Telomere length of donors influences granulocyte recovery in children after hematopoietic stem cell transplantation

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LETTER TO THE EDITOR

Telomere length of donors influences granulocyte recovery in children after hematopoietic stem cell transplantation

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Dear Editor,

Telomeres (terminal restriction fragments, TRF) are non-coding repetitive DNA sequences located at the end of chromosomes in order to prevent end-to-end fusion and rearrangements [1]. Telomere length is widely recognized as a critical factor in determining the replicative potential of mitotic cells. Accelerated TRF shortening due to excessive replicative stem cell turnover may represent a likely indicator of hematopoietic stem cell (HSC) premature aging, ultimately leading to increased incidence of graft failure and/or clonal disorders [2–6]. Since the clinical course of transplanted patients depends on the rapid recovery of hematopoiesis immediately after engraftment and because correlation between telomere length and granulocyte recovery remains controversial [7, 8], we measured HSC reconstitution of recipients as well as telomere length from donors and recipients at different intervals from hematopoietic stem cell transplantation (HSCT). Nineteen children with various disorders referred to the HSCT Unit of the "G. Gaslini" Children's Research Institute to receive allogeneic HSCT were entered in the study after approval of the protocol by the Institutional Ethics Committee. Relevant clinical findings are detailed in Table 1. Thirteen had acute or poor prognosis chronic leukemia and six had other diseases associated with bone marrow failure. Average age of patients at HSCT was 9.5 years (range 1–18) and that of donors 31.1 years (range 10–45). Five patients received HSCT from a human leukocyte antigen (HLA) identical sibling, 11 from an HLA-matched unrelated donor, and three from a mismatched relative; bone marrow was the source of HSC in all cases but three, who received peripheral blood stem cells. Children were conditioned with a myeloablative regimen based on total body irradiation plus cyclophosphamide–thiotepa. Among patients with malignancy, nine were in their first complete remission/chronic phase (CR/CP) at the time of transplant and six in second or subsequent CR/CP. Peripheral blood samples were separated into a light-density mononuclear cell (MNC) fraction and granulocyte (polymorphonuclear leukocyte, PMN)-rich pellets. Unfractionated MNCs were used because previous authors have shown that telomere loss of monocytes is similar to that in lymphocytes with aging [9]. Genomic DNA was isolated from PMN and MNC using a DNA purification kit (Amersham, Little Chalfont, UK). TRF were evaluated with a chemiluminescent assay kit (Telo-TAGGG telomere length assay, Roche, Mannheim, Germany) as described before [10]. Complete engraftment was obtained in all cases

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Table 1 Relevant clinical data

Patients	Disease	Age (years)	Sex	Donor			Granulocyte recovery (days)	BM $CD34^+$ ($\times 10^8$/kg)	aGVHD
				Age (years)	Relation to recipient	Stem cells source			
1	ALL	12	M	40	U	PB	21	2[b]	I
2	HD	12	F	14	IS	PB	10	13.7[b]	I
3	ALL	2	F	31	U	BM	15	13.2	II
4	CML	8	F	35	U	BM	19	7.17	II
5[a]	ALL	18	M	31	U	BM	27	5.6	II
6[a]	AML	6	F	34	U	BM	16	7.9	IV
7[a]	AML	2.5	M	45	PM Rel	BM	21	8.7	II
8	AML	12.5	M	18	IS	BM	15	4.6	II
9	SAA	13.5	F	18	IS	BM	19	3.6	0
10[a]	ALL	8	F	10	IS	BM	10	3.5	II
11	ALL	8	F	36	U	BM	22	5	0
12	AML	11	M	36	PM Rel	BM	20	5.2	III
13	ALL	12	F	41	PM Rel	BM	24	3.24	III
14	AML	12.5	M	24	IS	BM	14	3.9	II
15	ALL	13	F	32	U	BM	21	7.3	I
16	AML	1	F	38	U	PB	11	42.4[b]	III
17	ALL	6.5	F	29	IS	BM	18	2.09	II
18	SAA	12	F	38	U	BM	19	5.7	0
19[a]	ALL	8.5	F	42	U	BM	17	4.5	0

