BIOGERONTOLOGY

Telomerase Activity in Cells of 9-Day-Old Spleen Colonies Formed by Bone Marrow of Normal and Thymectomized Mice

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Telomerase activity was measured in spleen colonies, in progeny of individual 9-day-old splenic CFU formed by the bone marrow from normal (physiological aging) and thymectomized mice. Cells of spleen colonies expressed telomerase activity. No correlation was found between telomerase activity in spleen colony cells and age of animals. Thymectomy of bone marrow donors had no effect on telomerase activity. Our results suggest that the thymus plays a role in cell aging.

Key Words: *telomerase; aging; thymus; spleen colony-forming units; mice*

Replicative aging and death of cells during culturing and physiological aging are related to shortening of telomeric DNA in each cell cycle [1]. Activation of telomerase is a mechanism regulating telomere length. Telomerase plays a role of reverse transcriptase, synthesizes *de novo* telomeric sequences, and stabilizes the length or prevents shortening of telomeres in intensively dividing cells [4].

In most somatic cells of adult humans telomerase activity (TA) cannot be detected. TA is low in lympho- and hemopoietic cells and depends on the hierarchic age, stage of differentiation [9], and response to cytokines [11].

As differentiated from humans, in mice TA was found in most somatic tissues during postnatal ontogeny [8,14]. It can be hypothesized that the telomere-telomerase complex does not play an important role in wild-type laboratory mice. Considerable length of telomeres in mice (5-10 times longer than in humans) [12] and continuous expression of telomerase prevent shortening of telomeres to a critical size. These specific features prevent replicative aging of cells and aging of the whole organism in mice (*e.g.*, immunologic aging). T cell disturbances in mice occur in the early postnatal period (end of the 1st year of life). Although mice have considerable reserves of telomeric sequences, enzyme activity sharply increases under conditions of immortalization and mitogenic stimulation of lymphocytes [7,10]. In the offspring of mice deficient by the gene encoding the catalytic subunit of telomerase (TERT), the absence of telomerase and extremely short telomere length produce serious systemic disorders in intensively proliferating tissues (*e.g.*, blood and intestinal tissue) and reduce cell potential to neoplastic growth [5]. In the population of bone marrow (BM) cells from thymectomized mice TA is 2-5 times higher than in control mice of the same age [2]. Study of TA in mouse hemopoietic cells during physiological aging and experimental thymic deficiency is of interest.

The hemopoietic system is presented by cells differing in the hierarchic age, stage of differentiation,

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and maturity. As differentiated from the whole BM, the standard model of spleen colonies (SC) [15] allows studying of regulation of telomerase expression in cells that develop from individual spleen colony-forming units (CFUs) and have the same replicative age. CFUs is a heterogeneous population, which comprises precursor cells differing in the degree of maturity and other characteristics (*e.g.*, period of colony formation) [13]. Here we studied the dependence of TA on the age of normal (physiological aging) and thymectomized mice. Experiments were performed on SC formed by 9-day-old CFUs exhibiting maximum TA [3].

MATERIALS AND METHODS

The study was conducted on male (C57Bl×CBA)F_1 mice aging 1-23 months. Some 4-week-old mice (n=5) were thymectomized as described elsewhere [6]. Intact mice of the same age served as the control (n=5). BM was repeatedly aspirated from the right and left femurs of intact or thymectomized mice on months 2, 5, 9, 17, and 23 of life under light ether anesthesia to obtain SC [15]. Repeated aspiration of BM from the same animals allowed us to perform individual monitoring and minimize data scattering. SC were obtained from lethally irradiated mice intravenously receiving 5×10^4 BM cells. The animals were killed 9 days after treatment. The spleens were removed. Macroscopically visualized colonies were excised and their cell composition was evaluated. TA was measured in cell lysates prepared from individual colonies (10-15 colonies per sample) [3] and expressed in percents of the positive control. TA of telomerase-positive K-562 cells was taken as 100%.

