# APPEARANCE OF FOCI OF HEMATOPOIESIS AND OF HEMATOPOIETIC STEM CELLS IN THE MOUSE LIVER AFTER A SINGLE INJECTION OF CYCLOPHOSPHAMIDE

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On the 6th day after a single intraperitoneal injection of cyclophosphamide in a dose of 200 mg/kg body weight into mice of lines DBA/2, C57BL/6j, and $F_1$(C57BL/6j × CBA) foci of hematopoiesis appeared in the liver and lymph glands. This process was accompanied by the appearance of hematopoietic stem cells in the liver. Their number reached a maximum on the 6th and 9th days. On the 12th day their number fell, but it was still higher than in animals receiving an injection of a suspension of normal liver cells. After exhaustion of the pool of bone marrow and spleen stem cells by myleran, a subsequent injection of cyclophosphamide did not induce the appearance of foci of hematopoiesis and stem cells in the liver.

KEY WORDS: cyclophosphamide; myleran; focus of hematopoiesis; stem cells.

Alkylating agents, which are widely used in the chemotherapy of tumors, have a marked cytostatic action on hematopoietic cells also. Soon after being strongly inhibited, hematopoiesis starts to recover and the process is accompanied by marked hyperproduction of myeloid tissue and an increase in the number of hematopoietic stem cells in the bone marrow, spleen, and peripheral blood [3, 5-7, 9]. During this period hematopoietic stem cells also are found in the lymph glands [1, 2] and liver [9].

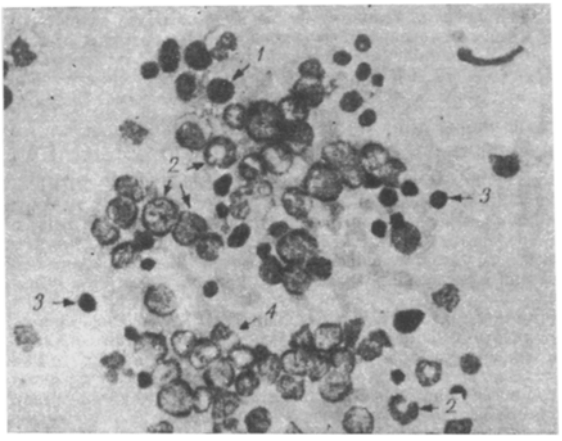


Fig. 1. Focus of hematopoiesis in mouse liver. Azure-eosin, 200 ×: 1) nuclei of hepatocytes; 2) cells of myeloid series; 3) cells of erythroid series; 4) plasma cell.

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TABLE 1. Cell Composition of Liver of Different Lines of Mice 6 days after Receiving an Injection of Cyclophosphamide (based on 500 cells)

| Line of mice | Group of animals | Promyelocytes | Myelocytes | Metamyelocytes | Neutrophils | | Blast cells | Reticulum cells | Erythroid cells | Lymphocytes | Monocytes | Hepatocytes |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | stab cells | polymorphs | | | | | | |
| DBA/2 | Exptl. ($n$=10) | 13,0±3,9 | 7,8±2,9 | 5,8±2,5 | 16,1±2,8 | 9,0±1,5 | 6,0±0,9 | 5,8±1,0 | 94,0±16,8 | 29,5±2,9 | 1,4±0,5 | 303,5±18,6 |
| | Control ($n$=14) | — | — | — | 0,8±0,2 | 9,9±0,9 | — | 3,7±0,9 | 0,2±0,2 | 34,5±3,8 | — | 449,3±4,6 |
| | $P$ | | | | <0,001 | >0,617 | | >0,110 | <0,001 | >0,317 | | |
| C57BL/6j | Exptl. ($n$=10) | 9,2±2,2 | 10,3±2,8 | 7,6±1,8 | 16,9±2,2 | 33,6±8,6 | 8,4±2,2 | 13,1±2,6 | 72,1±18,1 | 34,9±6,9 | 7,0±1,8 | 271,6±30,2 |
| | Control ($n$=10) | — | — | — | 0,1±0,1 | 6,4±1,0 | 0,2±0,1 | 4,1±0,8 | 0,1±0,1 | 46,7±3,4 | 0,4±0,2 | 439,10±4,9 |
| | $P$ | | | | <0,001 | =0,006 | =0,002 | =0,004 | <0,001 | =0,152 | =0,003 | |
| $F_1$ hybrids C57BL/6j ×CBA | Exptl. ($n$=10) | 0,5±0,1 | 2,9±0,6 | 1,2±0,3 | 18,3±2,6 | 25,8±3,2 | — | 0,8±0,5 | 35,8±13,2 | 24,6±3,5 | 6,8±1,0 | 383,3±21,9 |
| | Control ($n$=7) | — | — | — | 2,0±1,1 | 7,6±2,0 | — | 1,1±0,4 | 4,1±1,1 | 63,7±13,3 | 2,7±0,6 | 418,7±17,7 |
| | $P$ | | | | <0,001 | <0,001 | | <0,625 | <0,028 | <0,015 | <0,005 | |

