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Basic Research

Age-Related Decrease in the Number of Stromal Cell Precursors in Bone Marrow and Spleen May Be Due To both Cellular Pool Depletion and Regulatory Impact of the Body

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Abstract

The cloning efficiency (CFE-F) of stromal precursor cells (CFU-F) in cultures of heterotopic transplant cells and the quantity of CFU-F in bone marrow transplants from old donors to old recipients (O-O group) has decreased almost eight times, compared with transplants where the bone marrow from young donors has been transplanted to young recipients (Y-Y group). However, in the O-Y group, the CFE-F and the quantity of CFU-F in the transplants increased more than three times compared with the O-O group, however, staying 2.5 times lower than the Y-Y group level. Regarding transplanting bone marrow from young donors to old recipients (Y-O), the CFE-F of transplants decreased approximately two times lower compared with the CFE-F in the Y-Y group. The CFE-F in the cultures of the spleen transplant cells and the amount of CFU-F in the transplants of the O-O group have decreased four and six times, respectively, compared with the Y-Y group. However, if the spleen from the old mice was transplanted to young recipients (O-Y), the CFE-F in transplants was noted to increase almost seven times compared with the CFE-F in spleen transplants from old donors to old recipients (O-O), and exceeded the level of the Y-Y group. These data suggest that the age-related decrease in the CFE-F and the quantity of CFU-F in the bone marrow are the results of both changes in the stromal tissue (expressed in real reduction in number of CFU-F), and the regulating action of the organism on stromal tissue. Age-related decrease of CFE-F and the amount of CFU-F in spleen could occur, mainly due to the regulating action of the organism. IJBM 2011; 1(4):221-224.

Key words: *stromal cells, age-related changes.*

Stromal cells perform a wide range of very important functions in an organism, such as being involved in tissue renewal and repair, providing the microenvironment for hemopoietic and lymphoid cells, participating in creating the intercellular matrix and acting as antigen-presenting cells. Aging has been shown to be characterized by the loss of bone mass and a decrease in the regenerative ability of the tissues, as well as a decrease in the number and osteogenic activity of the stromal precursor cells [1, 3, 8,12, 14, 16]. However, it is still unclear to what degree thisdecrease is due to the drop in the number of stem stromal cells in an organism, and to what extent it is because of the regulating action that determines their number in the organism, based on age-related physiological needs. The study was conducted following the method of heterotopic transplantation of bone marrow and spleen of mice, and further determination was done of the cloning efficiency (CFE-F) and the number of stromal precursor cells (CFU-F) in the transplants, using the method of monolayer cultivation. During heterotopic transplantation under the kidney capsule of fragments or cellular suspensions of organs, as well as polyclonal strains or individual clones of the stromal fibroblasts, the stem stromal cells, contained in them, replicate the structure of the initial organ. For example, the osteogenic precursor cells of bone marrow form a bone colonized by hemopoietic cells, while spleen stromal stem cells form spleen [4-7, 15]. A transplant, therefore, represents a kind of a chimerical organ, where

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the stromal cells come from the donor, and hemopoietic and lymphoid cells, as well as macrophages and endothelial cells belong to the recipient [9]. Thus, by the cross-transplantation of bone marrow or spleen to old and young animals it becomes possible to determine whether the exhaustion of the CFU-F pool actually occurs with aging or the decrease in the number of these cells in the respective organs is a result of the regulating factors of the organism.

