ORIGIN OF CELLS IN THE REGENERATING MOUSE SPLEEN (EXPERIMENTS WITH LABELED CHROMOSOMES)

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Previous investigations have shown that the spleen is capable of regeneration after resection of a considerable proportion of its tissue [1, 2]. It has not been finally established from which cells this organ regenerates—whether by proliferation of cells of the residual part of the spleen or whether by division of cells migrating to the organ from the peripheral blood and other hemopoietic organs.

Experiments on parabionts have shown that the chromosome-labeled cells of one partner can be found in the hemopoietic organs of the other partner (spleen, bone marrow, lymph glands, and thymus) [6]. Metaphase plates with markers can be detected in the recipient's tissues after transplantation of labeled bone marrow [3] or spleen [4] cells. The results of these investigations show that cells circulating in the blood stream in reparative regeneration of the spleen was studied.

EXPERIMENTAL METHOD

Mice of lines CBA and CBA-T6T6 were used in the experiment. Parabiosis was achieved by joining mice of different lines (aged 1.5 months) by means of a skin-muscle-body cavity anastomosis. Two-thirds of the spleen was resected from the CBA mice at operation. Three pairs of mice were sacrificed three days after the operation, and one pair each 8, 17, and 20 days after. Marker was found in the spleen by the following method [5]: pieces of normal and regenerating spleen were placed in 1% sodium citrate and incubated for 30 min at 37°. The suspension was centrifuged three times at 400-500 rpm for 3 min. The residue was fixed three times in fresh portions of fixing solution (3 parts of absolute methanol and 1 part glacial acetic acid). Cells suspended in a fresh portion of fixing solution were placed on cold slides and dried in the flame of a gas burner. The preparations were stained with azure—eosin. Colchicine was not used in the preparation of most specimens so that as far as possible the structure of the chromosomes would not be changed.

All metaphase plates in which all chromosomes could be clearly differentiated were counted (diploid number 40). The 39th and 40th chromosomes were marked in the CBA-T6T6 mice: they were much smaller than the other chromosomes and had the appearance of three dense points.

EXPERIMENTAL RESULTS

Three days after the operation the weight of the residual part of the spleen showed a slight increase. Twenty days after resection the weight of the regenerating spleen was 63 mg and that of the intact spleen of the other parabionts was 75 mg, so that good regeneration of the organ was observed. Regeneration took place as the result of regeneration hypertrophy [1, 2].

The results of counting the metaphase plates of the parabionts are given in the table.

The results given in the table show that at all times after the operation the parabionts were chimeras containing cells with qualitatively different sets of chromosomes. Blood cells, evidently lymphocytes, settled in the spleen and proliferated actively. The degree to which circulating cells participated in regeneration was directly dependent on the intensity of the reparative processes: during physiological regeneration only 1-2% of mitoses belonged to cells of the other parabiont. During reparative regeneration this value reached 11.3%, the number of dividing cells migrating from the second parabiont increasing progressively as the period of survival of the parabionts became longer. The percentage of dividing labeled cells in the regenerating spleen increased from 1.6 to 11.3 during the 17 days of the experiment.

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Number of Marked and Unmarked Cells in Spleen of Parabiont Mice

Day after operation	Number of parabiotic pairs	Metaphase plates in regenerating spleen			Metaphase plates in normal spleen		
		of CBA mouse	of CBA-T6T6 mouse	percent of marked plates	of CBA mouse	of CBA-T6T6 mouse	percent of marked plates
	1	173	5	2.8	2	90	2.1
3rd	1	158	3	1.8	–	101	–
	1	160	1	0.6	1	70	1.4
8th	1	178	7	3.8	1	89	1.0
17th	1	145	13	8.9	–	–	–
20th	1	150	17	11.3	3	172	1.7

Lymphoid, erythroid, and myeloid hemopoiesis take place in the mouse spleen. It could not be decided to which hemopoietic series the discovered metaphase plates belonged. Most of the cells destroyed during preparation of the specimens, in which mitoses could still be seen, were cells of blast type. They were large, round cells with large chromosomes. It may therefore be postulated that most of the metaphase plates belonged to cells of blast type.

The results described above show that lymphocytes circulating in the blood and repopulating the lymphoid tissue play an active part in regeneration of the spleen. The extent of this participation is much greater than that indicated by the percentage of labeled mitoses (up to 11%), because this index reflects the participation of lymphocytes of only the second partner in the process of regeneration, and not that of lymphocytes belonging to the animal undergoing the operation.

Many mitoses in the regenerating spleen were unlabeled. This suggests that cells of the parabiont undergoing the operation take part in regeneration, but it does not determine conclusively whether these cells originate from the circulating blood or from the part of the spleen remaining after resection. The role of the lymphoid and reticulum cells of the residual spleen in the process of regeneration remains unclear and requires further study.

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