Polyclonal hematopoiesis with variable telomere shortening in human long-term allogeneic marrow graft recipients

George Mathioudakis, Rainer Storb, Peter A. McSweeney, Beverly Torok-Storb, Peter M. Lansdorp, Tim H. Brümmendorf, M. John Gass, Eileen M. Bryant, Jan Storek, Mary E. D. Flowers, Ted Gooley, and Richard A. Nash

Donor-derived hematopoiesis was assessed in 17 patients who received allogeneic marrow grafts from HLA-matched siblings between 1971 and 1980. Complete blood counts were normal or near normal in all patients except one. Chimerism analyses, using either dual-color XY-chromosome fluorescence in situ hybridization (FISH) or analysis of variable number tandem repeat loci, indicated that 15 out of 16 patients had greater than 97% donor-derived hematopoiesis, whereas 1 patient had indeterminate chimerism. All 12 recipients of grafts from female donors exhibited polyclonal hematopoiesis by X-linked clonal analysis with the use of molecular probes. Of the 17 recipients, 9 exhibited a less than 1.0-kilobase shortening of granulocyte telomere length compared with their respective donors, according to terminal restriction fragment analysis or flow-FISH with a fluorescein-labeled peptide nucleic acid probe. These data suggest that under standard transplantation conditions, the stem cell proliferative potential is not compromised during hematopoietic reconstitution. (Blood. 2000;96:3991-3994)

© 2000 by The American Society of Hematology

Study design

Patients

Seventeen patients who received transplants between 1971 and 1980 and their HLA-identical donors agreed to participate in the study (Table 1).

Mean telomere length analysis

Terminal restriction fragment length (TRF) analysis on peripheral blood granulocytes and flow-FISH were performed on granulocytes and mononuclear cells as previously described.21-23

© 2000 by The American Society of Hematology
a significant shift in the recipients’ clonal ratios of the X-linked donor-derived hematopoiesis (Table 1). Furthermore, there was not a lack of informative markers (Table 1), and one patient/donor pair consisted of monozygous twins. The majority of recipients 2 months to 8.5 years after transplantation had normal leukocyte counts and normal hemoglobin levels. This supports previous observations that long-term survivors of uncomplicated marrow transplantation.

Results and discussion

The absolute neutrophil counts (ANCs) of all the recipients except one were in the normal range. Patient 1 was pancytopenic with an ANC of 960/μL when contacted for the study. However, the average difference between recipients’ and donors’ ANCs was the donor’s TRF minus the patient’s TRF).

Statistical analyses

The Wilcoxon signed rank test was used to determine statistical significance of differences in recipient and donor complete blood count, mean corpuscular volume, and difference in TRF (dTRF; ie, the donor’s TRF minus the patient’s TRF).

Results from a representative TRF experiment are shown in Figure 1A, which depicts the analysis of 3 patient/donor pairs. We tested the null hypothesis that the average dTRF was equal to zero, where the variance of the estimated mean was adjusted to account for the fact that multiple experiments were done in individual patients. The average dTRF across all 50 experiments was estimated to be 0.94 kilobases (kb) (95% confidence interval, 0.69-1.20; P < .0001) (Figure 1B). When the null hypothesis that the average dTRF was equal to 0.369 kb was tested instead, the estimated average dTRF was still significantly different than 0.365 kb (P < .0001). Telomere length was also calculated by flow-FISH independently in recipient and donor pairs 1 and 9, and similar differences between donor and recipient telomere lengths were observed. Statistical analysis revealed no correlation between dTRF and total number of nucleated marrow cells infused, donor age, or time after transplant (data not shown).

Our findings indicate that, although telomere shortening was consistently seen in long-term marrow recipients, the degree of telomere shortening was variable among recipients and, on average, was not greater than what has been previously reported early after transplant.10-13,26,27 If we accept that telomere shortening reflects an increased number of stem cell divisions after transplantation, then the observed variability among patients suggests that either a variable number of HSCs contributed to engraftment and hematopoietic reconstitution or secondary demands in hematopoiesis vary among these recipients. Furthermore, assuming that telomeres lose 100 bp per cell division and given an average dTRF of 0.94 kb, transplanted HSCs would theoretically undergo an average of 9 to 10 extra divisions after transplant. In granulocytes, telomere length is estimated to decrease by 30 bp per year; thus a decrease of 0.94 kb would correspond to 30 years of normal hematopoiesis, consistent with previous theoretical estimates.28 The similar degree of telomere
shortening in both short- and long-term recipients is consistent with a model in which demand for HSC replication stabilizes following an initial accelerated period. Late after transplantation, the demand for stem cell replication appears to be no greater than in the normal donor. A more rapid loss of telomere length over time relative to the donor may occur in a setting in which donor-derived hematopoietic reconstitution is monoclonal or oligoclonal.

In summary, our findings suggest that many years after BMT, despite increased demands early after transplantation, donor-derived hematopoiesis can sustain normal counts and remains polyclonal. These observations emphasize the extensive replicative reserve of HSCs.

References


Polyclonal hematopoiesis with variable telomere shortening in human long-term allogeneic marrow graft recipients

George Mathioudakis, Rainer Storb, Peter A. McSweeney, Beverly Torok-Storb, Peter M. Lansdorp, Tim H. Brümmendorf, M. John Gass, Eileen M. Bryant, Jan Storek, Mary E. D. Flowers, Ted Gooley and Richard A. Nash