ROLE OF CELLS SENSITIVE TO ANTI-MOUSE BRAIN SERUM IN THE REGULATION OF CFU-S PROLIFERATION

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With the aid of rabbit anti-mouse brain serum (RAMBS) it is possible to remove cells that differ from CFU-S, that are necessary for colony formation in the spleen, from a bone marrow suspension [2, 3]. Addition of thymocytes replaces the eliminated population and restores colony formation. The number of splenic colonies from RAMBS-treated bone marrow is less than the control on the 7th-8th day of growth, but catches up with the control group by the 14th day of growth. It has been suggested that the serum inactivates a cell population present in bone marrow which regulates the proliferation of CFU-S [4, 9]. In addition, RAMBS acts on the lymphocyte population which determines differentiation of CFU-S (T_d lymphocytes) [1]. The aim of the present investigation was to test these hypotheses. For this purpose the kinetics of CFU-S regeneration in the bone marrow and spleen was determined after injection of RAMBS-treated bone marrow, with or without the addition of thymocytes, into primary recipients. The distribution of colonies by histological types after injection of bone-marrow suspension, incubated with RAMBS, also was analyzed on an extensive experimental material.

EXPERIMENTAL METHOD

Female (CBA × C57BL)F_1 mice aged 2.5 months were used. The recipient mice were irradiated with ^{60}Co γ-rays on a Luch-1 radiotherapy apparatus 18-24 h before receiving a transplantation of bone marrow. RAMBS was obtained by the method in [6]. The colony-forming activity of the bone marrow or spleen was determined by the splenic colonies method [8]. The bone marrow suspension was treated with RAMBS as described previously [2]. Thymocytes were injected intravenously 30-40 min before injection of bone marrow. From seven to 15 mice from each group were killed after 4, 7, 11, and 14 days, the spleen and femoral marrow were removed, cell suspensions were prepared, and these were injected into secondary recipients. The latter were killed on the 10th day after transplantation, the spleen was removed and fixed in Bouin's solution, and the number of macrocolonies was counted. To study the histological character of the colonies the spleens were embedded in paraffin wax and serial sections were cut along the length of the organ. Every 7th section was taken and stained with hematoxylin-eosin.

EXPERIMENTAL RESULTS

Data showing regeneration of CFU-S of bone marrow and spleen in the recipients of transplanted intact bone marrow, with or without the addition of thymocytes, and also RAMBS-treated bone marrow, with or without the addition of thymocytes, are given in Fig. 1. The rate of repopulation of CFU-S was identical in the spleens of mice receiving intact and RAMBS-treated bone marrow. The time taken for the number of CFU-S to double in both cases was 38 h. Additional injection of thymocytes into the recipients led to a decrease, although small, in the doubling time of CFU-S, to 31 h. The repopulation kinetics for CFU-S in the femur differed from that in the spleen. Treatment of bone marrow with RAMBS sharply delayed repopulation of medullary CFU-S. In this case the doubling time was almost twice that in the control (114.2 and 65.46 h respectively). Addition of thymocytes quickened the course of repopulation, and in this case the CFU-S doubling time was 60 h. Values of the CFU-S doubling time

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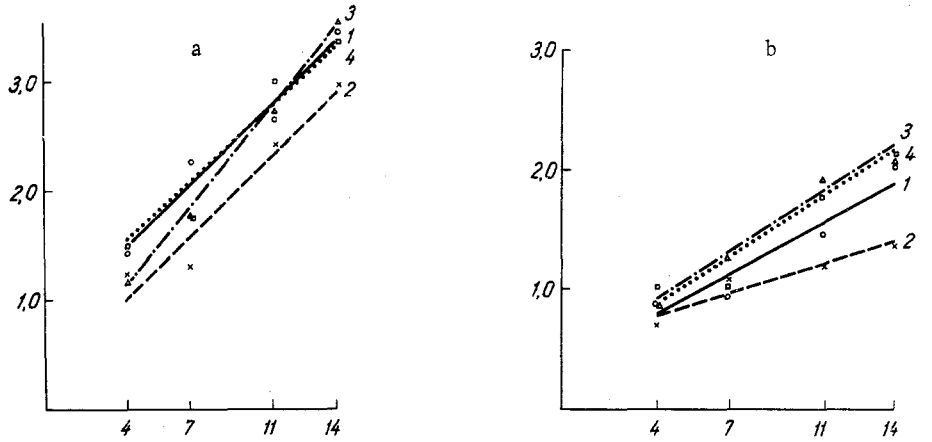

