ABILITY OF INTACT AND REGENERATING LIVER TO TRAP CFUs

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UDC 616.36-003.93-07:616.36-003.971

KEY WORDS: liver; partial hepatectomy; regeneration; CFUs; macrophages.

The number of clonogenic hematopoietic cells (CFUs) has been shown to be increased in the liver regenerating after partial hepatectomy [1, 3, 4]. The exact origin of these CFUs is unknown. The possibility cannot be ruled out that the liver can trap extrahepatic, circulating CFUs and that, under regeneration conditions, this ability is enhanced. The investigation described below was undertaken to test this hypothesis.

EXPERIMENTAL METHOD

Two-thirds of the liver was removed from male CBA, C57Bl/6, and F_1 hybrid mice aged 3 months, under ether anesthesia. Control animals underwent a mock operation (laparotomy followed by suture of the wound). After 3 days the hepatectomized and control animals were irradiated in a dose of 750 R (7.5 Gy) to suppress endogenous CFUs, and $2 \cdot 10^7$ and $4.5 \cdot 10^7$ bone marrow cells from F_1 hybrids were transplated intravenously into them. It was first shown that the increase in the CFUs concentration in the liver takes place 3-5 days after partial hepatectomy. The mice acting as primary recipients were killed (at least five at each time) 2 and 24 h after transplantation of suspensions of bone marrow cells and the concentration of CFUs in their liver was determined by the method in [8]. For this purpose, secondary recipients (only F_1 hybrids, females) received an intravenous injection of 50 mg of liver cells, washed in Eagle's medium, in the form of a suspension. Some of the primary recipients received an intravenous injection of 4 mg of quartz particles (DQ-12, from West Germany, particle size about 3 μm) 24 h before irradiation and transplantation of bone marrow cells; quartz has a specific toxic action on macrophages [2, 7]. The quartz particles were injected together with Hanks' solution. Other animals received Hanks' solution alone.

EXPERIMENTAL RESULTS

Both the intact and the regenerating liver is able to trap CFUs for a short time, and this ability is enhanced in the regenerating liver (Table 1). Irrespective of the number of transplanted bone marrow cells ($2 \cdot 10^7$ or $4.5 \cdot 10^7$), the CFUs-trapping ability of the liver was about equal and, consequently, it was limited under these experimental conditions. After 24 h the concentration of CFUs was reduced, but significant differences still remained between the intact and regenerating liver in most experiments. The CFUs-trapping ability of the liver also was exhibited relative to semiallogeneic CFUs, i.e., in $F_1 \rightarrow$ CBA and $F_1 \rightarrow$ C57Bl/6 combinations. When unirradiated primary recipients were used this ability was the same as in the irradiated recipients, at least after 2 h.

The use of quartz particles, with a toxic action on macrophages, inhibited this ability.

The regenerating liver of irradiated and unirradiated mice thus acquires enhanced ability, compared with the intact liver, to take up and (or) retain exogenous (transplanted) CFUs; this ability, moreover, is determined not by the number of transplanted CFUs, but by tissue or organ conditions of the liver that are sensitive to the action of quartz particles.

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Laboratory of Histogenesis, N. K. Kol'tsov Institute of Developmental Biology, Academy of Sciences of the USSR, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 98, No. 9, pp. 344-345, September, 1984. Original article submitted January 20, 1984.

 0007-4888/84/9809-1256$08.50

TABLE 1. Ability of Liver to Trap Exogenous CFUs 3 Days after Partial Hepatectomy or Mock Operation (M ± m)

Expt.	Primary recipients underwent	Line of mice	Number of bone-marrow cells transplanted × 10^7	Number of CFUs after 2 h	P	Number of CFUs after 24 h	P
1	Hepatectomy	F_1	4,5	10,00±0,90 (9)	<0,001	3,10±0,50 (11)	<0,01
	Mock operation			4,20±0,40 (8)		1,50±0,30 (10)	
2	Hepatectomy	F_1	2,0	8,67±0,51 (12)	<0,001	0,60±0,21 (10)	>0,1
	Mock operation			2,40±0,34 (10)		0,60±0,21 (10)	
3	Hepatectomy	F_1	2,0	12,70±0,70 (13)	<0,001	2,10±0,30 (15)	<0,01
	Mock operation			3,80±0,44 (10)		0,80±0,20 (15)	
4	Hepatectomy	F_1	2,0	—	—	1,60±0,40 (9)	<0,02
	Mock operation			—		0,30±0,10 (9)	
5	Hepatectomy	C57B *l*/6	2,0	18,85±1,02 (13)	<0,001	—	—
	Mock operation			6,83±0,62 (12)			
6	Hepatectomy	C57B *l*/6	2,0	20,42±0,69 (12)	<0,01	1,75±0,31 (8)	<0,01
	Mock operation			9,08±0,52 (12)		0,78±0,15 (9)	
7	Hepatectomy	CBA	2,0	6,58±0,47 (12)	<0,01	1,56±0,35 (9)	<0,01
	Mock operation			2,80±0,62 (10)		0,78±0,20 (9)	
8	Hepatectomy	CBA	2,0	—	—	1,08±0,09 (12)	>0,05
	Mock operation			—		1,88±0,29 (8)	
9	Hepatectomy	F_1	2,0	3,50±0,43 (10)	<0,01	—	—
	Mock operation			0,71±0,28 (7)	<0,01	—	
10	Hepatectomy	F_1	2,0	2,64±0,24 (11)	<0,001	—	—
	Mock operation			0,70±0,20 (10)	<0,02	—	

Legend. Number of secondary recipients shown in parentheses. In experiments Nos. 9 and 10 the primary recipients received an injection of quartz particles 24 h before irradiation and transplantation of bone marrow cells; the numerical values obtained were compared with the corresponding data for experiments Nos. 2 and 3. The number of endogenous colonies in irradiated mice injected with Eagle's medium or a suspension of liver cells from irradiated animals did not exceed 0.1-0.2 per spleen (38 animals).

