from early B cell precursors to mature surface immunoglobulin-bearing B cells, lack aberrant antigen expression and do not exhibit clonal immunoglobulin or TCR gene rearrangements.<sup>2,3</sup> Their increase has been observed in marrow regenerative states following chemotherapy or BM transplantation and may cause diagnostic confusion particularly after treatment for ALL because of hematogones' similarities to neoplastic lymphoblasts.<sup>3</sup> Expansion of hematogones may be persistent, for 2 years after completion of chemotherapy for ALL and for more than 1 year following BM transplantation.<sup>4,5</sup> No clear description of their increase after CB transplantation has been reported until now. Most recently, Shono *et al.*<sup>6</sup> reported a case of non-clonal expansion of blastic-appearing CD19+CD20+ lymphocytes in BM and PB of an adult recipient after unrelated CB transplant.

In our patient, increased hematogones were observed following chemotherapy and transplantation, clinical settings regarded as marrow regenerative states with enhanced B lymphocytogenesis.<sup>3,5</sup> Morphological evaluation of BM smears confirmed that at the level of 5% or more hematogones were conspicuous and likely to be confused with ALL relapse as reported previously.<sup>3</sup> However, their immunophenotypic features, reported in Table 2, were always consistent with typical B cell precursors. Taking into consideration immunophenotypic features, chimerism status, MRD results and X chromosome inactivation analysis, B cell precursor expansion appeared clearly as non-malignant state. The biological significance of hematogone expansion was unclear, but we regarded this phenomenon as a consequence of increased B lymphocytogenesis during marrow regenerative state caused by the high number and generative capacity of B lymphocyte precursors contained in CB.<sup>7,8</sup>

Differential diagnosis in this case was straightforward but distinguishing expansion of non-clonal hematogones from precursor B cell acute lymphoblastic leukemia relapse may be more problematic, mostly after chemotherapy or stem cell transplantation and especially when concomitant anemia and thrombocytopenia are observed.

In conclusion, we suggest that B cell precursor expansion should be considered when ALL relapse is suspected in CB recipients; in such cases, careful differential diagnosis is mandatory.

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