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PRECURSOR CELLS OF FIBROBLASTS DETECTED BY in vitro CLONING OF CELLS FROM HEMATOPOIETIC ORGANS OF NORMAL AND IRRADIATED MICE

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Colonies of fibroblasts are formed in monolayer cultures of bone marrow, spleen, and thymus cells of adult mice with an efficiency of colony formation (per 10^5 cells) of 2.2 for bone marrow, 0.20 for spleen, and 0.16 for thymus. On irradiation of mice with a dose of 150 R, about half of the fibroblast colony-forming units in the bone marrow die; during the next 6 days their number falls a further fivefold, with a return to the normal level 25 days after irradiation.

KEY WORDS: monolayer culture; efficiency of colony formation; fibroblast colonies.

Colonies, consisting of clones of fibroblasts, form in monolayer cultures of cells from hematopoietic and lymphoid organs [3, 7, 8]. The cells responsible for the formation of colonies of this type belong to the group of stromal precursor cells responsible for transferring the characteristic microenvironment for the appropriate hematopoietic organs – the bone marrow and spleen [7].

Data on the content of stromal colony-forming units in the bone marrow, spleen, and thymus of guinea pigs [4], the thymus and bone marrow of rabbits [1], and human bone marrow [2] were given previously.

The object of this investigation was to study the number of stromal colony-forming units (CFU-F) in the bone marrow, thymus, and spleen of mice and changes in the number of CFU-F in the bone marrow of mice irradiated in a dose of 150 R.

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EXPERIMENTAL METHOD

Adult (CBA × C57BL)F_1 mice weighing 18-20 g were used. Suspensions of cells from the femoral bone marrow, spleen, and thymus were prepared in the usual way [4] in medium 199. Cells taken from three or four mice were always mixed for explantation. Cultivation was carried out in 100-ml Roux flasks on double Eagle's medium with 20% calf embryonic serum. Every 3 days "nutrient cocktail" [10] was added to the medium. The medium was not changed throughout the cultivation period. In some experiments at the time of explantation syngeneic bone marrow cells previously irradiated in vitro in a dose of 4000 R were added to the cultures as an additional feeder. On the 10th-14th day the flasks were fixed with 96% ethanol and stained with azure–eosin. The number of colonies, i.e., the number of clusters of fibroblasts consisting of not less than 50 cells, was counted under a binocular loupe.

A cobalt source with a dose rate of 30 R/min was used to irradiate the mice and suspensions of bone marrow cells at 4°C, in a cell concentration of 10^7/ml.

EXPERIMENTAL RESULTS

A distinguishing feature of monolayer cultures of mouse bone marrow and spleen compared with guinea pig cultures is the persistence of numerous histiocytes during the first 2 weeks in culture. Besides the histiocytes starting from the third to fifth day colonies of fibroblasts develop: These are large cells with pale cytoplasm and a large nucleus, forming compact aggregations (Fig. 1).

No substantial number of histiocytes were found in the thymus cultures, but starting from the fifth to seventh day the number of isolated large elongated cells became appreciable, and later colonies of fibroblasts appeared in their place.

On explantation of the cells with an initial density of not less than $3 \cdot 10^5$ cells/cm^2 of the bottom of the Roux flask for bone marrow, $10 \cdot 10^6$ cells for spleen, and $5 \cdot 10^5$ for thymus, the number of colonies growing was a linear function of the number of explanted cells. The efficiency of colony formation (ECF-F) was virtually unchanged if the same initial cell density was attained by the addition of irradiated bone marrow cells as feeder. Within the limits of the experiment, i.e., after explantation of the same cell suspension, the number of growing colonies in individual flasks varied very little. Meanwhile the value of ECF-F and the number of CFU-F in the bone marrow of normal mice, according to results obtained in different experiments, varied much more strongly: The value of ECF-F for bone marrow varied from 0.7 to $4.5/10^5$ cells (mean $2.2/10^5$ cells), and the number of CFU-F in the bone marrow of one femur varied from 100 to 630 (mean 272). The mean value of ECF-F in the spleen and thymus was $0.20/10^5$ cells and $0.16/10^5$ cells respectively, and the number of CFU-F was 173 and 216 respectively.

