Age-Related Changes in Activation of Telomerase in the Bone Marrow of Normal and Thymectomized Mice

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Age-related and thymus-dependent regulation of telomerase activity was studied in the bone marrow of normal (physiological aging) and thymectomized (experimental aging) mice. There was no strong correlation between the age and telomerase activity in bone marrow cells of normal mice. We observed only small individual differences in telomerase activity. Thymectomy 2-fold increased telomerase expression in young (2-month-old) and old (24-month-old) animals. Individual differences in telomerase activity in the bone marrow of thymectomized mice were more pronounced and did not depend on the age. The role of the thymus in cell aging is discussed.

Key Words: *telomerase; bone marrow; aging; mice*

The mechanisms of age-related changes in normal hemopoiesis are still unclear. Among numerous theories of cell aging and death, the telomere-telomerase hypothesis of aging attracts much recent attention. According to this theory, the progressive shortening of telomeric DNA during cell division (including hemopoietic cells) is the chief cause of cell aging [1,3]. The length of telomeres is an indicator of cell proliferative history. On the other hand, shortening of telomeres to a critical size triggers some processes inhibiting cell division [3,8]. In vertebrates, telomere is a nontranscriptional terminal region of eukaryotic chromosomes containing simple tandem nucleotide repeats (TTA GGG)n, whose number varies between species [10].

The length of telomeres is controlled by telomerase. Normal embryonic and immortal cells display high telomerase activity (TA) [7,9], while leukocytes of the peripheral blood, umbilical cord, and bone marrow in normal donors [7], mouse splenocytes [14], and activated T cells of the thymus and lymph nodes exhibit low TA [12]. Expression of telomerase in T and B lymphocytes is regulated during their differentiation and activation [15]. TA was also detected in stem cells, including primitive hemopoietic precursors in the bone marrow of humans and old mice [6,11].

Most experiments were performed on human hemopoietic cells. At the same time, the analysis of telomerase regulation in mice (the general model for studying of hemopoietic cells) is of considerable interest. Human and mouse telomerases differ in their functional properties and regulatory mechanisms [13]. While human somatic cells with low or undetectable TA, mouse tissues express telomerase also in the postnatal period [5,9,14]. However, it is unknown whether TA in mice is constant or it changes during aging. It is known that aging is accompanied by involution of the thymus and imbalance between various growth factors, including thymic factors. Here we studied TA in bone marrow cells of normal (physiological aging) and thymectomized (TE, experimental aging) mice over their life.

MATERIALS AND METHODS

Experiments were performed on female (C57Bl×CBA) F1 mice aged 1-23 months. Some 4-week-old mice (n=5) were thymectomized as described elsewhere [4]. The completeness of thymectomy was verified before

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euthanasia. Intact mice of the same age served as the control (n=5). The bone marrow was repeatedly aspirated from the right and left femur of normal or TE mice on months 2, 5, 9, 17, and 23 of life under light ether anesthesia. Repeated aspiration of the bone marrow from the same animals allowed us to perform the individual monitoring and to minimize data scattering. TA was estimated by the method comprising TRAP analysis [9], polymerase chain reaction (PCR), and enzyme immunoassay using standard Boehringer Mannheim kits.

TA was measured in lysates of 2×10^6 bone marrow cells [2]. For PCR, 3 μl lysate (3×10^4 bone marrow cells) were used. The samples were treated with 1 μg/ml RNase A at 37°C for 20 min to inhibit telomerase. Lysates of K-562 cells with high TA served as the positive control. The lysing buffer was used as the negative control.

The intensity of telomerase expression was analyzed in a photometer at 450 nm. TA was expressed in percents of the control and then calculated per 10^4 bone marrow cells. TA in lysates of telomerase-positive K-562 cells was taken as 100%.

The results were analyzed by Student's t test. Simple and multiple linear regression analyses were used to evaluate the relationship between TA and the age of mice. The linear regression equation and correlation coefficient (r^2) were estimated. Kolmogorov-Smirnov nonparametric test was used to analyze the effects of thymic factors on telomerase activation. The differences were significant at $\lambda^2>1.84$.

RESULTS

TA in bone marrow cells from normal mice changed during aging. Young (2- and 5-month-old) and old (23-month-old) animals had low TA (2% per 10^4 cells), while in 9-17-month mice this parameter was 2 times higher (insignificant differences, Fig. 1). We revealed no strong correlation between mouse age and intensity of telomerase expression (r^2=0.16, Fig. 2, *1*). The asynchronous individual variations in TA were insignificant (data not shown).

Thymectomy in young adult mice led to considerable activation of telomerase (λ^2=3.208, p<0.01) compared to the basal level (TA in bone marrow cells of normal mice). TA in the bone marrow of young and old TE mice was similar and 2-4-fold surpassed that in normal mice of the same age. One month after surgery, the content of telomerase-positive cells in the bone marrow of TE mice 4 times surpassed the control (p<0.05). A peak of TA (19%) was noted in 12-month-old mice: this parameter 6-fold surpassed that in normal mice of the same age (p<0.05, Fig. 1). The asynchronous interindividual variations in TA in the

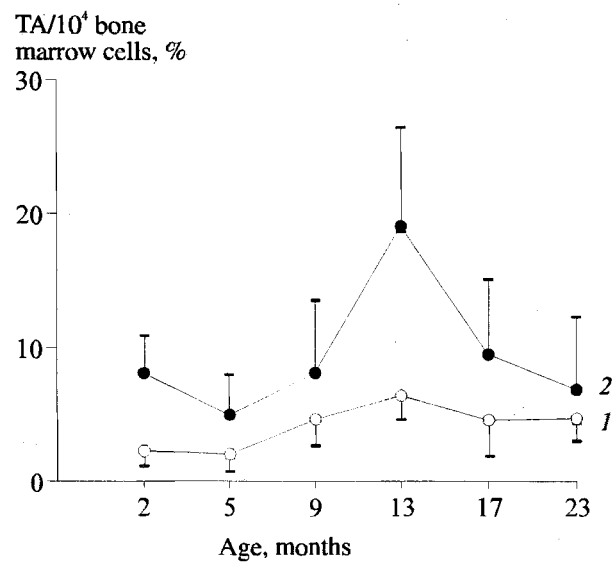

Fig. 1. Telomerase activity (TA) in the bone marrow of normal (*1*) and thymectomized (*2*) mice.

