EXPERIMENTAL BIOLOGY

HEMATOPOIETIC PRECURSOR CELLS IN RADIATION CHIMERAS RESTORED BY BONE MARROW OF THYMECTOMIZED MICE

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The role of the thymus and its hormones in regulation of the properties of hematopoietic stem cells (HSC) is most clearly demonstrated in radiobiological test systems. Thymectomy in adult mice disturbs the ability of 8-day CFUs (splenic colony-forming units) [1] but not of 11-day CFUs [3] to repair sublethal radiation lesions. The effect is reversible, for transplantation of the thymus and its derivatives (thymocytes, humoral factor) into thymectomized (TE) mice abolishes the effect of thymectomy [2]. It was shown previously [6] that bone marrow of neonatally thymectomized (neo-TE) mice possesses sharply reduced protective ability. To achieve equal restoration of hematopoiesis in irradiated recipients, the number of bone marrow cells required from neo-TE donors is two orders of magnitude greater than from intact animals.

In the investigation described below the protective ability of bone marrow from adult TE mice was studied on a model of radiation chimeras.

EXPERIMENTAL METHOD

Experiments were carried out on female (CBA × C57BL/6)F_1 mice. Thymectomy was performed on mice donating bone marrow at the age of 2 months by the method in [4]. Complete removal of the thymus was verified before bone marrow was taken. To obtain radiation chimeras intact mice were irradiated in a lethal dose (12 Gy, ^{137}Cs source, Blood Transfusion Institute), and their hematopoiesis was restored with $5 \cdot 10^6$ bone marrow cells from TE donors. The age of the donors after thymectomy was 8-11 months, Chimeras restored with normal bone marrow from mice aged 8-11 months in a dose of $5 \cdot 10^6$ cells served as the control. The choice of optimal dose of bone marrow cells, enabling complete restoration of hematopoiesis in the irradiated recipients, depended on the protective ability of the bone marrow of the TE mice. According to data in [6], complete restoration of hematopoiesis took place after injection of $3 \cdot 10^6$ bone marrow cells from TE mice. The number of bone marrow CFUs was determined by the method in [5]. The number of 8- and 11-day CFUs and their proliferative potential were determined 1-7.5 months after creation of the radiation-chimeras. To determine the proliferative potential of the CFUs, cells of the test bone marrow were injected intravenously into lethally irradiated mice. After 11 days the pool of homogenized spleen cells was injected in a dose of 0.1-0.2 colony per mouse. The number of colonies in the secondary recipients was determined 8 days later. By suitable calculations the proliferative potential was determined, i.e., the number of daughter CFUs per colony. The number of mice in the group of recipients was 10-15, The number of exogenous colonies did not exceed 0.2 per spleen. The results were subjected to statistical analysis by Student's t test.

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TABLE 1. Cell Content of Bone Marrow of Radiation Chimeras Restored by Bone Marrow from Intact and Thymectomized Mice, M ± m

Duration of chimerism, months	Number of daughter CFUs per 11-day restored by bone marrow of TE mice	Number of daughter CFUs per 11-day restored by bone marrow of TE mice
1	11,80±2,80	8,75±1,12
3,5	13,06±0,90	14,12±1,37
5	11,75±1,25	16,62±1,16
7,5	15,75±0,75	15,37±0,20

TABLE 2. Proliferative Potential of CFUs from Bone Marrow of Radiation Chimeras

Duration of chimerism, months	Number of daughter CFUs per 11-day colony in chimeras restored by bone marrow of intact mice	Number of daughter CFUs per 11-day colony in chimeras restored by bone marrow of TE mice
1	83	16
1	64	12
3,5	90	28
3,5	74	24
3,5	68	—
5	88	29
7,5	88	15
7,5	77	13
$M \pm m$	79±3	20±3

EXPERIMENTAL RESULTS

Restoration of hematopoiesis in the radiation chimeras was judged from the cell content of the bone marrow, values of which also were compared with the number of myelokaryocytes in 2-6-month old unirradiated mice. The results are given in Table 1. The cell content of the bone marrow of the chimeras at all times of investigation was on average identical and was independent of the source of bone marrow. The values obtained also agreed with the number of myelokaryocytes in 2-6-month old, nonirradiated intact and TE mice, namely 15.1 ± 0.8 and 16.6 ± 1.2 million cells respectively.

The content of 8- and 11-day CFUs in the femur of the radiation chimeras (duration of chimerism 1-5 months) was independent of the source of bone marrow used to restore the irradiated mice (Fig. 1). The values obtained agree with those in nonirradiated intact (CFUs-8 3377 ± 396; CFUs-11 3351 ± 392) and TE (CFUs-8 4504 ± 401: CFUs-l 1 3956 ± 437) mice.

A sharp fall (by more than 75%) in the number of CFUs-8 and CFUs-11 in chimeras restored with bone marrow from TE mice was recorded only 7.5 months after irradiation and restoration of hematopoiesis (Fig. 1) due to a decrease in the relative number of CFUs (data not given).

The proliferative potential of the CFUs, determined as the number of daughter colonies formed from 11-day primary colonies, was reduced by 75% already in 1-month experimental chimeras (restored with bone marrow from TE mice) compared with the controls (restored with bone marrow from intact mice) (Table 2). Since the proliferative potential of CFUs is not linked to the duration of chimerism, the results were averaged and are shown in Fig. 2 compared with values of proliferative potentials of CFUs from bone marrow of nonirradiated and intact TE mice. A clear difference in proliferative potential of CFUs from bone marrow of TE mice, used to restore hematopoiesis of lethally irradiated recipients, compared with the remaining groups of animals, can be seen in Fig. 2.