The survival rate of bone-marrow CFU-F after irradiation in vitro was determined in experiments in which the same suspension of bone marrow cells was divided into parts, one of which acted as the control whereas the rest were irradiated. After irradiation in doses of 50, 150, 300, and 400 R about 70, 59, 36, and 16% of the CFU-F respectively were preserved (Fig. 2). The value of D_{37} for CFU-F was thus about 200 R. Special experiments were carried out to study the feeder activity of bone marrow cells of mice irradiated in

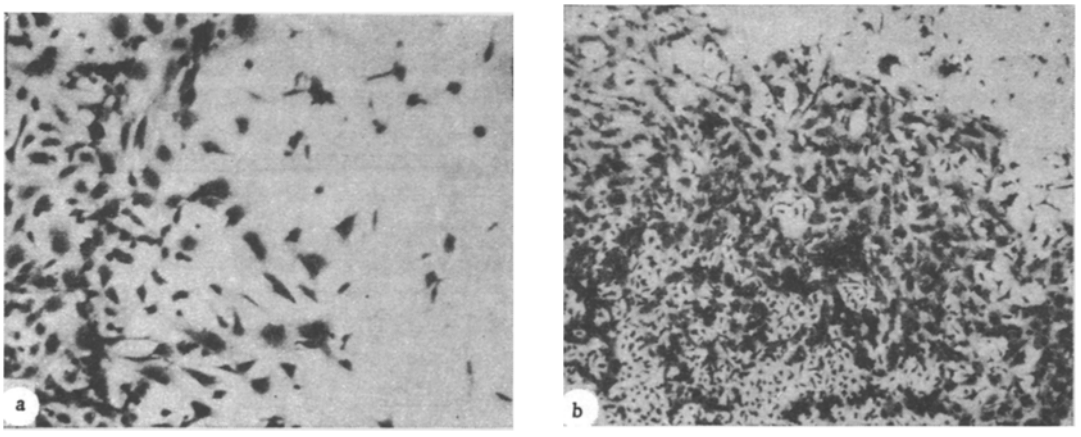

Fig. 1. Colonies of fibroblasts in monolayer cultures of mouse bone marrow. Azure–eosin, 20 × (a), 10 × (b).

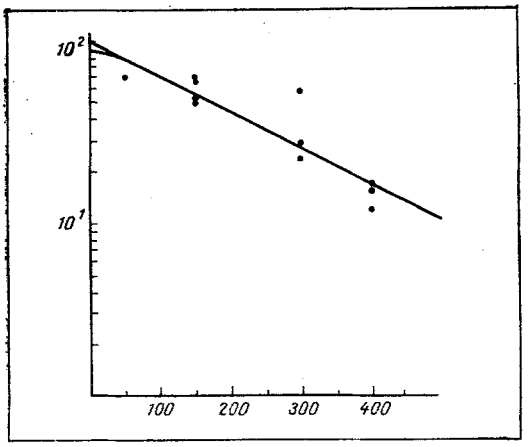

Fig. 2.

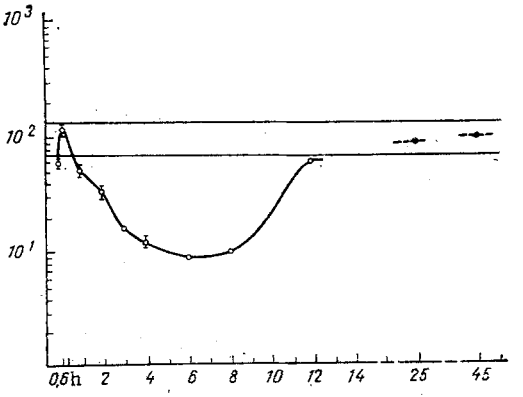

Fig. 3.

Fig. 2. Radiosensitivity curve of CFU-F from mouse bone marrow. Abscissa, dose of irradiation (in R); ordinate, log of percentage survival of CFU-F.

Fig. 3. Changes in number of CFU-F in femoral marrow of mice irradiated in a dose of 150 R. Abscissa, time after irradiation (in days); ordinate, percentage survival of CFU-F. Parallel lines mark normal limits.

a dose of 150 R. For this purpose the bone marrow of normal and irradiated (150 R) mice was explanted separately and as a mixture. The efficiency of colony formation in the mixed cultures proved to be the additive efficiency of colony formation of each component of the mixture.