Fig. 1. Restoration of CFU-S in spleen (a) and femur (b) of mice receiving intact bone marrow (1), intact bone marrow + thymocytes (4), RAMBS-treated bone marrow (2), and bone marrow treated with RAMBS + thymocytes (3). Abscissa, time after transplantation, days; ordinate, $\log_{10}$ of number of CFU-S per organ.

of the femoral marrow obtained in two independent experiments, together with the corresponding data for splenic CFU-S, are given in Table 1. These results are in good agreement with data in [9], when the authors cited observed a sharp increase in doubling time for CFU-S from enriched bone marrow compared with intact, and considered that during the enrichment procedure, a population of "accessory" cells, regulating CFU-S proliferation, is removed from it. The present writers suggested previously that during incubation of bone marrow with RAMBS the latter inactivates an accessory cell population that is necessary for regulation of CFU-S proliferation [4]. The data given in this paper provide arguments in support of this hypothesis. It appeared extremely important to test yet another hypothesis, put forward by the writers previously, namely that treatment of bone marrow with RAMBS damages lymphocytes of the T series, which determine the direction of differentiation of hematopoietic stem cells along the etythroid path. This hypothesis was based on data obtained on a relatively small amount of experimental material. However, analysis of extensive cumulated results forced the abandonment of this hypothesis. It will be clear from Table 2 that treatment of bone marrow with RAMBS did not change CFU-S differentiation in the erythroid direction. Under these circumstances, however, the relative percentage of colonies of the granulocytic type was reduced, although not significantly, with the result that the E/G ratio slightly exceeded its value in the control. It must also be pointed out that as a result of the action of RAMBS on the bone marrow suspension the relative percentage of undifferentiated colonies increased, probably as a result of slowing of CFU-S proliferation after removal of the accessory cells from the graft.

Incubation of bone marrow with RAMBS thus leads to the elimination of a population of "accessory" cells regulating CFU-S proliferation but not participating in differentiation processes or, more exactly, not affecting the direction of differentiation. Cells similar to or identical with these are present in the thymus in very small numbers. They are probably essential in the early stages of development of CFU-S, when the latter are either polypotent or in the first stages of commitment. In this case proliferation may be controlled at the level of intercellular interactions.

Recently, published data [7, 10, 11] provide evidence that factors controlling division and differentiation of hematopoietic stem cells are produced in bone marrow. The most interesting information concerns one of them, namely a stem cell activating factor, or interleukin-3, which can increase the number of CFU-S by several times [10, 11]. It seems likely that the "accessory" cells are able to produce this factor. The role of accessory cells may also be claimed by cells secreting the short-range stimulator of CFU-S proliferation which Lord and Schofield found in mouse bone marrow [7].

Elucidation of the nature of the accessory population required for normal CFU-S proliferation is extremely interesting. Some progress has already been made along this path. For example, an $SC\text{-}I^{+}$-population, whose removal from bone marrow considerably weakens its colony-

TABLE 1. Doubling Time (in h) of CFU-S in Femoral Marrow and Spleen (results obtained by calculating repopulation kinetics within a time interval of 4-14 days)

Group	Bone marrow		Spleen	
	1	2	1	2
1 Control	55.2	65.46	38.0	37.2
2 RAMBS	111.1	114.2	38.0	36.5
3 RAMBS + thymus	51.6	60.0	31.0	34.8
4 Control + thymus	73.7	55.4	41.0	42.2

Legend. 1, 2) Nos. of experiments.

TABLE 2. Distribution of Splenic Colonies by Histological Types from Recipients of Transplanted RAMBS-Treated Bone Marrow

Incubation of bone marrow	Number of spleens	Number per spleen		Percentage of colonies					
		macrocolonies	microcolonies	E	G	Sm	U/D	Mg	E/G
Control	44	11.2±0.8	16.3±1.7	57.1±4.5	31.5±4.2	2.6±0.7	5.7±1.9	3.1±1.5	1.8
RAMBS	50	2.3±0.5	4.9±1.0	60.5±6.8	24.3±5.7	1.4±0.9	11.4±4.9	2.4±1.4	2.4

Legend. Mean number of colonies given per 10^5 bone marrow cells injected.

forming activity, has been partly characterized [5]. However, a final decision on this matter can be reached only after careful research, using pure cell populations.

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