The increase in the relative number of CFUs in the regenerating liver cannot be explained by their multiplication, for 2 h was clearly insufficient for that purpose. In addition, after 24 h the value of this parameter fell. An additional experiment showed that their number remained low even 7 days after transplantation, and was about the same in the intact and regenerating organs: 3.80 ± 0.39 (n = 10) and 3.91 ± 0.44 (n = 11) respectively. A change in the conditions of the microcirculation can be proposed as one factor trapping CFUs. However, the role of this factor is evidently not significant and it cannot be estimated. The results of the present experiments and data in the literature provide no grounds for ascribing an important role to this factor. Finally, fixation of exogenous CFUs in the liver can be regarded as a manifestation of their interaction with macrophages (Kupffer cells). In other words, the CFUs-retaining capacity of the liver is based on a concrete cellular mechanism. This, in our opinion, the most probable suggestion is supported by the fact that the CFUs-retaining capacity was considerably inhibited by quartz particles, which have a specific toxic action on macrophages. The arguments put forward can also be supported by data in the literature. It has been shown that processes of Kupffer cells which project into the lumen of sinusoidal capillaries sometimes make contact with hematopoietic cells [5]. The increase in the CFUs concentration which we observed in the regenerating liver also coincides in time with hyperplasia and hypertrophy of the Kupffer cells [6].

The intact and, in particular, the regenerating liver can thus trap circulating CFUs, and inhibition of macrophages (Kupffer cells) reduces this ability drastically. Although it is not clear whether this indicates direct interaction between macrophages and CFUs or whether inactivation of macrophages involves a change in certain other factors of the microenvironment directly controlling the retention of CFUs in the liver. This is a problem for further research.

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SELF-MAINTENANCE CAPACITY OF HEMATOPOIETIC CFUs IN THE LATE STAGES AFTER LONG-TERM IRRADIATION

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UDC 616.419-001.29-092.9-07:616.419-003.971-018.15

KEY WORDS: colony-forming units; spleen; irradiation.

Long-term exposure to harmful factors such as ionizing radiation or cytostatic agents leads to an irreversible decrease in the stem-cell pool of the hematopoietic system [2, 9] and also, perhaps, of other cell renewal systems. The mechanism of this residual damage is not yet clear. The size of the stem-cell population is determined primarily by the self-maintenance capacity of these cells [4, 6]. It has been shown that this unique property of polypotent CFUs (units forming colonies in the spleen) may be damaged as a result of chronic irradiation [5, 10].

The aim of this investigation was to study the ability of mouse bone marrow CFUs to maintain their own population in the late stages after long-term external irradiation.

EXPERIMENTAL METHOD

CBA mice aged 9-11 weeks were irradiated daily on an experimental ^{137}Cs γ-ray source in a dose of 0.5 Gy (exposure dose rate $0.34 \cdot 10^{4}$-$0.44 \cdot 10^{-4}$ A/kg) up to a total dose of 10 Gy. The number of CFUs in the bone marrow of the irradiated mice was determined by the exocolonization method [11]; the recipients were irradiated on an ÉGO-2 apparatus in a dose of 9.2 Gy. The self-maintenance capacity of the CFUs was determined by the number of CFUs in individual colonies formed by stem cells of the animals studied [8, 12]. For this purpose, a cell suspension was prepared from the bone marrow of mice of the experimental and control groups and injected into lethally irradiated recipients in a dose of 8-9 CFUs per mouse. After 10 days 12 to 15 colonies were isolated from the spleens of these primary recipients, and each was carefully freed from surrounding tissue. A cell suspension was prepared from the contents of each colony in 0.6-0.8 ml of medium, and this was injected in equal amounts into three or four lethally irradiated secondary recipients. Nine days later the number of colonies growing on the surface of their spleens was counted, and the number of CFUs in each separate colony was determined. Self-maintenance capacity of the CFUs was studied 3, 6, and 12 months after irradiation in a total dose of 10 Gy. Three repetitions of the experiments were done at each time of the investigation, and three donors (experimental mice and intact mice of the same age) and 10 to 12 primary recipients were used in each of them. Ability of CFUs of the bone marrow to maintain their own population was compared in the experimental and control animals, and also in mice of the different experimental groups, by calculating the mean number of CFUs per colony and their distribution among the colonies studied.

In a separate series the proliferative activity of bone marrow CFUs was studied during and after long-term irradiation. The number of CFUs in the period of DNA synthesis was found with the aid of [^{3}H]thymidine with high specific activity, as described previously [3]. To determine the percentage of CFUs taking part in mitosis, three or four experiments were carried out at each time of the investigation.

The results were subjected to statistical analysis by Student's t test ($P \leq 0.05$).

EXPERIMENTAL RESULTS

Daily irradiation of CBA mice in a total dose of 10 Gy led to a statistically significant decrease in their mean life span (624 ± 24 days compared with 749 ± 34 days in the control; $P < 0.05$). Pools of morphologically

(Presented by Academician of the Academy of Medical Sciences of the USSR R. V. Petrov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 98, No. 9, pp. 346-348, September, 1984. Original article submitted November 23, 1983.

 0007-4888/84/9809-1258$08.50