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Content of Stromal Precursor Cells in Heterotopic Transplants of Bone Marrow in CBA Mice of Various Ages

Yu. F. Gorskaya, A. I. Kuralesova, E. Yu. Shuklina, and V. G. Nesterenko

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Efficiency of colony formation of stromal precursor cells in cultured bone marrow transplants from old (24 month) CBA mice implanted to young (2-month-old) mice almost 3-fold surpassed that in cultured transplants implanted to old recipients. The content of nucleated cells in bone marrow transplants from senescence accelerated mice SAMP increased more than 2-fold, if SAMR mice with normal aging rate were used as the recipients instead of SAMP mice. Bone marrow taken from old and young CBA mice endured the same number of transplantations if the recipient mice were of the same age (5 month). It was concluded that stromal tissue considerably changes with age and is under strict control of the body.

Key Words: *bone marrow stromal cells; heterotopic transplantation; age-related changes*

The content of stromal fibroblast colony-forming cells (CFU-F) in hemopoietic and lymphoid organs of CBA mice and guinea pigs decreases with age [2]. This age-related decrease affects the entire population of CFU-F including the fraction of weakly bound CFU-F that can be isolated by mechanical disaggregation and CFU-F fraction that can be isolated with trypsin only. Therefore, the age-related decrease in CFU-F content cannot be explained by cell redistribution between CFU-F fractions [1]. We previously showed that CFU-F content in hemopoietic and lymphoid organs decreases both in SAMP mice (senescence accelerated prone) and in control SAMR mice (senescence-accelerated resistant) [8], but in SAMP mice this decrease occur more earlier compared to SAMR mice (at the age of 9-11 months *vs.* 16-19 months) [1]. Bone marrow stromal tissue contains committed osteogenic precursors (COP). Transplantation of bone marrow fragments under renal capsule gives rise to a hemopoietic organ populated with hemopoietic cells [4]. It was demonstrated that the number of nucleated cells in the bone marrow transplants taken from old animals (mice or rats) is lower by 40% than in transplants taken from young animals [6]. It probably indicates that the stromal tissue is involved in general aging and these age-related changes probably underlie (at least partially) osteoporosis. It was hypothesized that aging is accompanied by depletion of the COP pool, however, this assumption was not confirmed experimentally. The relative contributions of age-related changes in the stromal tissue and general body influences into stromal tissue deficiency also remain unclear. Our aim was to study the dynamics of COP and hemopoietic cell populations in bone marrow transplants taken from young and old CBA mice and implanted under the renal capsule to CBA mice of various age. The same process was examined in transplants taken from rapidly aging SAMP mice and control SAMR mice after cross-transplantation. In addition, we studied whether the donor's age affects the number of serial transplantations of its bone marrow.

Laboratory of Cell Immunology, Laboratory of Immunology Stromal Control, N. F. Gamaleya Institute of Epidemiology and Microbiology, Russian Academy of Medical Sciences, Moscow

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MATERIALS AND METHODS

Experiments were carried out on male and female guinea pigs aging 4-5 months, CBA mice aging 2-24 months (Kryukovo Nursery), and on 2-19-month-old SAMR and 2-11-month-old SAMP mice (Department of Embryology in M. V. Lomonosov Moscow State University).

For heterotopic transplantation of the bone marrow, a half of the content of mouse femoral cavity was placed under renal capsule of these animals [4]. The following combinations of donors and recipients were tested: CBA mice, young-young (Y→Y), young-old (Y→O), old→old (O-O), old→young (O-Y); SAMP and SAMR mice, SAMP→SAMP, SAMP→SAMR, SAMR→SAMP, SAMR→SAMR.

Serial retransplantations of the bone marrow were performed as follows: a half of the femoral cavity content of young (2-3 months) and old (12-24 months) CBA mice was implanted under the renal capsule of 5-month-old CBA mice. After 1.5-2.0 months the transplants were extracted and retransplanted under renal capsule of 5-month-old CBA mice. This procedure was repeated 4 times until complete depletion of the source. The content of nucleated cell was determined in the serial transplants of various passages.

Suspensions of guinea pig bone marrow cells were prepared by passing through a syringe [7]. Suspensions of bone marrow grown in the transplants were prepared as follows [4]: the content of marrow capsule was scraped with a scalpel into α-MEM medium with 5% FCS (Paneco), several times passed through a syringe with needles of decreasing diameters and filtered through a 4-layer capron filter.

