EXPERIMENTAL BIOLOGY

Age-Specific Features of Formation of Hemopoietic Microenvironment by Stromal Precursors from Bone Marrow of Thymectomized Mice

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Heterotopic transplantation of bone marrow demonstrated that the content of stromal precursor cells capable of hemopoietic microenvironment transfer does not depend on thymus function. Thymectomy of bone marrow donors involves a decrease in the size of foci formed in young donors and an increase in old recipients. The results indicate a thymus-dependent regulation of proliferation of stromal precursors and/or their factor-sensitive category, determining the proliferation of recirculating stem hemopoietic cells. The size of ectopic hemopoiesis focus depends on the age of recipient. Transplantation of syngeneic thymus under renal capsule of thymectomized mice abolished the effect of thymectomy. Osteogenic activity of stromal precursors correlates with the age of bone marrow donors.

Key Words: *thymectomy; hemopoietic stroma precursor cells; ectopic hemopoiesis focus; osteogenic activity; age*

The presence of humoral regulators in an organism is the basic condition of hemopoietic microenvironment and lymphoid organ regulation.

Heterotopic transplantation of bone marrow helps study the function of stromal cells transferring the hemopoietic microenvironment; it demonstrated that the size of ectopic focus (EF) of hemopoiesis is age-dependent. There are different opinions on the factors determining it (bone marrow recipient or donor age). The size of ectopic hemopoiesis focus decreases with age of bone marrow donors [7]. The content of stromal precursors in the bone marrow of old animals is reported [1]. On the other hand, old recipients possess factors stimulating the growth of implanted hemopoetic stroma precursors [1] but causing thymus involution and preventing ectopic growth of thymus transplanted from young animals [6]. The relationship between thymus involution and regulatory factors stimulating the growth of bone marrow stroma and inhibiting the growth of thymus microenvironment is not clear.

This study was devoted to assessing the effect of thymectomy (TE) in adult mice on the bone marrow content of stromal precursor cells and on their osteogenic activity.

MATERIALS AND METHODS

Female (CBA×C57BL/6) F_1 mice aged 6-10 weeks (at the beginning of experiment) to 25-16 months were used. Bone marrow donors were thymectomized at the age of 6 weeks under 1% hexenal narcosis [3]. Completeness of thymus removal was verified before bone marrow collection.

Foci of ectopic hemopoiesis were induced by implanting bone marrow from thymectomized mice under the capsule of intact recipients 1-23 months

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after the operation. Intact age-matching donors served as controls. The size of formed EF was determined 2 months after bone marrow implantation from the total count of hemopoietic cells in them. Osteogenic activity of stromal cells in EF was estimated by the bulk of newly formed bone shell. The number of EF in a group was 8-12; at least 2 experiments were performed. Results were statistically processed using Student's *t* test.

RESULTS

The effect of TE of bone marrow donors was studied 1, 11, and 23 months after removal of the thymus, i. e., by the beginning of experiment the mice (operated and intact) were 2-, 12-, and 24-month-old, respectively. After heterotopic transplantation of bone marrow, hemopoietic cells left the implant, and stromal precursors constructed hemopoietic microenvironment repopulated by recirculating recipient cells [2,4]. Hence, under the chosen experimental conditions, EF differed by cellular composition only in that the stromal precursors were from thymectomized or intact donors, while recirculating stem hemopoietic cells were similar (belonged to intact recipients). The number of hemopoietic cells in newly formed EF characterizes the number of stromal precursors in them [2,4].

Stromal precursors from bone marrow of thymectomized donors construct hemopoietic microenvironment which by the size of hemopoiesis foci formed by them was comparable to those formed by bone marrow stromal cells of intact age-matching mice (Table 1). This characteristic did not change for 2 years.

The size of hemopoietic microenvironment was determined by the age of bone marrow recipient but not of the donor. In one-year-old recipients the EFs

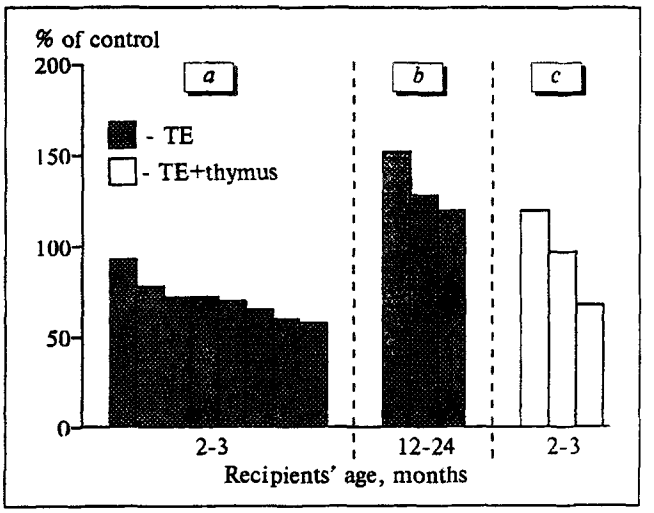

Fig. 1. Size of ectopic hemopoiesis focus formed by implantation of bone marrow from thymectomized (TE) mice to intact recipients of different age. Ordinate: size of ectopic hemopoiesis formed by bone marrow of TE mice (focus formed by bone marrow of intact donors). Bone marrow of TE mice was implanted to 2-3-month-old (*a*) and 12-24-months-old (*b*) recipients; *c*) bone marrow of TE mice restored by the thymus of newborn syngeneic mice implanted to 2-3-month-old recipients.