TABLE 2. Number of CFU in Liver of $F_1$ Hybrid (C57BL/6j × CBA) Mice at Various Times after Injection of CP

| Expt. No. | Time of investigation of liver (days) | No. of recipients | No. of liver cells injected | Mean number of colonies | Level of significance compared with number of | |
|---|---|---|---|---|---|---|
| | | | | | endocolonies | colonies in "intact liver" group |
| | | | | | P | |
| 1 | 4 | 9 | $2\times10^6$ | 1,8±0,7 | >0,7 | <0,02 |
| | 6 | 9 | $2\times10^6$ | 33,2±2,3 | <0,001 | <0,001 |
| | 9 | 5 | $1\times10^6$ | 30,0±1,1 | <0,001 | <0,001 |
| | 12 | 10 | $2\times10^6$ | 8,0±0,9 | <0,001 | =0,004 |
| | Intact liver | 10 | $2\times10^6$ | 5,0±0,9 | =0,02 | — |
| | Endocolonies | 9 | — | 2,2±0,7 | — | — |
| 2 | 6 | 7 | $6\times10^6$ | Confluent | — | — |
| | Intact liver | 5 | $6\times10^6$ | 6,2±1,3 | =0,007 | — |
| | Endocolonies | 6 | — | 0,2±0,2 | — | — |

In the investigation described below the dynamics of the number of stem cells and the cell composition of liver tissue after injection of cyclophosphamide (CP) were studied.

## EXPERIMENTAL METHOD

Female mice of lines C57BL/6j and DBA/2 and $F_1$(C57BL/6j × CBA) hybrids weighting 18-22 g were used. CP was dissolved in bidistilled water in a concentation of 20 mg/ml and injected intraperitoneally in a dose of 200 mg/kg body weight. The animals were decapitated 6 days after the injection of the compound and squash preparations were made of the liver and mesenteric lymph glands, fixed in methanol for 30 min, and stained with azure-eosin. In preparations from the liver 500 cells were identified, and 1000 cells in those from the lymph glands.

To determine the number of colony-forming units (CFU) pieces of liver were washed with medium No. 199 containing a standard concentration of penicillin and streptomycin, minced in a glass homogenizer, and filtered through four layers of gauze and three layers of kapron. The number of living nucleated cells was counted in 1 ml of cell suspension and between $2\times10^6$ and $6\times10^6$ cells were injected intravenously into syngeneic recipients 24 h after lethal irradiation. On the 8th day after injection of CP the spleens of the recipients were fixed in Bouin's solution and the number of colonies was counted [8]. A suspension of myleran in 3% gelatin was injected via gastric tube into the C57Bl/6j mice in a dose of 150 mg/kg body weight. The $\alpha$-fetoprotein (AFP) in the sera of the mice was determined by the microprocipitation test in agar by the method used in Abelev's laboratory [14]. The test system for determination of AFP was kindly provided by Dr. A. K. Yazova.

## EXPERIMENTAL RESULTS

The cell composition of the liver after the injection of CP was studied in three lines of mice (Table 1). Myeloid cells at different stages of maturity were found in the mice of all three lines (Fig. 1). The total number of proliferating cells of the neutrophil series was about the same in the DBA/2 and C57BL/6j mice and a little lower in the $F_1$ hybrid. These cells were completely absent from intact mice. Mature neutrophils were found in the animals of all groups, including the control, but their level was much higher in the experimental groups.