The results of determination of the content of CFU-F in the bone marrow from 3 min to 45 days after irradiation of mice in a dose of 150 R are given in Fig. 3. Immediately after irradiation the number of CFU-F in the bone marrow of one femur clearly fell to 58% of the mean level for unirradiated mice. On the following days the number continued to fall to reach a minimum (9% of the normal level) on the sixth to eighth day, after which it rose and was close to the normal level by the 25th day. Changes in the value of ECF-F for bone marrow cells of irradiated mice were generally similar in character. It is interesting to note that during the first day after irradiation the number of CFU-F in the bone marrow changed in different directions. Between 3 min and 6 h after irradiation the number of CFU-F was approximately doubled (from 58% 3 min after irradiation to 114% 6 h after irradiation); toward the end of the first day the number of CFU-F fell again to the level corresponding to their number immediately after irradiation.

During explantation of mouse bone marrow, thymus, and spleen cells into monolayer cultures, colonies of fibroblasts are thus formed in the same way as has been shown for guinea pig, rabbit, and human cells [1-4, 7, 8]. Within the limits of specified initial cell densities, colony formation takes place with consistent efficiency for all the cell populations, i.e., the number of growing colonies was a linear function of the number of explanted cells. The same result was obtained after the addition of a standard feeder – syngeneic bone marrow cells irradiated in a dose of 4000 R; under these circumstances the efficiency of colony formation characteristic of each of the above-mentioned populations was maintained. This shows that differences in the efficiency of colony formation by bone marrow, spleen, and thymus cells reflect differences in the concentration of colony-forming cells in these cell populations, and the decrease in ECF-F of bone marrow in irradiated mice depends on a reduction in the number of CFU-F it contains.

Although irradiation in a dose 150 R kills only 42% of CFU-F, from the second day after irradiation their number began to fall below this level, and by the sixth day it was only 9% of normal. The number of CFU then gradually increased, so that by the 25th day it was almost back to normal, but did not exceed normal. This dynamics of the change in the number of CFU-F in the bone marrow of the irradiated mice almost coincides with the dynamics of the change in the number of hematopoietic stem cells in bone marrow after irradiation in the same dose [9]. It is difficult to imagine that this coincidence is accidental. It would be more natural to suggest that the postradiation restoration of stromal and hematopoietic cells is interconnected. Stromal precursor cells (CFU-F) are evidently used up in creating a new microenvironment for proliferation of the hematopoietic stem cells or their progenies. This seems all the more probable because during proliferation of immunocompetent cells in lymph nodes, induced by injection of antigens, and proliferation of bone marrow cells

after blood loss the number of CFU-F rises sharply [5]. It is noteworthy that in the first 6 h after irradiation in a dose of 150 R the number of CFU-F in the bone marrow increased to twice their number immediately after irradiation; the nature of the sources from which the CFU-F are recruited remains unexplained.

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CHANGES IN MITOTIC ACTIVITY OF HEPATOCYTES AND RESORPTION OF NECROTIC AREAS DURING INDUCTION OF CIRRHOSIS OF THE LIVER

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Cirrhosis of the liver was induced in rats weighing 120-140 g by administration of CCl_4 for 2 months. Iodinated oil was injected through the spleen of the experimental and intact animals to produce embolism of the branches of the portal vein and foci of necrosis in the liver tissue. The volume of the necrotic foci and the mitotic activity of the hepatocytes were determined. In cirrhosis of the liver the necrotic foci were resorbed more rapidly. The increase in the mitotic index on the second day after injection of the iodinated oil was greater in the control rats. The results suggest the appearance of a new cell clone in the liver which is responsible for resorption of the necrotic tissue and the reduction in mitotic activity of the hepatocytes in animals during development of cirrhosis of the liver.

KEY WORDS: cirrhosis of the liver; necrotic foci; proliferation in the liver.

Many investigations indicating the high proliferative potential of liver tissue have now been published. For instance, after removal of two-thirds of the liver the weight of the residual part doubles after 50 h. After partial hepatectomy repeated 12 times on rats, with the removal of 71 g liver tissue in the course of 1 year (natural weight of the liver 17.5 g), the histological structure of the organ was fully restored and the mean weight of the liver in these rats was 13.8 g [4, 5]. Meanwhile, during the development of experimental cirrhosis of the liver induced by CCl_4, after the precirrhotic period (about 2 months in rats) the proliferative power of the hepatocytes becomes inadequate and, although the volume of the necrotic areas is reduced, a marked increase is observed in the amount of connective tissue, with the formation of irreversible cirrhosis of the liver [2, 3].

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