Abbreviations: *ALL* acute lymphoblastic leukemia, 9; *AML* acute myeloid leukemia, 6; *CML* chronic myeloid leukemia, 1; *SAA* severe aplastic anemia, 2; *HD* Hodgkin's disease, 1; *U* unrelated; *IS* identical sibling; *PM Rel* partially matched relative; *BM* bone marrow; *PB* peripheral blood; *aGVHD* acute graft-versus-host disease

[a] Deceased

[b] PB $CD34^+ \times 10^6$/kg

and full donor chimerism confirmed by short tandem repeat analysis on peripheral blood and bone marrow samples obtained periodically after HSCT. Granulocyte recovery (PMN$>0.5\times10^9$/L) was achieved after a median time of 17 days (range 10–27), whereas platelets reached values $>50\times10^9$/L after a median time of 23 days (range 947). At the time of this letter, 13 patients are alive in CR with a median follow-up of 37 months after HSCT (range 27–47), one is alive with disease, and five died (three of progressive disease, one of secondary AML, and one of septic shock 7 months post transplant). In this letter, we analyze TRF length dynamic changes occurring in two hematopoietic cell populations purified from peripheral blood of 19 children during the first 6 months reconstitution period after HSCT. Figure 1a shows mean changes in telomere length, with respect to donor values, in the recipients' PMN and MNC at various intervals after HSCT. Overall analysis reveals a distinct pattern of telomere length dynamics in the two cell populations, with a statistically significant length reduction for PMN only ($p<0.05$) without attaining homeostasis at 6 months from HSCT [11–14]. More interesting, regression analysis of data show a significant relationship between TRF length of donors and time to recovery: longer donor TRF affected faster granulocyte recovery in the recipient (Fig. 1b; $r=0.48$; $p<0.05$). Although on a small sample, sex and age of donors or infections and graft-versus-host disease did not apparently affect the outcome of this study [7]. Unlike what was previously reported [15], in this preliminary study, we found no significant correlation between the number of $CD34^+$ cells infused and granulocyte recovery. In conclusion, the present observations support the concept that HSC derived from donors with longer TRF may offer a replicative advantage to the recipients by more efficiently counteracting the premature exhaustion of HSC due to the high demand of hematopoiesis reconstitution.

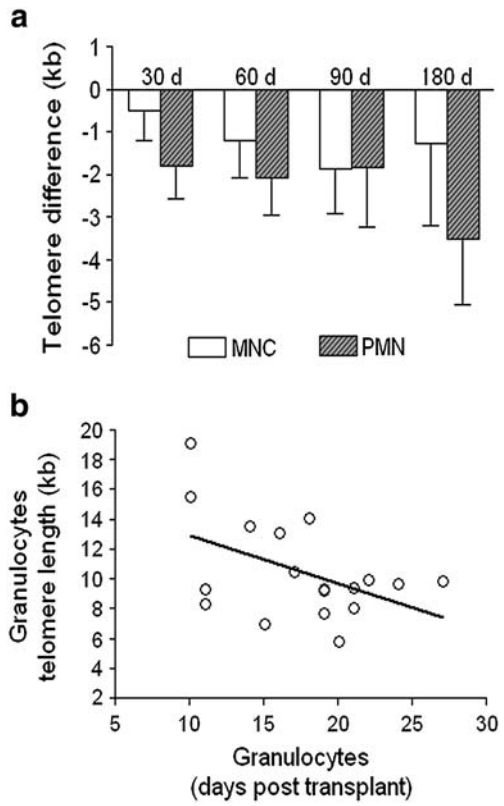

Fig. 1 Telomere length changes and granulocyte recovery. **a** Mean changes in telomere length (with respect to donor values) in the recipients at 30, 60, 90, and 180 days after HSCT for PMN and MNC. The recipient's TRF minus the donor's TRF gives rise to the difference. *Vertical bars* indicate the mean ± SD. **b** Regression analysis of hematopoietic stem cell recovery, i.e., PMNs>0.5×10^9/L of recipients, according to TRF length of donors in granulocytes, a cell population believed to reflect stem cell turnover

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