ELIMINATION OF AN ACCESSORY POPULATION INVOLVED IN PROLIFERATION OF PLEURIPOTENT HEMATOPOIETIC STEM CELLS FROM BONE MARROW BY MOUSE ANTIBRAIN SERUM

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The writers showed previously that mouse antibrain serum (MABS) removes a cell population from bone marrow which performs an accessory role during the formation of splenic colonies, and which can be replaced by thymus cells [7]. MABS also eliminates cells which play a definite role in differentiation of hematopoietic stem cells toward erythropoiesis [2]. These observations were made on the 8th-9th day of growth of splenic colonies. In 1982 it was reported [11] that only a limited cell population, namely precursors of erythropoiesis, has to be considered at these times.

With the above facts in mind it was decided to study changes in the number of splenic exocolonies and also postradiation repopulation of hematopoietic organs in recipients after receiving an injection of bone marrow treated with MABS, with or without the addition of thymocytes, in the later stages, viz., on the 12th-14th day.

EXPERIMENTAL METHOD

Male (CBA × C57BL)F_1 hybrid mice aged about 1.5 months were used. The recipient mice were irradiated with ^{60}Co γ-rays in a dose of 8.5-8.0 Gy, on the Luch-1 radiotherapeutic apparatus 18 h before receiving the injection of bone marrow and thymocytes. Colony-forming activity of the bone marrow was determined by the splenic exocolonies method [14]. Recipient mice were killed 8, 12, and 14 days after transplantation of cell suspensions into them, and their spleens were removed and fixed in Bouin's fluid. MABS was obtained by the method in [11]. Bone marrow cells were treated with MABS as described previously [7]. Intact thymocytes ($2 \cdot 10^7$ cells per mouse) were injected intravenously into the recipients 30-40 min before the injection of bone marrow. To improve the survival rate of the experimental animals, a larger number of bone marrow cells than usually ($2 \cdot 10^5$-$4 \cdot 10^5$ cells per mouse) was injected into them, and as a rule the ratio between bone marrow cells and thymocytes was between 1:70 and 1:100, whereas to obtain the optimal effect, this ratio must be 1:200 [13]. Consequently the addition of thymus cells in the above proportion led to only partial stimulation of colony formation. To determine the time course of postradiation repopulation of the bone marrow and spleen, the mice were irradiated in a dose of 8.5 Gy and, 4 h later, they received an injection of intact or MABS-treated bone marrow in a dose of $5 \cdot 10^5$ cells per mouse. Besides bone marrow, mice of one group received an injection of thymocytes in a dose of $2 \cdot 10^7$ cells per mouse. On the 4th, 8th, 11th, and 14th days the number of karyocytes in the spleen and femur was counted. Each point in Figs. 1 and 2 represent averaged data for 15 animals.

EXPERIMENTAL RESULTS

The results of counting splenic macrocolonies on the 8th, 12th, and 14th days of growth after injection of bone marrow and thymocytes are given in Table 1. In the control group a very small decrease in the number of colonies was observed from the 8th until the 14th days of growth, possibly due to fusion of separate colonies. Within the experimental groups

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TABLE 1. Number of Exogenous Splenic Colonies Formed Depending on Time of Observation

Experimental conditions	Time of observation, days	Number of mice	Mean number of colonies per 10^5 bone marrow cells
Control	8	22	9,9±0,6
	12	34	7,7±0,6
	14	8	6,5±0,4
MABS	8	32	1,5±0,3
	12	27	1,8±0,4
	14	5	13,8±0,4
MABS + thymus	8	29	4,8±0,5
	12	41	4,6±0,5
	14	10	11,0±0,2

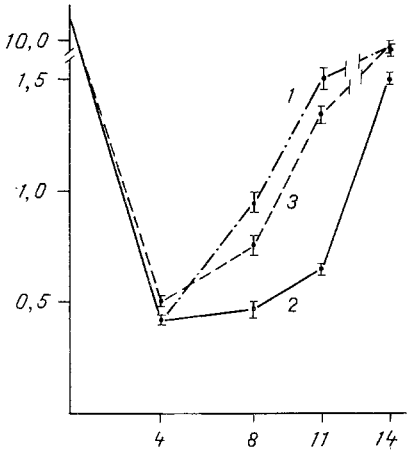

Fig. 1

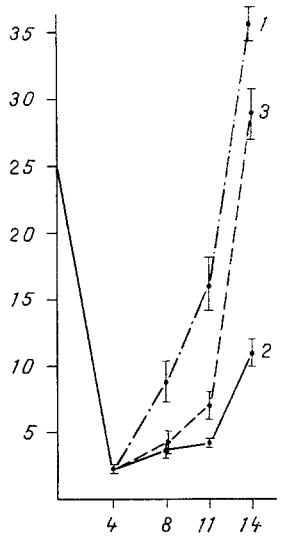

Fig. 2

Fig. 1. Dynamics of femoral marrow cell population in mice irradiated in a dose of 8.5 Gy and restored by intact bone marrow (1), by MABS-treated bone marrow (2), and by MABS-treated bone marrow plus thymocytes (3). Here and in Fig. 2: abscissa, time after injection of bone marrow cells (in days); ordinate, number of karyocytes in femur ($\cdot 10^{-6}$).

