# CHANGES IN THE NUMBER OF ANTIBODY-FORMING AND STEM CELLS IN THE SPLEEN OF UNILATERALLY NEPHRECTOMIZED MICE

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UDC 612.411.017.1-06:612.46-089.873

A suspension of spleen cells from intact and unilaterally nephrectomized mice, obtained in the latter case 19-21 h after the operation, was injected into lethally irradiated (CBA × C57BL/6) $F_1$ mice 24 h after irradiation. On the 8th day after injection of the cells the number of colony-forming and plaque-forming cells in the spleen of the irradiated recipients was determined. To determine the number of plaque-forming cells, a mixture of spleen cells and sheep's red cells was injected into the irradiated recipients. The number of colonies in the recipients' spleen 19-21 h after the operation either was unchanged or was significantly reduced. Stimulation of the production of antibody-forming cells was observed at these same times, and it coincided in time with the period of manifestation of the ability of the splenic lymphoid cells of the unilaterally nephrectomized mice to induce proliferation in the kidney of the intact recipients.

KEY WORDS: spleen; unilateral nephrectomy; colony-forming cells; plaque-forming cells.

Previous experiments showed that the splenic lymphocytes of partially hepatectomized mice become capable of stimulating proliferation in the liver of intact recipients [2] at the same time as production of antibody-forming cells in the spleen of the intact animals is stimulated [1, 4]. Stimulation of the production of antibody-forming cells synthesizing antibodies against sheep's red cells did not correlate with the change in the number of stem cells in the spleen of partially hepatectomized mice. Splenic lymphocytes of unilaterally nephrectomized mice 19-21 h after the operation are also known to have marked ability to stimulate proliferation in the kidney of intact recipients [3].

The next step was to discover to what extent the changes in the cell composition and function of the spleen in experiments with compensatory hypertrophy of the kidney follow the same pattern as those discovered in experiments with regeneration of the liver. Accordingly the number of antibody-producing and colony-forming cells or units (CFUs) in the spleen of unilaterally nephrectomized mice was studied 19-21 h after the operation, at the time of greatest stimulating activity of the splenic lymphocytes.

## EXPERIMENTAL METHOD

The experimental animals were 119 male (CBA × C57BL/6) $F_1$ mice from the Stolbovaya nursery of the Academy of Medical Sciences of the USSR.

The donor mice were sacrificed 19-21 h after unilateral nephrectomy. A cell suspension was prepared from the spleen of 3-7 nephrectomized mice (experimental group) or of intact animals and mice undergoing a mock operation (control group). The spleen cells were injected intravenously into the lethally irradiated recipients 24 h after irradiation. The recipients were irradiated on the Stebel' 3A apparatus in a dose of 900 R (dose rate 900 R/min; $LD_{100/13}$). To determine the number of CFUs, spleen cells were

Laboratory of Experimental Genetics, Institute of Medical Genetics, Academy of Medical Sciences of the USSR. Laboratory of Growth and Development, Institute of Human Morphology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR, N. A. Fedorov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 78, No. 9, pp. 69-72, September, 1974. Original article submitted November 29, 1973.

TABLE 1. Number of Colonies and Antibody-Forming Cells in the Spleen of Irradiated Mice Receiving an Injection of Spleen Cells Obtained from (CBA × C57BL/6) $F_1$ Mice 19-21 h after Unilateral Nephrectomy (M ± m)

| Group of recipients | No. of expt. | No. of mice | Number of colonies in spleen | P | No. of mice | Number of plaques | P |
|---|---|---|---|---|---|---|---|
| Control | | 10 | 8,9±1,4 | | — | Not determined | — |
| Control (suspension from donors undergoing mock operation) | 1 | 9 | 6,8±1,2 | 0,28 | — | " " | — |
| Experimental | | 16 | 4,7±0,7 | 0,003 | — | " " | — |
| Control | 2 | 7 | 12,0±1,3 | — | 7 | 1730±446 | |
| Experimental | | 9 | 11,1±0,6 | | 20 | 7750±600 | 0,0001 |
| Control | | — | Not determined | | 8 | 3117±180 | |
| Control (suspension from donors undergoing mock operation) | 3 | — | " " | — | 10 | 2963±323 | 0,1 |

injected in a dose of $1 \times 10^6$. On the 8th day after injection of the cells the number of colonies in the recipients' spleen was determined by the method of Till and McCulloch [6]. To determine the number of antibody-forming cells, spleen cells of unilaterally nephrectomized mice were injected into the recipients in a dose of $1 \times 10^7$ mixed with $2 \times 10^8$ sheep's red cells. The recipients were killed on the 8th day and the number of plaque-forming cells in the spleen determined by Jerne's method [5]. Irradiated mice injected with spleen cells of intact mice or mice undergoing the mock operation, mixed with sheep's red cells, acted as the control. The number of spontaneous colonies of hematopoietic cells in the spleen of irradiated mice receiving no injections averaged 0.4.

The significance of differences between the numerical values was determined by the Fisher–Student method.

## EXPERIMENTAL RESULTS

The results of the calculation showed that the properties of the lymphocytes in the spleen changed during compensatory hypertrophy of the kidney (Table 1). This change was clearly revealed 19-21 h after unilateral nephrectomy. The number of antibody-forming cells in the spleen of the recipients receiving a suspension of spleen cells of unilaterally nephrectomized donors mixed with sheep's red cells was more than four times greater than in the control ($P = 0.0001$). The question whether any operation might be followed by changes of this type in the properties of the lymphocytes had next to be considered. A special series of experiments showed that the mock operation did not give rise to any significant changes in the antibody-forming capacity of the mouse spleen 19-21 h after trauma. The number of antibody-forming cells in the recipients of the suspension of spleen cells from mice undergoing the mock nephrectomy was virtually indistinguishable from their number in the recipients of a suspension of spleen cells from intact mice (Table 1, Expt. No. 3).

The changes in the number of stem cells in the spleen of the unilaterally nephrectomized mice varied in type. In one experiment the number of stem cells in recipients of the spleen cells of the nephrectomized mice did not differ significantly from the control, but in another experiment it was significantly lower than in the control recipients ($P = 0.003$). On the whole the changes in the number both of antibody-forming cells and of stem cells in the spleen of the animals after unilateral nephrectomy were similar with the changes observed in the corresponding experiments in which partially hepatectomized mice were used as donors [1, 4]. The operation on the kidney, like the operation on the liver, was accompanied by an increase in the antibody-forming capacity of the spleen in response to injection of sheep's red cells at times after the operation at which the ability of the spleen cells of mice undergoing the operation to stimulate proliferative processes in intact recipients is increased. Just as after the operation on the liver, no correlation could be detected between the ability of the lymphocytes to transmit "regeneration information" and the change in the number of stem cells in the spleen of the unilaterally nephrectomized animals.

Since the mock operation caused no change in the antibody-forming properties of the spleen, removal of the tissue of an organ must therefore stimulate this specific action of the antigen (sheep's red cells).

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