constructed on the stroma from intact and thymectomized donors were 2 and 3 times larger, respectively, than in the reference group of 2-month-old donors and recipients. In 2-year-old recipients this value was still higher: 3 times higher on the stroma from intact donors and 4 times higher on the stroma from thymectomized mice.

An interesting regularity was noted. After bone marrow from thymectomized donors was implanted to young recipients aged 2-3 months, the size of heterotopic hemopoiesis focus was 72±4% of the control, while in old (12-24 months) recipients cell count in the new focus was 133±11% of control (Fig. 1). Figure 1 represents EF according to a decrease

Table 1. Relationship between Mouse Age and Thymectomy of Bone Marrow Donors and the Size of heterotopic Hemopoiesis Focus (*M±m*)

Mouse age by the beginning of experiment, months		Size of ectopic hemopoiesis focus formed by implantation of mouse bone marrow, cell count per focus, $\times 10^6$	
recipients	donors	intact	thymectomized
2	2 (reference)	11.8±1.6 (3.5)	8.7±0.4 (3.4)
	12	9.0±0.2 (3.8)	6.9±0.5 (3.7)
	24	9.4±1.5 (5.4)	11.4±1.6 (7.0)
12	2	19.2±2.0* (3.3)	24.2±2.0** (4.0)
	12	20.1±0.2* (4.0)	25.8±2.3** (4.4)
24	2	17.8±1.4* (3.2)	27.3±0.6** (5.0)
	24	32.8±1.9* (5.5)	38.4±2.9** (7.5)

Note. Bone weight (mg) is given in parentheses. $p<0.05$: *vs. reference group in the control (intact bone marrow donors); **vs. reference group in experiment (thymectomized bone marrow donors).

in their size expressed in percent of control (EF formed by bone marrow of intact mice). In all 7 experiments the size of EF was less than in the control, only in one case it approximated, but was not equal to the control. This indicates a systemic decrease in the capacity to form EF by stromal precursor cells caused by TE of bone marrow donors (Fig. 1, *a*). A similar trend, but toward higher values (higher than the control in all cases), was noted for EF formed by bone marrow of thymectomized donors in old recipients (Fig. 1, *b*). Transplantation of syngeneic thymus under the renal capsule of thymectomized donors abolished the effect of TE (Fig. 1, *c*).

Transplantation of a bone marrow fragment under renal capsule after initial necrosis and reorganization of the transplant resulted in the osteoblast growth. Only bone capsule was left after 1.5-2 months, surrounding a newly formed hemopoietic organ [4,7]. The process of formation of heterotopic hemopoiesis foci does not depend on the presence of the thymus and age of bone marrow donors, while osteogenic potentials of stromal precursors are much more actively realized in the bone marrow of old (24 months), intact, and thymectomized mice; the latter case the bulk of newly formed bone is larger than in the former (5.4 and 5.5 mg *vs.* 7 and 7.5 mg, respectively, Table 1).

Thus, we demonstrated for the first time that the content of stromal precursor cells in the bone marrow capable of transferring and constructing hemopoietic microenvironment, does not depend on the presence of the thymus and does not decrease with age. Thymectomy of bone marrow donors involves a decrease in the size of EF formed in young recipients (not depending on the age of donors) and their increase in old recipients in comparison with the control. This indicates thymus-dependent regulation of stromal precursor proliferation and/or their factor-sensitive category [2,4], which eventually determines proliferation of recirculating stem hemopoietic cells. We confirmed previous data [1,8] that the size of ectopic hemopoiesis is determined by recipient's age, i. e., older animals produce factor(s) stimulating the growth of implanted stromal precursor cells.

Osteogenic potentials of stromal precursors correlate with the age of bone marrow donors. The process of aging involving involution of the thymus promotes activation of osteogenic precursor proliferation. Their stimulation is most expressed in old (24-month) thymectomized mice, in which the thymus was removed at the period of its maximum activity (at the age of 6 weeks). However, these results do not permit us to assert that osteogenesis directly depends on the functional activity of the thymus, because, among other reasons, the cause of thymus involution and aging of an organism in general is still unknown.

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