Hemopoietic Microenvironment-Transferring Units and Inducible Hemopoietic Stromal Precursors in the Bone Marrow of Thymectomized Mice

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The method of heterotopic transplantation of the bone marrow was used to study the effect of thymectomy on clonogenic and inducible hemopoietic stromal precursors in adult rats. The self-maintenance or clonogenic capacity of stromal precursors was evaluated by retransplantation of primary hemopoietic foci. The kinetics of the formation of ectopic foci from thymectomized rats is similar to that of normal bone marrow. The presence of inducible stromal hemopoietic precursors was evaluated by the stimulation index (the ratio of the size of hemopoietic focus formed in irradiated to that in nonirradiated recipient). It is found that the growth of ectopic focus in chimeras is stimulated by a nonthymic factor, which suggests thymus-independent regulation of hemopoietic microenvironment precursor cells.

Key Words: *thymectomy; age; stroma; heterotopic hemopoietic focus; clonogenic and inducible hemopoietic stromal precursors; radiation chimeras*

The method of heterotopic bone marrow transplantation is an instrument for studying the properties of stromal cells or hemopoietic microenvironment-transferring units (HMTU). Bone marrow cells inoculated under the kidney capsule form new hemopoietic foci, in which stroma is formed by donor cells, while hemopoietic cells are formed due to proliferation and differentiation of recirculating hemopoietic precursors of the recipient [4]. The formation of new hemopoietic microenvironment (HME) depends on clonogenic stromal cells (HMTU), whose clone-formation capacity was proved by the radiobiological methods [7]. Serial retransplantations of ectopic foci in intact and irradiated recipient revealed no decrease in the size of newly formed foci over at least 9 passages [1], which confirms unique HME-transferring capacity of HMTU. Ectopic foci after retransplantation are constructed anew. The ability of HMTU to form new HMU is referred as self-maintenance.

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The stromal precursor population sensitive to distant regulatory factors was determined in radiation chimeras. Preliminary (before bone marrow transplantation) irradiation of the recipients led to the formation of large ectopic foci (2-3-fold greater than in nonirradiated recipients) [2,6]. Unlike HMTU, inducible HME precursors are neither transferable nor self-maintaining. Moreover, HMTU are regulated primarily by local, while inducible HME precursors by distant regulatory factors [4].

HME regulates proliferation and differentiation of hemopoietic stem cells via immediate cell-cell contacts and production of growth factors, in particular, hemopoietins [8]. Stromal cells do not only secrete growth factors but also respond to some humoral factors. For instance, in old recipients, similarly to irradiated ones, some factors are synthesized that promote the growth of implanted stromal precursors [3], but also inducing thymus involution and inhibiting the ectopic growth of the thymus graft from young animals [9].The relationship between thymus involution and some distant regulatory factors (hemopoietic, sti-

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mulating stromal growth, and suppressing thymic microenvironment) remains unclear.

In light of this, we studied the effect of thymectomy (TE) of adult mice on the clonogenic capacity of HNTU and the sensitivity of inducible stromal cells to distant regulators.

MATERIALS AND METHODS

The study was carried out on female (CBA×C57Bl)F_1 mice aged from 6-10 weeks (at the start of the experiment) to 7-10 months. Donor mice were thymectomized under Hexenal (1%) narcosis at the age of 6 weeks [5]. The quality of TE was verified before bone marrow isolation.

Ectopic hemopoietic foci were induced by inoculation of the test bone marrow samples under the kidney capsule of the corresponding recipient mice.

For evaluation of the clonogenic capacity of stromal precursors the bone marrow from sham-operated, TE mice and TE mice with transplanted thymus was transferred under the kidney capsule of sham-operated and TE recipients. One month after transplantation, some recipients were sacrificed, the size of newly formed foci was measured, and some foci were retransplanted to TE mice. A total of 4 transplantations were carried out.

For identification of inducible stromal precursors, bone marrow from experimental mice was implanted to irradiated (8 Gy) or nonirradiated recipients. The stimulation index was calculated as the ratio of hemopoietic focus size in irradiated to that in nonirradiated recipients 1 month after transplantation.

