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Telomerase Activity in Mouse Bone Marrow and Subpopulations of Spleen Colony-Forming Units

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Telomerase activity is studied in mouse bone marrow and in splenic colonies produced by individual CFU characterized by different proliferative potential and time of colony formation (7-14 days). It is found that splenic CFU forming colonies on day 9 in culture exhibit maximum telomerase activity that 5-fold surpassed telomerase activity in the original bone marrow.

Key Words: *telomere; telomerase; bone marrow; splenic CFU*

Hemopoietic differon consists of stem hemopoietic cells (SHC), committed precursors, and mature cells. Mature cells undergo terminal differentiation and degrade. According to the telomere hypothesis, cell aging is associated with loss of telomeric DNA during cell divisions [1,8]. Telomere is a highly conserved terminal region of eukaryotic chromosomes. In mice, humans, and other vertebrates telomere consists of simple tandem sequences (TTAGGG)n.

Analysis of the length of telomeric tandem repeats showed that somatic cells lose 50-200 nucleotides in each cell cycle [3,8]. Age-dependent reduction of telomere length was observed in skin epidermis and derma [3], peripheral blood leukocytes [16], rectum epitheliocytes [9], but not in sperm DNA [3]. It was found that senescent cells contain approximately the same amounts of telomeric DNA. It was hypothesized that reduction of telomere length to a critical value terminate cell division, i.e., telomere length is a factor of aging and, probably, cell maturity [2,8].

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In the majority of organisms stability of terminal chromosome regions are maintained by telomerase, a ribonucleoprotein complex acting as reverse transcriptase from internal RNA template. Until recently it was considered that embryonic and immortalized cells exhibit telomerase activity (TA), while most somatic cells lack this activity. However, highly sensitive PCR-based TRAP assay (Telomeric Repeat Amplification Protocol) [12] detected TA in normal cells and benign tumors. TA was also found in stem cells, in particular, hemopoietic cells. Total peripheral blood leukocytes, leukocytes from umbilical blood and bone marrow of healthy donors [5,7], as well as monocytes and granulocytes [5] exhibit low TA, while in stimulated *in vitro* T and B lymphocytes TA increases [10]. Hemopoietic precursors in the bone marrow are characterized by different TA: expression of TA in committed cells is higher than in primitive precursors [10]. Proliferation and differentiation of committed precursors are associated with an upsurge of TA.

In humans, TA was primarily studied in transitory hemopoietic populations. At the same time, verification of the telomeric hypothesis of aging for human SHC is of special interest. The well-studied model of splenic CFU (sCFU) best suits this purpose [15]. It has

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been found that the length of telomere and TA are different in mice and men, in particular, telomere length in different mouse strains varies from 5 to 150 kb, while terminal ends of human chromosomes, for instance in peripheral blood cells contain 10-15 kb [13]. In human somatic cells TA is low or absent, while in mice most tissues express telomerase in the postnatal period [6].

It has been demonstrated that the SHC population is heterogeneous: it is presented by hemopoietic precursors at different stages of differentiation. This is also true for sCFU population: it includes sCFU forming early (7-9 days) and later (11-14 days) colonies in the spleen of irradiated mice ($sCFU_{7-9}$ and $sCFU_{11-14}$, respectively) [14]. These sCFU subpopulations differ not only by the time of formation of macrocolonies, but also by their proliferative potential, response to cycle-specific agents [4,11], position in the cell cycle [4], and histological types of colonies [14]. It was however unclear, whether sCFU possess TA. This was the subject of our study.

MATERIALS AND METHODS

The study was carried out on female (CBA×C57Bl) F_1 mice aged 4-5 months. Splenic colonies were obtained as described previously [15]. Lethally irradiated mice were injected with 4×10^4 bone marrow cells and sacrificed after 7, 9, 11, 13, and 14 days. Individual splenic colonies were suspended in 1 ml cold phosphate buffer, cells were counted, and 10^6 cells from each suspension were lysed.

In cell lysates, TA was measured by the TRAP assay and PCR-immunoassay using a standard Boehringer Mannheim kit. To this end, 10^6 cells from each suspension were twice washed with 500 μl cold phosphate buffer, the pellet was resuspended in 200 μl cold lysing buffer containing 0.5% CHAPS, 10 mM Tris HCl (pH 7.5), 1 mM $MgCl_2$, 1 mM EGTA, 0.1 mM benzamidine, 5 mM β-mercaptoethanol, and 10% glycerol (Oncor). After 30-min incubation on ice the samples were centrifuged (12,000 rpm for 20 min at 4°C) and stored at -70°C.