It can be concluded from the results that thymectomy reduces the proliferative potential of HSC, which can be detected only in a system with an extra passage. When hematopoiesis is on demand, i.e., during regeneration of hematopoiesis in irradiated recipients, HSC of TE mice cannot adequately replenish the pool of CFUs subjected to rapid proliferation and differentiation. The fall of 75% in the proliferative potential of HSC, which corresponds to

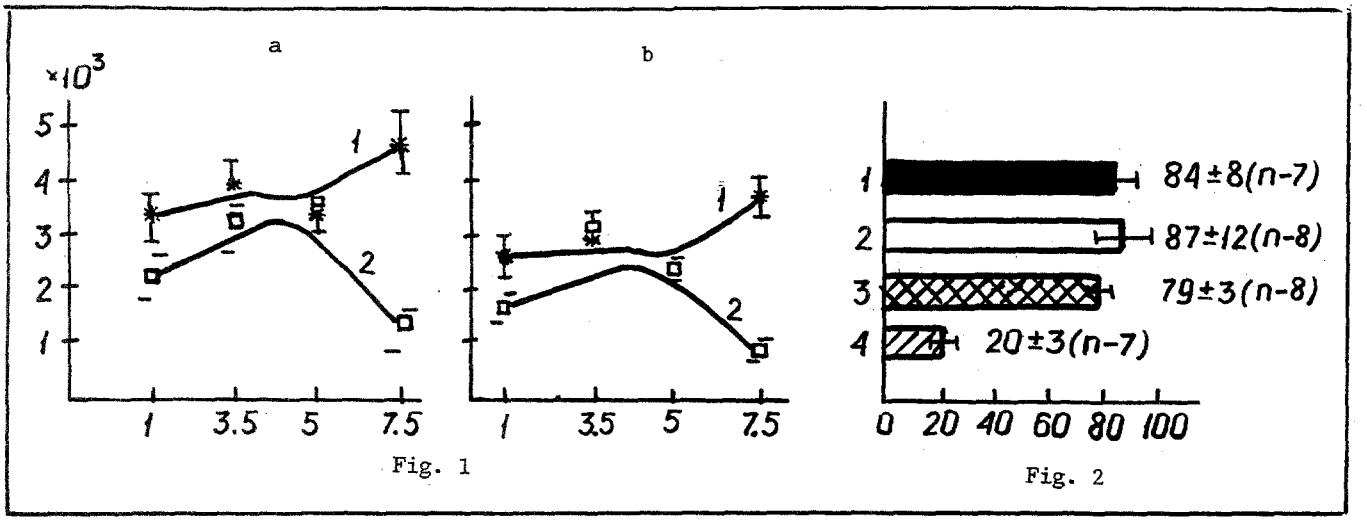

Fig. 1

Fig. 2

Fig. 1. Content of two subpopulations of CFUs in bone marrow of radiation chimeras. Abscissa, duration of chimerism (in months); ordinate, content of CFUs-8 days per femur (a) and content of CFUs-11 days per femur (b). 1) Content of CFUs per femur in radiation chimeras restored with bone marrow of intact mice; 2) content of CFUs per femur in radiation chimeras restored with bone marrow from TE mice, Vertical lines with symbols indicate ± standard deviations.

Fig. 2. Proliferative potential of CFUs from bone marrow of nonirradiated mice and radiation chimeras. Abscissa, proliferative potential of CFUs; ordinate, experimental groups of animals. 1) Nonirradiated mice, intact; 2) nonirradiated mice, thymectomized (TE); 3) radiation chimeras, restored with bone marrow from intact mice; 4) radiation chimeras, restored with bone marrow from TE mice. Numbers on right of columns show levels of proliferative potential (M ± m); n) number of experiments.

two mitoses, was recorded 4 weeks after creation of the experimental chimeras, and preceded a sharp decline in the number of 8- and 11-day CFUs in 7.5-month chimeras. The decrease in the number of CFUs, however, was not reflected in the total cell content of the bone marrow, the high level of which is evidently maintained by proliferation and differentiation of more mature precursors.

An important conclusion regarding activity of T lymphocytes or T-cell growth factors in the maintenance of homeostasis of the hematopoietic system or, in particular, of the proliferative potential of HSC, can thus be drawn from the present investigation. Disturbance of this important property of HSC, not detectable under conditions of stable hematopoiesis, i.e., in thymectomized mice (Fig. 2), is manifested only under conditions of hematopoiesis on demand. In an in vivo system, on a model of radiation chimeras, further proof was thus obtained of the involvement of the thymus in the regulation of the properties of HSC, and the protective ability of HSC was shown to correlate with its proliferative potential.

REFERENCES

1. T. V. Todriya, Byull. Éksp. Biol. Med., No. 11, 584 (1978).
2. T. V. Todriya, All-Union Symposium on Clinical and Experimental Bone Marrow Transplantation [in Russian], Moscow (1984), pp. 25-26.
3. T. V. Todriya, Byull. Éksp. Biol. Med., No. 5, 597 (1988).
4. E. A. Boyse, L. J. Old, and C. A. Iritani, Transplantation, **12**, 93 (1971).
5. J. E. Till and E. A. McCulloch, Radiat. Res., **14**, 213 (1961).
6. D. Zipori and N. Trainin, Exp. Hemat., **3**, 1 (1975).