Besides the myeloid cells, squash preparations of the liver of the experimental mice contained many cells of the erythroid series, mainly normoblasts. Preparations from the liver of the experimental and control animals also contained reticular cells, lymphocytes, and monocytes. The foci of hematopoiesis in preparations of the mesenteric lymph glands consisted chiefly of myeloid cells.

The results of determination of CFU in the liver of the $F_1$(C57BL/6j × CBA) hybrids receiving CP are given in Table 2. In the first experiment the lethally irradiated syngeneic recipients were given an intravenous injection of $6\times10^6$ liver cells. The colonies were confluent in character in all seven recipients. In the second experiment, to determine the precise number of CFU and the dynamics of their appearance a suspension of liver cells was injected into irradiated recipients in a dose of $2\times10^6$ at various times after the injection of CP.

On the 4th day after the injection of CP the number of CFU did not exceed the level of endocolonies in the control recipients, but on the 6th day it rose sharply, it remained at that level until the 9th day, and it had not fallen completely by the 12th day. The number of CFU in the liver at all these times, starting from the 6th day, was considerably higher than in the lymph glands [1].

To study the origin of the foci of hematopoiesis in the mouse liver experiments were carried out with myleran, a selective inhibitor of myeloid hematopoiesis and of proliferation of hematopoietic stem cells. Altogether 32 C57BL/6j female mice were used, 15 of which died during the first few days after the injection of myleran. Five days after injection of the lethal dose of myleran 9 mice were given an intraperitoneal injection of 200 mg/kg CP; 6 animals which survived until the 6th day after injection of CP were killed in a moribund state and were investigated morphologically and for the presence of CFU in the liver and spleen. Microscopic examination showed depopulation of the bone marrow, absence of myeloid and erythroid cells in the spleen, and a complete absence of nucleated cells in the blood films. In squash preparations of the liver no traces of hematopoiesis could be found. Similar results were obtained by the investigation of animals receiving myleran alone. After injection of a suspension of liver and spleen cells of both groups of mice into lethally irradiated syngeneic recipients, no sign of colony formation was observed in their spleen.

It can be concluded from these preliminary data that foci of hematopoiesis are formed by cells which migrate from the hematopoietic organ (bone marrow or spleen). When these organs are inhibited by myleran, CP cannot induce extramedullary hematopoiesis. No AFP could be found in the sera of the mice receiving cyclophosphamide.

## LITERATURE CITED


1. D. A. Bodyagin, "Reaction of the lymph glands of mice with tumors to injection of sarcolysin," Author's Abstract of Candidate's Dissertation, Moscow (1972), p. 22.
2. D. A. Bodyagin and I. A. Svet-Moldavskaya, "Hematopoietic stem cells in the lymph glands of mice during myeloid metaplasia of lymphoid tissue induced by sarcolysin," Farmakol. i Toksikol., No. 3, 349 (1973).
3. S. K. Germane, "The effect of cyclophosphamide on hematopoiesis. Communication III," in: Cyclophosphamide [in Russian], Riga (1965), p. 67.
4. A. I. Gusev and V. S. Tsvetkov, "Technique of the microprecipitation reaction in agar," Lab. Delo, No. 2, 43 (1961).
5. A. A. Zidermane et al., "The effect of cyclophosphamide on hematopoiesis. Communication I," in: Cyclophosphamide [in Russian], Riga (1965), p. 51.
6. M. A. Yumasheva et al., "Morphological and functional changes arising in the spleen of mice under the influence of cyclophosphamide," Byull. Éksperim. Biol. i Med., No.5, 54 (1973).
7. W. Fried and C. Johnson, "The effect of cyclophosphamide on hematopoietic stem cells," Radiat. Res., 36, 521 (1968).
8. J. E. Till and E. A. McCulloch, "A direct measurement of the radiation sensitivity of normal mouse bone marrow cells," Radiat. Res., 14, 213 (1961).
9. S. L. Scaro et al., "Hematopoietic recovery after a single high dose of cyclophosphamide in the mouse," Acta Physiol. Lat.-Amer., 22, 258 (1972).