EFFECT OF REGENERATION OF HEMATOPOIETIC ORGANS ON THE NUMBER AND TYPE OF SPLENIC COLONIES

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Two thirds of the spleen was resected or the bone marrow from one tibia was removed in CBA mice. On the 8th day after irradiation and injection of a suspension of intact spleen cells into the animals hematopoietic colonies were obtained and examined microscopically. In the animals of the experimental groups some increase was observed in the number of colonies compared with the control, and the number of granulocytic colonies was significantly higher although the number of colonies belonging to other hematopoietic series remained unchanged. The authors suggest that these changes may be due both to the local effect of the proliferating stroma of the spleen and to the action of a factor secreted by the regenerating stroma of the hematopoietic organs.

KEY WORDS: hematopoietic colonies; myeloid hematopoiesis; regeneration of the spleen; regeneration of the bone marrow.

It was shown previously that the number of hematopoietic colonies in the spleen increases significantly in mice with regenerating hematopoietic organs (spleen and bone marrow) [1]. This has led the writers to postulate that the regenerating stroma of hematopoietic organs influences the survival and proliferation of colony-forming units (CFU).

The object of the present investigation was to test the above hypothesis by a microscopic study of the number and types of hematopoietic colonies.

EXPERIMENTAL METHOD

Sexually mature male mice of the CBA strain weighing 18-20 g were used. Two thirds of the spleen was removed from the mice of one group [2] and the bone marrow from the femur or tibia, equivalent to 8.5% of the total mass of bone marrow of the mouse [6], was removed from mice of another group. Intact mice served as the control. To obtain hematopoietic colonies the method of Till and McCulloch was used [7]. The experimental and control animals were irradiated with x rays in a dose of 800-900 R (dose rate 50 R/min) on the RUM-15 apparatus 2 days after the operation. An intravenous injection of a suspension of intact spleen cells was given 3-6 h later to the mice in a dose of $1 \cdot 10^6$ nucleated cells per mouse. On the morning of the 8th day after injection of the cells the animals were killed and the spleens fixed with Carnoy's mixture, embedded in paraffin wax, and histological analysis of the colonies in a central section was carried out. The time of 8 days was chosen so as to avoid mutual overlapping of the colonies [8].

Sections 4-5 μ thick were stained with hematoxylin–eosin and methyl green–pyronine. The total number of colonies and the number of colonies of each type separately (erythroid, myeloid, megakaryocytic, and undifferentiated) were counted. The basic direction of differentiation was judged from the ratio between the numbers of erythroid and myeloid colonies (E/M). The results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

The results of histological analysis of the splenic colonies are given in Table 1. They show that the total number of hematopoietic colonies was a little higher (but not significantly) in the experiment with regenerating bone marrow (35.1 ± 2.6) than in the control (26 ± 4.9).

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TABLE 1. Number and Type of Hematopoietic Colonies in Mice (M ± m)

Group of animals	Number of animals	Number of colonies					E/M	Number of clusters of lympho-cytes
		total	E	M	Meg	Un		
Control (whole spleen)	5	26±4,9	15,4±2,43	6,4±0,67	0,5±0,39	3,7±1,59	2,4±0,18	15,4±1,6
Control (half of spleen)	15	13±2,73	7,7±1,21	3,2±0,34	0,25±0,05	1,85±0,79	—	7,7±0,8
Mice with regenerating spleen	12	19,1±1,25	6,9±0,45	10,3±2,8	0,6±0,2	1,3±0,5	0,73±0,027	10,6±1,04
Mice with regenerating bone marrow	9	35,1±2,6	13,0±0,6	18,6±1,44	0,4±0,2	3,1±1,24	0,71±0,018	15,4±1,7

Legend. E) Erythroid, M) myeloid, Meg) megakaryocytic, Un) undifferentiated colonies.

Analysis of the number of colonies belonging to the different types of hematopoiesis showed a significant increase (by 2.9 times) only in the number of myeloid colonies compared with the control. Not only their number, but also their size and degree of differentiation were changed. Myeloid colonies of small size consisting mainly of immature cells (promyelocytes and myelocytes), located mainly in the central parts of the red pulp, were characteristic of the control. In the experimental group large and well-differentiated colonies appeared, consisting mainly of metamyelocytes and stab cells; these colonies were usually located beneath the capsule, lifting it up; so that they could be detected macroscopically.

