Counts of Stromal Precursor Cells in Heterotopic Splenic Transplants in CBA Mice of Different Age

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The content of stromal precursor cells in heterotopic splenic transplants from old and young mice changed appreciably after cross transplantation to old and young animals. The content of CFC-F in the young→old transplants decreased almost 1.5 times in comparison with the young→young transplants, the counts of CFC-F in old→old transplants were minimum in comparison with all other groups (2.5±0.1), while in the old→young group transplants this value increased almost 8-fold (to 19.0±1.3) and surpassed the control level. Age-associated shifts in the splenic stromal tissue were determined by regulatory influences of the host, rather than by decreased count of stromal precursor cells in the tissue.

Key Words: *splenic stromal precursor cells; heterotopic transplantation; age-associated changes*

Splenic tissue includes stromal precursor cells (CFC-F) providing specific microenvironment for proliferation and differentiation of lymphoid and hemopoietic cells. After retransplantation of splenic fragments, stromal precursor cells present in the transplant and responsible for the transplantability of the stromal tissue reproduce the structure of the initial organ. In heterotopic cell transplants of lymphoid and hemopoietic organs stromal cells belong to the donor, while lymphoid and hemopoietic cells belong to the recipient [4]. Today transplantation of the stromal tissue as a method for the treatment of many diseases is widely used in practice [5,7], and possible effects of the recipient hemopoietic and lymphoid cells and the body in general on the transplanted stromal tissue acquire special importance. An important aspects of the problem of the interactions between the stromal tissue and the organism is phenomenon of age-associated decrease in CFC-F counts in hemopoietic and lymphoid organs of humans and animals [6]. During aging the number of CFC-F in the mouse femoral bone marrow decreases 2-fold and in the spleen more than 8-fold [3].

Experiments on heterotopic bone marrow transplants showed that though bone marrow stromal tissue undergoes appreciable age-specific changes, it is regulated by the host [1]. However, it was never studied to which measure this conclusion is true for age-specific changes in the splenic stromal tissue.

The aim of our study was to find out whether the count of CFC-F in splenic transplants is host-regulated or is determined (within the same age group) by the number of stromal stem cells present in the transplant and organizing it, *i. e.* whether linear relationship exists between the bulk of transplanted splenic tissue and CFC-F content in the transplants, and to clear out how the content of CFC-F is changing in heterotopic splenic transplants from old (O) and young (Y) donors in cross-transplantation of splenic tissue to old and young recipients.

MATERIALS AND METHODS

Experiments were carried out on CBA mice aged 2-3 and 24 months and male guinea pigs (4-5 months) from Kryukovo Breeding Center. Heterotopic trans-

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plantation was carried out as follows: $^1/_5$ (or $^1/_5$ and $^1/_{15}$) of mouse spleen was transplanted under the renal capsule of these animals [1] in the following donor-recipient combinations: Y→Y, Y→O, O→O, and O→Y.

Cell suspensions of splenic tissue in 2-month transplants was prepared as follows: the transplant content was scraped with a scalpel into α-MEM with 5% FCS (Paneko), passed several times through a syringe with needles of decreasing diameters, and filtered through four Capron layers. Splenic cells ($5\text{-}10\times10^6$) were explanted into 25 cm^2 flasks in 5 ml α-MEM (Sigma) with 5% FCS (Paneko). After 2 h the medium with nonadherent cells was discarded, the cultures were washed twice with α-MEM, and complete culture medium was added (80% α-MEM, 20% FCS, and antibiotics penicillin and streptomycin, 100 μg/ml each). Bone marrow cells (1×10^7) from guinea pigs irradiated in a dose of 60 Gy (Co 60, 10 Gy/min) were added into all cultures as a feeder. Guinea pig bone marrow cell suspensions were prepared with a syringe [2]. All cultures were incubated for 12 days in a CO_2 incubator at 37°C, fixed in ethanol, stained with Azur-eosin, and colonies containing at least 50 fibroblasts were counted. Cloning efficiency (CFE-F), *i. e.* number of colonies formed by the explanted cells (10^6), was evaluated.