Material and Methods

In this study, CBA male mice, 2-24 months old, as well as male guinea pigs, 4-5 months old, from Kryukovo Central Nursery of Laboratory Animals were used. All the animals were handled according to Animal Welfare Act. For heterotopic transplantation 1/2 of the content of femoral medullary cavity or 1/5 of the spleen of 2-month-old or 24-month-old mice was placed under the kidney capsule of recipient mice, as described earlier [11, 13]. The following combinations of donors and recipients: young-young (Y-Y), young-old (Y-O), old-old (O-O), old-young (O-Y) were used. Totally, eight transplants were registered for each group. Cell suspensions of mice bone marrow and spleen and guinea pigs bone marrow were prepared, as described earlier [10]. Suspensions of the cells contained in the transplants of the bone marrow and spleen were prepared using the following method: two months post transplantation, cells from the transplants were scrubbed out into α-MEM medium (Sigma, USA) with 5% fetal calf serum (FCS) (Paneco, Moscow), passed several times through a syringe with needles of narrowing diameter and finally filtered [11, 13]. Next, 1×10^6 bone marrow cells, and 5×10^6 spleen cells were explanted into 25 cm^2 plastic flasks (Nunc) in 5 ml of α-MEM medium with 5% FCS. After two hours, the medium with the nonadherent cells was poured off, cultures were washed twice with α-MEM, and full culture media containing α-MEM (80%), FCS (20%) and antibiotics (penicillin and streptomycin in 100 μg/ml concentration) were added. Then, 1×10^7 guinea pigs' bone marrow cells irradiated with 60 Gy (Co^{60}, 10 Gy/min) were added to all the cultures as feeder. Cultivation was performed at 37°C in a humidified mixture of 5% CO_2 with air. On days 10 to 12, the cultures were washed with Hanks balanced salt solution (Invitrogen), fixed with ethanol and stained with azure-eosin. CFU-F colonies containing 50 or more cells were counted and the CFE-F (number of colonies per 1×10^5 (for bone marrow) or 1×10^6 (for spleen) explanted cells) was calculated.

Results

Table 1 shows that CFE-F in the monolayer cultures of bone marrow and spleen cells of the CBA mice and, correspondingly, the CFU-F content in these organs decreased with the age of the animals - in bone marrow, to a maximum of two times, and in spleen, to a maximum of eight times in 24-month-old mice compared with 2-month-old animals. The content of the nucleated cells in the bone marrow and spleen transplants in all the groups studied showed a slight change (Tables 2 and 3).

If the bone marrow from old mice was transplanted to young recipients (O-Y group), the CFE-F and content of CFU-F in transplants increased more than thrice compared with the O-O group, but were 2.5 times lower than in the Y -Y group. When bone marrow was transplanted from young donors to old recipients (Y-O), the CFE-F of transplants decreased approximately twice as much compared with CFE-F in the Y-Y group. The CFE-F in the cultures of transplant spleen cells and the number of CFU-F (Table 3) in transplants of the O-O group decreased four and six times, respectively, compared with the Y-Y group.

If spleen from the old mice was transplanted to young recipients (O-Y), CFE-F in the transplants increased almost seven times compared with the CFE-F in the spleen transplants of old donors to old recipients (O-O) and even

Table 1
CFE-F and CFU-F content in bone marrow and spleen of mice of different ages (M±m).

Age of mice (months)	Bone marrow			Spleen		
	Number of nucleated cells in organ ($\times10^6$)	CFE-F ($\times10^5$)	Number of CFU-F in organ	Number of nucleated cells in organ ($\times10^6$)	CFE-F ($\times10^6$)	Number of CFU-F in organ
2	12.4±2.1	3.7±0.8	467±98	133.3±7.7	1.72±0.18	229±27
5	15.1±2.0	3.4±0.5	525±105	144.8±8.8	0.38±0.05	55±10
10	17.8±1.2	3.1±0.6	532±63	180.4±15.6	0.52±0.24	89±37
24	17.9±1.7	1.4±0.2	242±65	168±10.2	0.17±0.04	28±16

Table 2
CFE-F and CFU-F content in bone marrow transplants of mice of different ages (M±m).

Age group of transplants (donor-recipient)	Number of nucleated cells in transplant ($\times10^6$)	CFE-F ($\times10^5$)	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 3
CFE-F and CFU-F content in spleen transplants of mice of different ages (M±m).

Age group of transplants (donor-recipient)	Number of nucleated cells in transplant ($\times 10^6$)	CFE-F ($\times 10^6$)	Number of CFU-F in transplant
Y-Y	4.7±1.0	3.2±0.1	15.0 ±4.2
Y-O	5.2±0.8	2.0±0.3	10.4±3.4
O-O	2.9±0.1	0.8±0.1	2.5±0.1
O-Y	3.7±1.0	5.4±1.1	19.0±1.3

slightly exceeded the level of the Y-Y group. In transplants of the Y-O group, the CFE-F and number of CFU-F decreased nearly 1.5 times compared with the Y-Y group.

