HEMATOPOIETIC STEM CELLS IN THE INTACT AND REGENERATING ADULT MOUSE LIVER

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It was shown by the splenic colonies method that hematopoietic stem cells are present in the intact liver of adult CBA mice. Their number increases during regeneration of the liver after partial hepatectomy.

KEY WORDS: *hematopoietic stem cells*; *intact and regenerating liver*; *splenic colonies*.

Hematopoiesis in the mammalian liver takes place in the embryonic and early postnatal periods but ceases completely in adults [1, 6]. However, some qualification is perhaps required of this generally accepted state of affairs. Reports have recently been published that hematopoietic cells capable of giving rise to colonies in agar cultures and in the spleen of lethally irradiated mice are present in the intact and also the regenerating mouse liver [4, 5].

It was these observations which motivated the present investigation.

EXPERIMENTAL METHOD

Adult (weight 22-24 g) male CBA mice were used. The recipient mice were irradiated in a dose of 800 rad, which practically completely suppresses the formation of endogenous colonies in the spleen. The conditions of irradiation were: RUM-17 apparatus, 190 kV, 15 mA, filters 0.5 mm Cu + 0.75 mm Al, dose rate 55 rad/min. The number of hematopoietic stem cells (CFUs) was determined by counting macroscopic colonies in the spleen 9 days after irradiation and injection of a suspension of the test cells. The following cells were tested: 1) from intact liver, 2) from regenerating liver (4, 6, and 11 days after removal of two-thirds of the organ), 3) from bone marrow of intact animals, 4) from bone marrow of animals with a regenerating liver, 5) from regenerating liver of animals (4 days after removal of two-thirds of the liver) irradiated in a dose of 1500 rad before sacrifice, 6) from the bone marrow of these same animals, 7) from the kidney, 8) peripheral blood leukocytes.

A cell suspension was prepared from the intact and regenerating liver as follows. Usually to remove blood the liver was perfused in situ with 40 ml of medium No. 199. The tissue was then cut into pieces measuring 1-2 mm and washed 3 times with a large volume of medium No. 199. The pieces were placed in warm 0.25% trypsin (Difco) solution in medium No. 199 and dispersed on a magnetic mixer for 12 min at 37°C. The fragments were trypsinized twice or three times until they had completely dissolved. The supernatant was collected, filtered through 3 layers of Kapron filter, and centrifuged for 5 min at 1000 rpm. The residue was suspended in medium No. 199 and again filtered and centrifuged. The developing residue was diluted with medium No. 199 and the concentration of nucleated cells (except hepatocytes) was counted. To obtain a cell suspension tissue from 5 to 7 donors usually was used. Under these circumstances the maximal number of cells obtained was $30 \cdot 10^6$.

A suspension was prepared in the same way from the decapsulated kidney.

To prepare a suspension of peripheral blood leukocytes the heparinized blood from 5 to 7 donors was obtained by perforation of the orbit. The blood collected was diluted with an equal volume of medium No. 199 and centrifuged for 5 min at 3000 rpm. The residue was diluted in medium No. 199 and the concentration of nucleated cells was counted.

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TABLE 1. Number of Colonies in Spleen of Lethally Irradiated CBA Mice after Syngeneic Transplantation of Suspensions of Liver Cells

Source of transplanted cells	No. of recipients	Number of cells injected	Number of colonies (M ± m)
Intact liver	40	$5 \cdot 10^5$	9,4±0,6
Regenerating liver			
4 days	14	$1,5 \cdot 10^5$	7,9±1,2
4 "	20	$5 \cdot 10^5$	18,1±1,2*
4 "	12	$1,5 \cdot 10^6$	Confluent growth
6 "	17	$5 \cdot 10^5$	23,8±1,2*
11 "	20	$5 \cdot 10^5$	20,3±0,7*

*$P < 0.001$ compared with intact animals.

TABLE 2. Number of Colonies in Spleen of Lethally Irradiated CBA Mice after Syngeneic Transplantation of $0.5 \cdot 10^5$ Bone Marrow Cells

Source of bone marrow cells	Number of colonies (M ± m)
Intact mice	10,0±0,9
Mice at various times after partial hepatectomy	
4 days	8,4±0,9
6 "	25,0±1,1*
11 "	11,0±1,7

*$P < 0.001$ compared with intact animals.