Two microliters of the lysate of about 10^4 cells (bone marrow or colonies) were used for PCR. Control samples before PCR were treated with RNase (1 μg/ml, 20 min, 37°C) for inhibition of TA. Lysates of K-562 cells possessing high TA and lysing buffer served as positive and negative controls, respectively.

TA was measured by immunoenzyme assay on a photometer at 450 nm.

Recipient mice were irradiated in a dose of 10 Gy (0.165 Gy/min dose power) on a γ-apparatus (Cs) constructed for Institute of Blood Transfusion.

The data were processed statistically using Student's *t* test.

RESULTS

The age-dependent size of splenic colonies was determined by the number of cells (Fig. 1). The number of cells in early $sCFU_7$ colonies was 10-fold lower than in $sCFU_9$ and 18-fold lower than in $sCFU_{11-13}$; 11-13-14-day colonies were the largest and consisted of $3.7\pm0.46\times10^6$ cells. Differences between $sCFU_{11}$ and $sCFU_{14}$ in this parameter were insignificant.

Maximum TA (65% of control) was found in $sCFU_9$ (TA of 10^4 K-562 cells was taken as 100%). Minimum TA (6% of control) was observed $sCFU_7$. Bone marrow, 13- and 14-day colonies exhibited similar TA: 14, 15, and 18%, respectively. Relatively high TA (48%) was observed in $sCFU_{11}$ (Fig. 2).

Irrespectively of the time of formation and specialization of the initial sCFU, individual colonies contained precursors with high and low proliferative potential and mature cells, but their proportion depended on the time of appearance in the spleen of irradiated recipients, which is determined by the hierarchic age of the initial sCFU. As expected, $sCFU_7$

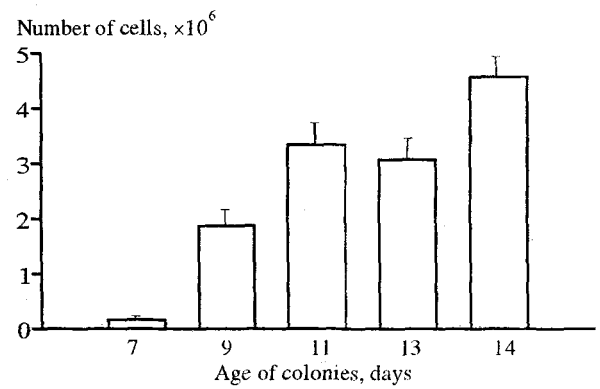

Fig. 1. Number of cells in 7-14-day splenic colonies (each value is a mean of 8 colonies).

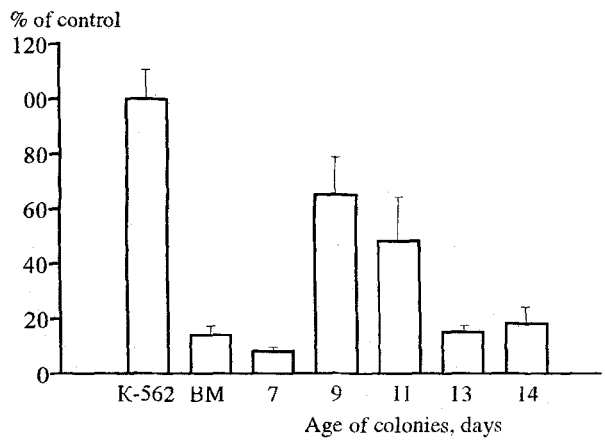

Fig. 2. Telomerase activity in bone marrow cells (BM) and 7-14-day colonies. Ordinate: telomerase activity per 10^4 cells (each value is a mean of 4-5 samples).

belonging to the self-maintaining precursor population [14] contain primarily mature cells and only a minor TA-positive fraction. Although $sCFU_{11-14}$ also contain self-maintaining precursors [14], the relative content of TA-positive cells among $sCFU_{13-14}$ progeny decreases due to intense proliferation and differentiation of committed precursors to mature cells. $sCFU_9$ form 2-fold smaller colonies in comparison with those formed over 11-14 days and contain maximum number of TA-positive cells.

Thus, SHC, in particular sCFU, possess TA. It is found that nonself-maintaining sCFU ($sCFU_7$) contain little TA-positive cells. The cells with high proliferative potential express telomerase, but the relative content of TA-positive cells in 11-14-day colonies is low due to accumulation of mature cells. Maximum TA was found in progeny of $sCFU_9$. It can be hypothesized that high TA in intensively proliferating SHC prevents reduction of telomere length thus protecting these cells from death.

The method proposed by us allows to determine TA in the progeny of individual sCFU. This parameter is more informative than integral TA of bone marrow cells, which can be underestimated because the bone marrow contains precursors differing in their proliferative potential and the degree of differentiation.

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