Discussion

The amount of CFU-F in hemopoietic and lymphoid organs showed significant decrease with aging [1, 8, 12, 16]. The results of this study confirmed these data (Table 1). Our earlier observations indicate that the age-related decrease in the number of CFU-F in the bone marrow, spleen and thymus in fast-aging SAMP mice occurs significantly earlier - in the age group of 9-11 months, while in SAMR mice (in line with the normal aging speed) – it is seen only in the age group of 16-19 months [12]. These data apparently demonstrate that stromal tissue is involved in the general process of organism aging and undergoes changes within this process. It appeared that though CFE-F and the content of CFU-F in the femoral bone marrow of old CBA mice compared with young ones decreased by a minimum of two times (Table 1), the decrease of these values in bone marrow transplants in the respective groups (Y-Y compared with O -O) was eight times (Table 2). These findings indicate the abrupt decrease in the transplantability of stem stromal cells i.e. referring to their ability to create new microenvironment in old animals. However, if the bone marrow from old mice was transplanted to young recipients (O-Y), the CFE-F and amount of CFU-F in the transplants increased more than three times compared with bone marrow transplants in the O-O group, however, still staying 2.5 times lower than the Y-Y group level. Thus, an eight-times age-related decrease in the CFE-F and CFU-F content in bone marrow transplants of the O-O group was, apparently, mediated both by real age-related reduction of CFE-F and the number of CFU-F 2.5-3 times (Y-Y:O-Y and Y-O:O-O) and by the action of organism, additionally decreasing these amounts about three times (Y-Y:Y-O and O-Y:O-O). Therefore, it may be supposed that the possibility of exhaustion of osteogenic bone marrow stromal cells with aging exists. These data must be considered when selecting the donor age for the transplantation of human bone marrow stromal tissue.

Our earlier work showed that if the volume of the spleen implanted tissue is sufficiently small (1/15 - 1/5 of organ), the same as in the case of bone marrow, then there is a linear dependence between the size of the transplanted spleen fragment and content of CFU-F in the transplant and number of nucleated cells in it; the amount of CFE-F in the transplants then remains unchanged. [13] This fact indicates that spleen heterotopic transplantation is a suitable method for evaluating age-related changes in the number of CFU-F in this organ.

A decrease in CFE-F and in the amount of CFU-F in old mice spleen compared with the young ones (Table 1) corresponds generally to a decrease in these values in the spleen transplants of the respective groups (Y-Y and O-O). The CFE-F in the cultures of spleen transplant cells and the content of CFU-F in the transplants of the O-O group decreased four and six times, respectively, compared with the Y-Y group. However, if spleen from the old mice was transplanted to young recipients (O-Y), the CFE-F in transplant cell cultures increased almost seven times compared with the CFE-F in the transplants of the O-O group, and even slightly (1.3 times) exceeded the level of the Y-Y group. These observations show that when the stromal tissue of old donors is influenced by the young recipients' organisms, this influence alone determines the amount of the CFU-F population in transplant territory. Thus, the character of age-related shifts of mice spleen stromal tissue differs from that of their bone marrow, where, apparently, there is an age-related defect of the stromal tissue that does not allow a restoration of the CFU-F population of the bone marrow of old donors in the young recipients. The fact that the number of CFU-F in spleen transplants in the Y-O group compared with the Y-Y group decreased only 1.5 times, while in the O-Y group, this number increased almost eight times compared with the O-O group, apparently provides the evidence that the decrease in the number of spleen CFU-F in old animals is mediated not by producing inhibiting factors but rather by the lack of factors stimulating it.

The data in this study were confirmed by the work of IM Conboy et al., [2] where it was found that heterochronic parabiosis restored the activation of Notch signaling, as well as the proliferation and regenerative capacity of aged satellite cells (skeletal muscle stem cells). The exposure of the satellite cells from old mice to young serum enhanced the expression of Notch ligands (Delta), increased Notch activation, and enhanced the proliferation in vitro. Furthermore, heterochronic parabiosis increased aged hepatocyte proliferation and restored the cEBR-α complex to levels observed in young animals.

In brief, the work done in this study suggests that age-related decrease in the number of stromal precursor cells can be mediated both by a reduction in the number (exhaustion) of the cell pool (CFU-F of bone marrow) and by the regulating action of the organism (CFU-F of bone marrow and spleen).

The data obtained appear to be significant for understanding the role of the osteogenic stromal precursor cells in the development of age-related defects of bone tissue, particularly senile osteoporosis.

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