Fig. 2. Dynamics of spleen cell population in mice irradiated in a dose of 8.5 Gy and restored by intact bone marrow (1), by MABS-treated bone marrow (2), and by MABS-treated bone marrow plus thymocytes (3).

(bone marrow treated with MABS, with or without thymocytes) no appreciable differences in the number of colonies on the 8th and 12th days could be detected, but by the 14th day there was a sharp rise in the number of colonies counted in both groups. The impression was obtained that incubation of bone marrow with MABS delays proliferation of splenic colony-forming units (CFUs) in the recipient, which continues for 5-6 days. It can be tentatively suggested that this delay of CFUs proliferation and, correspondingly, of growth of splenic colonies may be due to elimination by the MABS of an accessory population of helper cells necessary for colony formation. The delay was abolished by the 14th day, possibly due to restoration of the number of this population. Addition of thymocytes probably replaces to some degree the loss of accessory cells.

A similar time course also was observed when repopulation of the bone marrow and spleen was studied in irradiated mice, restored by MABS-treated bone marrow with or without the ad-

dition of thymocytes (irradiated mice restored with intact bone marrow served as the control). It will be clear from Figs. 1 and 2 that until the 4th day after irradiation and injection of bone marrow cells no difference could be found between the groups. On the 8th and 11th days the number of karyocytes in the bone marrow and spleen of mice receiving MABS-treated bone marrow was much less than in the control animals, although on the 11th day a small increase in the number of nucleated cells was noted. By the 14th day their number reached the control level. Transplantation of thymocytes accelerated repopulation of the hematopoietic organs in mice restored with MABS-treated bone marrow. It can be tentatively suggested that a cell population capable not only of normalizing splenic colony formation, but also of accelerating postradiation repopulations of hematopoietic organs in these animals, was restored between the 11th and 14th days in recipients receiving an injection of bone marrow, incubated with MABS. In the earlier stages the absence of this population was compensated by the addition of thymocytes. Restoration of the proliferative status of donors' CFUs in an allogeneic system also has been observed [10] to take place by the 14th day. The reasons for this were interpreted as the appearance of histocompatibility molecules, similar to the H-2 determinants of the F_1 recipient, by that time on the donors' bone marrow cells. However, observations by a number of workers [6, 12] have shown that injection of thymocytes syngeneic for the bone marrow donor creates conditions for restoration of growth of CFUs at earlier stages in an allogeneic recipient.

It can be postulated on the basis of the facts described above that treatment with MABS removes from bone marrow a population of cells essential for maintaining the normal level of proliferation in it. This hypothesis is confirmed to some extent by data obtained during a study of the kinetics of regeneration of bone marrow enchriched with CFUs [15]. The rate of regeneration of CFUs from enriched femoral marrow was only half that for CFUs from unfractionated bone marrow. Addition of thymocytes to a CFUs-enriched bone marrow suspension restores the normal rate of repopulation. The authors cited suggested that during enrichment of bone marrow with pleuripotent stem cells, a population of "accessory" cells, essential for maintenance of the normal level of CFUs proliferation, is removed from it. Evidently similar events also take place when bone marrow is treated with MABS.

It is now a firmly established and well-documented fact that differentiation of hematopoietic stem cells is a thymus-dependent process. A definite role in it is played both by humoral and by cellular factors of the thymus [1, 3-5, 9]. It has also been postulated that the processes of CFUs proliferation are also under control of the thymus; this control is effected, moreover, through long-range factors, and they differ for proliferation and differentiation [13]. The possibility of abolishing the inhibitory action of MABS on colony formation and of abolishing allogeneic inhibition by means of fraction 5 of thymosin [8] suggests that it contains a factor which promotes proliferation. However, like differentiation, proliferation of CFUs is probably controlled not only by humoral, but also by cellular factors of the thymus, supporting evidence for which is given by the facts described in this paper and data in the literature [15].

It can thus be postulated that MABS inactivates a population of cells in the bone marrow which are involved in the control of CFUs proliferation. This population is restored in the animal by the 14th day after irradiation and of injection of MABS-treated bone marrow.

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