Recipient mice were irradiated 2 times in a dose of 5 Gy at a 3-h interval (total dose 10 Gy, dose power 0.165 Gy/min). A γ-device (Cs) was specially constructed for the Institute of Blood Transfusion.

The results were analyzed by Student's t test.

RESULTS

TA depends on the age of SC (*i.e.*, hierarchic age of parent CFUs). Among 7-13-day-old SC, the progeny of 9-day-old CFUs from BM of intact mice had maximum TA [3]. We studied changes in TA in cells of SC formed by BM from thymectomized mice. The relative number of cells with maximum TA was highest in 9-day-old SC (9.5±0.8%).

Thymectomy had little effect on TA expression in 9-day-old SC (Fig. 1). TA in 9-day-old SC practically did not differ in mice aging 5-23 months (5.6-9.6%), but was 2.5 times higher in young animals (2 months). TA in cells of 9-day-old colonies formed by BM from intact and thymectomized mice aging 2 months was 25 and 22%, respectively.

Our results show that under conditions of stable hemopoiesis the regulation of TA in SC cells is not associated with physiological activity of the thymus. However, thymectomy activates expression of telomerase in the population of parent BM cells [2]. These differences are probably related to the cell composition of colonies. As distinct from lymphoid colonies, myeloid colonies (erythroid, granulocytic, and megakaryocytic colonies) were formed in the spleen of irradiated recipients. It can be hypothesized that even in senescent mice the percentage of telomerase-positive cells in myeloid colonies not containing lymphoid cells is higher than in BM (2-5%). Probably, the ratio of SC cells with different degree of maturity is maintained at a constant level independently on the age of animals.

Physiological stress produced by thymectomy is associated with abnormal differentiation of stem cells into T lymphocytes. These changes contribute to progressive impairment of the immune status of mice. However, T memory cells exhibit activity throughout the life. The increase in expression of telomerase in thymectomized mice results from activation of the enzyme in lymphoid cells. This assumption is indirectly confirmed by published data on high TA (19%) in the population of BM cells containing lymphocytes and isolated from thymectomized mice [12] and study of TA in splenocytes and thymocytes of 3-9-month-old animals. Lymphocytes constitute the main population of cells in the spleen and thymus. TA was similar in spleen cells (35.9±5.6%), but higher in thymocytes (68.3±5.3%). It remains unclear, why TA is high in 9-day-old SC of young mice, but decreases more than by 2.5 times in animals aging 5-23 months.

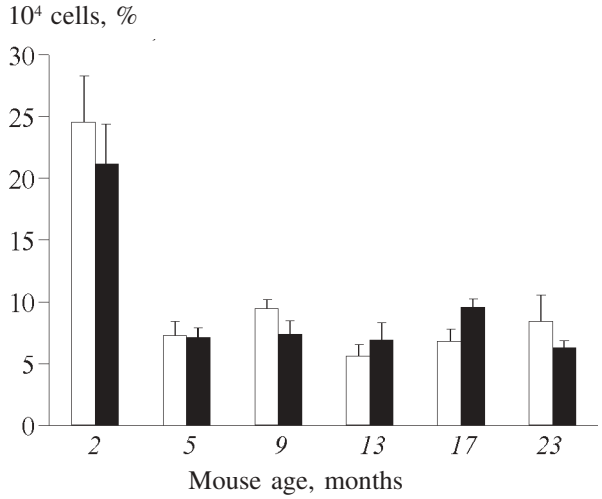

Fig. 1. Age-related changes in TA in cells of 9-day-old spleen colonies formed by bone marrow from intact (light bars) and thymectomized mice (dark bars).

Our results indicate that the thymus plays a role in the regulation of immune activity and cell aging in adult mice, which is mediated by transient activation of telomerase in lymphoid cells. However, TA is autonomously regulated in cells of SC.

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