RESULTS

A linear relationship was found between the size of the transplanted splenic fragment, number of CFC-F in the transplant, and number of nuclear cells in it after transplantation of $^1/_5$ and $^1/_{15}$ of the young mouse spleen to young or from old to old animals (within the same age group of recipients) (Table 1). The number of nuclear cells in the transplants from $^1/_{15}$ of the spleen surpassed the estimated values ($^1/_5$:3) by 20%. The CFE-F remained unchanged in the transplants. Hence, if the volume of transplanted tissue is sufficiently small, the number of CFC-F in splenic transplants within the same age group of recipients (young or old) is largely determined by the number of stromal stem cells organizing the transplant. This fact indicates that the method of heterotopic transplantation of the spleen can be used for evaluation of age-associated changes in the CFC-F number in this organ.

The content of nuclear cells in the transplants of Y→Y, Y→O, and O→Y groups changed negligibly; the content of nuclear cells was minimum in the O→O group of transplants (1.5 times lower than in Y→Y and Y→O groups). The CFE-F changed markedly in the transplants: dropped 1.5 times in the Y→O group *vs.* Y→Y group and 4-fold in O→O group *vs.* Y→Y group (Table 2). This decrease can be explained by the host effect on the stromal tissue. The CFE-F in the O→Y group transplants increased almost 7-fold in comparison with the O→O group and surpassed the control level (Y→Y group). The counts of CFC-F also changed appreciably in heterotopic splenic transplants after cross-transplantation to old and young animals. The content of CFC-F dropped 1.5 times in the Y→O group transplants *vs.* Y→Y group, while in O→O group transplants the CFC-F count was the minimum in comparison with other groups, but this value increased almost 8-fold in the O→Y group transplants and surpassed the control level (Y→Y; Table 2). Hence, the

TABLE 1. Number of Nuclear Cells and CFC-F in CBA Mouse Spleen Transplants after Transplantation of $^1/_5$ and $^1/_{15}$ of the Spleen

Recipient, volume of transplanted spleen		Number of nuclear cells per transplant, $\times10^6$	CFE-F, 10^{-6}	Count of CFC-F per transplant
Y	$^1/_5$	5.9±0.7	2.1±0.4	12.4±1.1
	$^1/_{15}$	2.4±0.3	2.1±0.5	5.0±0.5
O	$^1/_5$	6.9±1.0	0.3±0.0	2.1±0.2
	$^1/_{15}$	2.8±0.5	0.3±0.0	0.8±0.2

TABLE 2. Counts of CFC-F in Splenic Transplants in CBA Mice

Donor-recipient pair	Number of nuclear cells per transplant, $\times10^6$	CFE-F, 10^{-6}	Number of CFC-F per 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

Note. $^1/_5$ of the spleen was transplanted. Each group consisted of 10 transplantates.

content of CFC-F in heterotopic splenic transplants in young recipients is determined by the recipient age, but not donor age. Stromal tissue of old donors in young recipients is influenced by the recipient organism, this influence, presumably, determining the size of CFC-F population at the transplant territory. Stromal tissue from old mouse spleen in this case provides the needed size of CFC-F population. The type of age-specific shifts in mouse splenic stromal tissue differs from that in the bone marrow of these animals, in which the possible age-specific defect of stromal tissue prevents the recovery of CFC-F population transplanted from old donors in young recipients: though the counts of CFC-F in bone marrow transplants in the O→Y group are 3-fold higher than in the O→O group, still, they are 2.5 times lower than in the Y→Y group transplants [2]. The count of CFC-F in heterotopic splenic transplants in old recipients depends on recipient and donor age. The count of CFC-F in splenic transplants in the Y→O group decreased only 1.5 times *vs.* Y→Y group, while in the O→Y group this value increased almost 8-fold in comparison with the O→O group. This indicates that the decrease in the count of splenic CFC-F in old animals is determined by th absence of stimulatory factors (*e. g.* factors stimulating CFC-F proliferation or the so-called recruiting, when nonclonogenic stromal precursors become clonogenic and supplement the CFC-F population), rather than the presence of inhibitors.

Hence, age-specific changes in the splenic stromal tissue essential for its capacity to transfer specific microenvironment and maintain a certain count of CFC-F in newly organized tissue are largely determined by regulatory effects of the recipient, but not the decrease in the count of stromal CFC-F in new tissue.

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