Newly formed hemopoietic foci were evaluated 1 month after bone marrow transplantation by the total number of nucleated hemopoietic cells. The size of ectopic focus is directly proportional to the content of HMTU [2]. Eight to twelve ectopic foci developed in each group, each series consisted of at least 2 experiments.

Recipient mice were irradiated in a dose of 8 Gy (^{137}Cs, 16.5 rad/min dose power).

The data were processed statistically using the Student t test.

RESULTS

The ability of stromal precursors to repeated formation of HME (HMTU self-maintenance) was evaluated by inoculating the bone marrow fragments from TE donors to sham-operated or TE recipients followed by retransplantation of newly formed foci to TE recipients. First transplantation was carried out when the donors and recipients aged 3 months. The experimental conditions were so chosen that recipient mice were 1 month elder to each subsequent retransplantation (i.e., to the end of the first implantation and passages 2, 3, and 4 the age of recipient TE mice was 4, 5, 6, and 7 months, respectively). The recipient age should be taken into account since it determines the size of HME [3].

In young TE recipients, heterotopic hemopoietic foci developed more slowly irrespectively of the origin of stromal precursors (Table 1). One month after bone marrow inoculation, the size of HME was about 60% of the control (transplantation of the bone marrow from sham-operated donors to sham-operated recipients) and then the kinetics of ectopic foci development was similar to that previously described for normal bone marrow [1]. In particular, the size of HME correlated with recipient age, whereas the differences between all donor groups were insignificant.

Thus, the fact that HME develops anew after each transplantation followed by its secondary repopulation with actively proliferating hemopoietic stem cells from recipient's circulation suggests that stromal precursors from the bone marrow of TE donors are characterized by high self-maintenance potential, i.e., the ability of transferring and reconstructing HME.

Bone marrow fragments from TE donors were inoculated to irradiated and nonirradiated recipients

TABLE 1. Kinetics of HME Reconstruction after Retransplantation of Hemopoietic Focus

Bone marrow donors	Recipients	Size of heterotopic foci by the content of hemopoietic cells, $\times 10^6$			
		passage			
		1	2	3	4
Sham-operated	Sham-operated	19.2			
TE+thymus	Sham-operated	18.4			
TE	Sham-operated	13.4			
Sham-operated	TE	10.0	16.6	17.2	21.2
TE+thymus	TE	11.9	19.9	21.5	23.1
TE	TE	12.3	12.8	26.9	32.7

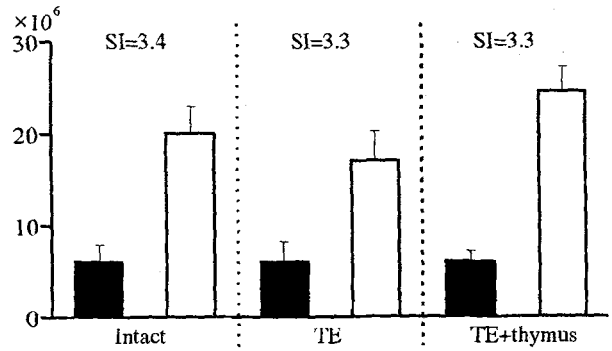

Fig. 1. Size of ectopic hemopoietic foci in normal (dark bars) and irradiated in a dose of 8 Gy (light bars) recipients. Ordinate: number of hemopoietic cells in heterotopic foci. SI: stimulation index (ratio of the size of ectopic hemopoietic focus in irradiated recipient to that in nonirradiated one).

(intact and TE mice). In TE mice we found inducible stromal precursors sensitive (as in intact donors) to unidentified factors produced by irradiated recipient (Fig. 1). Stromal precursor stimulation index for TE mice was similar to that for intact donors (3.3 and 3.4, respectively). The growth of ectopic hemopoietic foci in chimeras is promoted by a nonthymic factor, since stimulation index in the group of TE donors with implanted thymus was similar to those in the groups of intact and TE donors.

These findings confirm similar hierarchical structure of HME in thymus-deficient and normal mice. It consists of transferable hemopoietic stromal stem cells characterized by high clonogenic capacity and inducible stromal precursors sensitive to distant regulatory factors. The stroma in TE mice maintains proliferation of hemopoietic stem cells and hemopoiesis as a whole. Regulation of hemopoietic stromal precursors is thymus-independent.

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