Then $1\text{-}5\times10^5$ bone marrow cells were transferred into 25-cm^2 culture flasks with 5 ml α-MEM (Sigma) containing 5% FCS (Paneco), 100 μg/ml penicillin, and 100 μg/ml streptomycin. After 2 h the medium with non-attached cells was removed, the cultures were washed two times with α-MEM, and complete culture medium containing 80% α-MEM, 20% FCS, and antibiotics were added. Irradiated (60 Gy, ^{60}Co, 10 Gy/min) guinea pig bone marrow cells (1.5×10^7) were added to the cultures as a feeder. All cultures were grown for 12 days in a CO_2 incubator at 37°C. Then the cells were fixed with ethanol, stained with azure and eosin, and colonies containing no less than 50 fibroblasts were counted. The efficiency of colony formation (ECF-F) was determined as the number of colonies formed by 10^5 explanted cells.

RESULTS

In cultured bone marrow transplants taken from old donors and implanted to old recipients, ECF-F markedly decreased (almost 7-fold) compared to that in transplants taken from young donors and implanted to young recipients (Table 1). However, this decrease can be explained the effect of the organism, rather by a the decrease in CFU-F count. Indeed, when bone marrow from by old mice was transplanted to young recipients, ECF-F in such transplants increased more than 3-fold compared to bone marrow transplants from old donors transplanted to old recipients. Moreover, transplantation of the bone marrow from young donors to old recipient 2-fold decreased ECF-F of transplants in comparison with Y→Y transplantation (Table 1). These findings attest to age-related alternations of stromal tissue and considerable regulatory influences from the organism on the stroma.

The number of nucleated cells in bone marrow transplants from SAMP mice increased by more than 2 times, if the recipients were SAMR mice instead of SAMP mice (Table 2). The number of nucleated cells in the transplants of bone marrow from SAMR mice decreased almost 10-fold, if the recipients were SAMP mice instead of SAMR mice. However, stromal tissues of SAMP and SAMR possess approximately equal potential to form bone marrow organs of a certain size. Indeed, the counts of bone marrow cells in SAMP→SAMP and SAMR→SAMR transplants were approximately equal. Therefore, in this case the effect of recipient organism on the transplant was also quite significant. It should be taken into consideration that stromal tissue are characterized by a huge bone-forming potential. Indeed, CFU-F contained in 0.5

TABLE 1. Content of Nucleated Cells and CFU-F in Bone Marrow Transplants of CBA Mice of Various Age (*M*±*m*)

Transplant type	Number of nucleated cells in transplant, 10^6	ECF-F	Number of CFU-F in transplant
Y→Y	3.5±0.7	6.2±1.3	217±43
Y→O	3.2±0.6	2.6±0.6	79±16
O→O	3.0±0.7	0.9±0.2	27±5
O→Y	2.9±0.3	2.8±0.6	84±14

TABLE 2. Content of Nucleated Cells in Bone Marrow Transplants of SAMP and SAMR Mice (*M*±*m*)

Transplantation and transplant types		Mean number of nucleated cells in transplant, 10^6
SAMP—SAMP	Y→Y	1.3±0.3
	Y→O	0.5±0.1
SAMP—SAMR	Y→Y	2.9±0.1
	Y→O	4.2±0.3
SAMR—SAMP	Y→Y	0.15±0.00
	Y→O	0.18±0.03
SAMR—SAMR	Y→Y	1.6±0.1
	Y→O	1.3±0.2

TABLE 3. Content of Nucleated Cells in Bone Marrow Repeated Transplants of CBA Mice of Various Ages (*M*±*m*)

Order number of repeated transplantation	Donor age	Number of nucleated cells in transplant, 10^6
1	Y	5.6±1.1
	O	3.2±0.5
2	Y	5.7±0.9
	O	3.0±0.2
3	Y	3.2±0.3
	O	2.8±0.4
4	Y	0.2±0.0
	O	0.5±0.1

g guinea pig bone marrow is sufficient to produce 30 kg bone [5].

Mouse bone marrow tissue endures no less than 3-4 successive transplantations under renal capsule accompanied by gradual decrease in the number of nucleated cells and in the size of bone marrow organs [4]. Taking into account the fact that the number of CFU-F in the bone marrow decreases with age, one could expect that the bone marrow of old donors should endure lower number of retransplantations compared to bone marrow from young donors. However, in our experiments bone marrow transplants from old and young donors endured the same number of retransplantations, when the recipients were 5-month-old animals (Table 3).

The difference in the count of nucleated cells (about 40%) between the transplants from young and old donors persisted up to passage 3 and then disappeared (probably, due to aging of the initial bone marrow, whose age during passage 4 was more than 8 months).

Thus, the age-related modifications of the stromal tissue reducing its capacity to transfer bone marrow microenvironment and to maintain a certain number of stem stromal cells (CFU-F) in newly formed bone marrow tissue result from changes in the stromal tissue and regulatory influences from the body, the contribution of the latter seems to be essential.

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