bone marrow of TE mice were more pronounced than in the control. At the same time, TA in TE mice was maximum at the age of 12 months, then progressively decreased, and dropped to minimum by the 24th month of life. However, even in old TE mice TA was much higher than in normal animals of the same age.

Multiple linear regression was used to estimate the relationship between the age of TE mice and TA activity. This method improves statistical significance of linear regression analysis, because it allows more

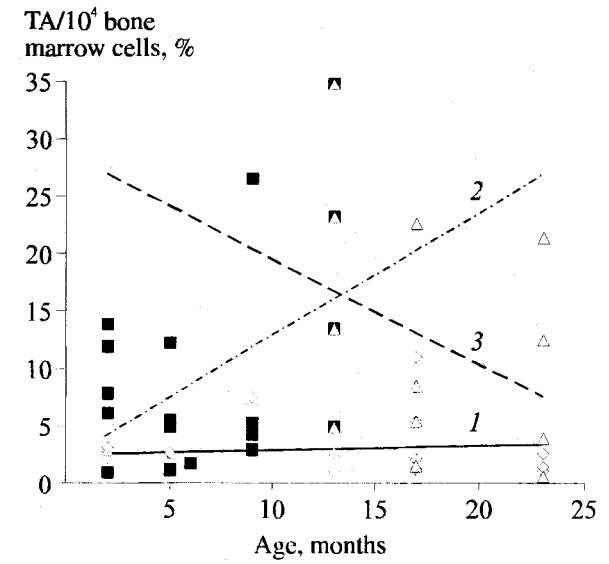

Fig. 2. Correlation between telomerase activity (TA) and age of mice. Individual TA: normal mice (rhombuses); 2-13- and 13-22-month-old thymectomized mice (squares and triangles, respectively). Linear regression equations: normal mice (y=0.03x+2.72, r^2=0.16, *1*), 2-13-month-old thymectomized mice (y=1.74x-2.5, r^2=1.35, *2*), and 13-23-month-old thymectomized mice (y=-1.22x+33.4, r^2=-0.91, *3*).

accurate arrangement of critical points relative to a theoretical curve plotted by the least squares method. TA in the bone marrow correlated with the age of mice. This parameter increased with age, reached maximum to the 12th month (Fig. 2, *2*), and then progressively decreased during the 2nd year of life (Fig. 2, *3*).

Under conditions of stable hemopoiesis and physiological aging, telomerase expression does not depend on animal age and functional state of the thymus. However, thymectomy considerably elevates TA in the population of bone marrow hemopoietic cells. Therefore, the thymus not only possesses immune functions, but also regulates cell aging. It is known that the most pronounced changes induced by thymectomy primarily concern differentiation of bone marrow stem cells to T lymphocytes resulting in progressive impairment of the immune system. At the same time, memory T cells function over all period of life. Under conditions of physiological stress (*e.g.*, thymectomy), TA increases primarily due to activation of the enzyme in lymphoid cells. These data demonstrate a surprising effect of T cells depletion on TA regulation in mouse hemopoietic cells.

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REFERENCES

1. A. M. Olovnikov, *Dokl. Akad. Nauk SSSR*, **201**, 1496-1499 (1971).
2. T. V. Todriya, N. I. Drize, I. L. Chertkov, *et al.*, *Byull. Eksp. Biol. Med.*, **127**, No. 3, 311-313 (1999).
3. R. C. Allsopp, H. Vaziri, C. Patterson, *et al.*, *Proc. Natl. Acad. Sci. USA*, **89**, 10, 114-10, 118 (1992).
4. E. A. Boyse, L. J. Old, and C. A. Iritani, *Transplantation*, **12**, 93-95 (1971).
5. C. Chadeneau, P. Siegel, C. B. Harley, *et al.*, *Oncogene*, **11**, 893-898 (1995).
6. C.-P. Chiu, W. Dragowska, N. W. Kim, *et al.*, *Stem Cells*, **14**, No. 2, 239-248 (1996).
7. C. M. Counter, J. Gupta, C. B. Harley, *et al.*, *Blood*, **95**, No. 9, 2315-2320 (1995).
8. C. B. Harley, H. Vaziri, C. M. Counter, *et al.*, *Exp. Gerontol.*, **27**, 375-382 (1992).
9. N. W. Kim, M. A. Piatyszek, K. R. Prowse, *et al.*, *Science*, **266**, 2011-2015 (1994).
10. D. Kipling and H. J. Cooke, *Nature*, **347**, 400-402 (1990).
11. S. J. Morrison, K. P. Prowse, and I. L. Weissman, *Immunity*, **5**, 207-216 (1996).
12. M. Ogoshi, A. Takashima, and R. S. Taylor, *J. Immunol.*, **158**, 622-628 (1997).
13. K. R. Prowse, A. A. Avilion, and C. W. Greider, *Proc. Natl. Acad. Sci. USA*, **90**, 1493-1497 (1993).
14. K. R. Prowse and C. W. Greider, *Ibid.*, **92**, 4818-4822 (1995).
15. N.-P. Weng, K. S. Hathcock, and R. J. Hodes, *Immunity*, **9**, 151-157 (1998).