The E/M ratio in the control spleen was 2.4 ± 0.18, in good agreement with data in the literature [5]. In the experiment with regenerating bone marrow this ratio was changed to 0.71 ± 0.018.

It is difficult to compare the data for the number of hematopoietic colonies obtained for the regenerating spleen with the control series because, even by the time of sacrifice (the 11th day after the operation) the spleen had regenerated only to half (by weight) of the intact organ [1].

Wolf and Trentin [9], who transplanted eight syngeneic spleens into mice, showed that under these circumstances the same number of colonies was formed in their own spleen as in the control and that, in addition, a substantial number of colonies developed on the transplanted spleens. These workers accordingly consider that the number of colonies depends on the presence of a suitable substrate on which the stem cells can settle.

It thus follows that twice as many stem cells could settle on the whole spleen (in the control) as on the half-spleen (in the experiment). For that reason, an additional line is given in the Table – the number of colonies on half of the control spleen – with which the data obtained for the regenerating spleen can be compared. The total number of colonies in the regenerating spleen under these conditions was almost 1.5 times higher than in the control.

The number of myeloid colonies in the experiments (10.3) was 3 times greater than in the control (3.2). For all other types of colonies the differences between the number of colonies in the experimental and control series was not significant. Just as in the experiments with the regenerating bone marrow, among the myeloid colonies some were found which were very large, lying under the capsule of the spleen, where they could be counted macroscopically.

The E/M ratio in the regenerating spleen (0.73 ± 0.27) was considerably altered compared with the control (2.4 ± 0.18).

Both in the experimental and in the control series, besides hematopoietic colonies, clusters of lymphocytes also were observed; these were counted separately and the results are given in the last column of Table 1. On staining with methyl green–pyronine, besides lymphocytes, cells of blast type and plasma cells of different degrees of maturity also were found in the lymphoid clusters. Sometimes mitotically dividing cells of blast type also were found in the lymphoid clusters (the cells were identified from the size and shape of their mitotic plate).

The question arises: What is responsible for the increase in number of hematopoietic colonies, detectable both macroscopically and microscopically, during regeneration of the hematopoietic organs (spleen and bone marrow)?

The total number of colonies per spleen, counted microscopically, was 4 to 5 times greater than their number counted macroscopically, for megakaryocytic, undifferentiated, and most of the myeloid colonies can be detected only microscopically. On the other hand, macroscopic counting of the colonies [1] gave a greater increase in the number of colonies in the experimental series than microscopic counting compared with the

control. The number of colonies on the regenerating spleen was 2.6 times greater (macroscopically) than their number on the intact spleen, but only 1.5 times greater microscopically; during regeneration of the bone marrow the corresponding increases were 3.25 and 1.35 times. This disagreement between the results of macroscopic and microscopic counting was possibly due to the appearance of large myeloid colonies. The increase in the number of colonies on the regenerating stroma of the spleen can be explained by the direct action of excited proliferating reticular cells, stimulating the survival and proliferation of CFU.

The number of myeloid colonies in the experiments with the regenerating spleen increased by 3.2 times and in the experiments with bone marrow by 2.9 times.

During regeneration of the spleen and bone marrow stimulation of granulocytic hematopoiesis thus takes place, but evidently as a result of the activation of reserve CFU and not on account of other types of differentiation.

Because of the marked intensification of myeloid hematopoiesis, with no accompanying change in the erythroid series, the E/M ratio fell from 2.4 in the control to 0.73 and 0.71 in the experimental groups, to reach values characteristic of hematopoiesis in normal bone marrow (0.5-0.7) [9].

In the opinion of many workers [3, 7, 9], the direction of differentiation of hematopoietic stem cells is determined by the microenvironment, of which the principal component is evidently the stroma. It is perhaps on account of the appearance of proliferating stromal elements in the regenerating spleen that the increase in the number of zones of the microenvironment stimulating myeloid hematopoiesis takes place. On the other hand, regeneration of the bone marrow tissue has a similar action on the number and type of colonies, although in this case the CFU interact with the normal stroma of the spleen. These observations suggest the presence of a factor secreted by the regenerating stroma of the hematopoietic organs and possessing a distant action on the CFU.

It is difficult to say whether the direct action of the regenerating stroma and the distant effect of the factor are two different aspects of the same process or whether they are not interconnected, for as yet the mech anism of action of the microenvironment is unknown. It has been suggested that stromal cells act on the stem cells not by direct contact, but through a chemical factor with a very small radius of action secreted by them [6].

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