A suspension of bone marrow cells was obtained by passing medium No. 199 repeatedly through the diaphyseal cavity of the femur.

The cell suspensions were injected intravenously 1-4 h after irradiation.

For histological analysis of the tissue used to prepare the cell suspension, pieces of the right lobe of the donors' liver were fixed. The recipients' spleens were fixed in formalin–alcohol–acetic acid. To study sections of the spleen and liver and the suspensions of injected cells the ordinary histological methods were used.

In some experiments the liver was screened. A lead plate 6 mm thick shielded the central and right lateral lobes of the liver from irradiation. Control (10 animals) and partly screened mice (14 animals) were irradiated simultaneously in a dose of 800 rad. The number of endogenous colonies in their spleen was determined 9 days after irradiation.

EXPERIMENTAL RESULTS

The experiments showed that hematopoietic stem cells are present in the intact and regenerating liver of adult CBA mice (Table 1). The concentration of CFUs in the intact liver was 1-2 per 10^5 nonparenchymatous cells. Partial hepatectomy appreciably increased the number of CFUs in the liver tissue. The concentration of CFUs in the liver 4, 6, and 11 days after the beginning of regeneration was considerably greater than in intact animals. Microscopic analysis showed that injection of a cell suspension from either normal or regenerating liver led to the formation of colonies of all types — erythroid, granulocytic, and megakaryocytic — in the spleen.

When the results are assessed and compared with data in the literature, differences in the method used to prepare suspensions of liver cells must be taken into account. Trypsinization of the tissue led to destruction of virtually all the hepatocytes. Their number in the suspension did not exceed 5%. Preliminary perfusion of the organ and rinsing the pieces of liver removed the peripheral blood cells. The chief components of the cell suspension obtained

in these experiments were lymphocytes (50-70%), macrophages, and monocytes (15-40%). There were not more than 1-3% of granulocytes. On microscopic examination of the normal and regenerating liver from which the suspension was obtained, no foci of hematopoiesis could be detected in either case.

Subsequent experiments were aimed at determining the nature (original) of the CFUs found in the liver. However, it was first necessary to rule out the possibility of a nonspecific stimulating action of transplantation of a large number of nonhematopoietic cells of the regeneration of endogenous CFUs. For this purpose, a suspension of cells from the kidney, and also from the regenerating liver and bone marrow, the CFUs of which were known to be inactivated by irradiation in a dose of 1500 rad was injected into irradiated recipients. No increase in the intensity of endogenous colony formation was observed in these control experiments.

Hematopoietic stem cells of the peripheral blood filling the liver could serve as the source of CFUs. However, this hypothesis was not confirmed. Preliminary removal of the blood by perfusing the organ with medium No. 199 did not affect the number of CFUs in the liver. Furthermore, the concentration of CFUs in the suspension of peripheral blood leukocytes was considerably lower than in the suspension obtained from the regenerating or intact liver.

Convincing proof of the presence of hematopoietic stem cells in the liver could be provided by the experiments with screening of the organ. Screening of the liver in these experiments led to a sharp increase in the intensity of endogenous colony formation. However, the fact that part of the bone marrow of the ribs inevitably lies in the screened region makes it difficult to interpret these observations.

From the remarks made above it can be concluded that hematopoietic stem cells are present in the liver of adult mice and that their number is increased during regeneration of the organ.

A very important question still remains: On account of what does the number of CFUs in the regenerating liver increase? Is it the local population of hematopoietic stem cells or are all the CFUs in the liver emigrants from the hematopoietic organs (bone marrow etc.). In the first case proliferation of hematopoietic stem cells actually in the liver during its regeneration can be postulated, and in the second case additional migration of these cells from outside. No direct experimental data confirming either of these hypotheses were obtained. The parallel increase in the number of CFUs in the regenerating liver and bone marrow (Table 2) points indirectly to the possibility of stimulation of migration of hematopoietic stem cells into regenerating liver tissue.

The functional significance of the local hematopoietic stem cells of liver likewise remains unexplained. It has been suggested [5] that the CFUs of the liver serve as the source for the formation of new Kupffer cells (the liver macrophages). It would also be interesting to study the role of the local stem cells during the appearance of extramedullary foci of hematopoiesis in the liver, which is rarely observed under pathological and experimental conditions [2, 3, 5].

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