FORMATION OF FIBROBLAST COLONIES IN MONOLAYER CULTURES OF THE SPLEEN OF IRRADIATED AND NORMAL MICE

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Colonies of fibroblasts, arising from precursor cells, are formed in monolayer cultures of spleen cells of normal mice. The effectiveness of colony formation is 0.3 per 10^6 cells or 20 colonies per spleen. A linear relationship exists between the number of growing colonies and the number of explanted cells. On the fifth to 20th day after irradiation of the mice in a dose of 150 R, the effectiveness of colony formation of the spleen cells is increased by three to six times. Irradiation in a dose of 600 R or more leads to a sharp decrease in the effectiveness of colony formation.

The formation of colonies consisting of fibroblasts in monolayer cultures of the hematopoietic tissue of guinea pigs was described in a previous paper [1]. The material then presented showed that each tissue of the colony is a cell clone, and that colonies are formed from a limited number of precursor cells present in the original populations of hematopoietic cells.

The object of the investigation described below was to study the formation of colonies of fibroblasts from spleen cells of normal mice and of mice irradiated with x rays.

EXPERIMENTAL METHOD

Experiments were carried out on adult mice of both sexes belonging to lines CBA and A. Whole-body irradiation of the animals with Co^{60} γ rays was given at a dose rate of 30 R/min. Cell suspensions were made from the spleens of normal and irradiated mice by expressing the cells from the two halves of the cut organ into medium No. 199. The suspension was then passed through needles of decreasing diameter from a syringe and filtered through four layers of Kapron. The cell concentration was adjusted to 10^7/ml. The prepared cell suspension was poured into Leighton's tubes with cover slips ($2 \cdot 10^7$ cells per tube) and into flat-bottomed flasks with a base area of 60 cm^2 ($1 \cdot 10^8$-$2 \cdot 10^8$ cells per flask). Cultivation took place in medium No. 199 with 20% bovine serum. To each 100 ml of medium, 5000 units streptomycin and the same dose of penicillin were added. The medium was changed first 48 h after setting up the culture, and half the volume was then changed every 2-4 days. Films of the original cell

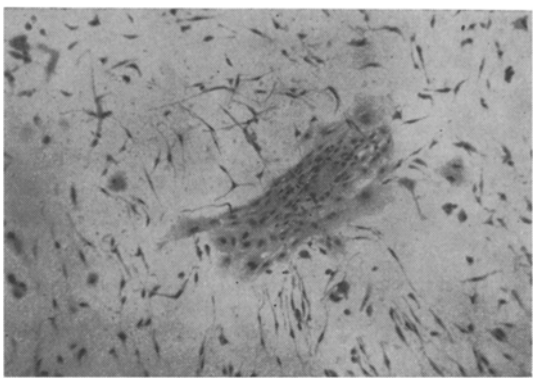

Fig. 1. Culture of spleen cells of a normal mouse. Duration of cultivation 10 days. Hematoxylin, 9×. Focus of fibroblasts among histiocytes.

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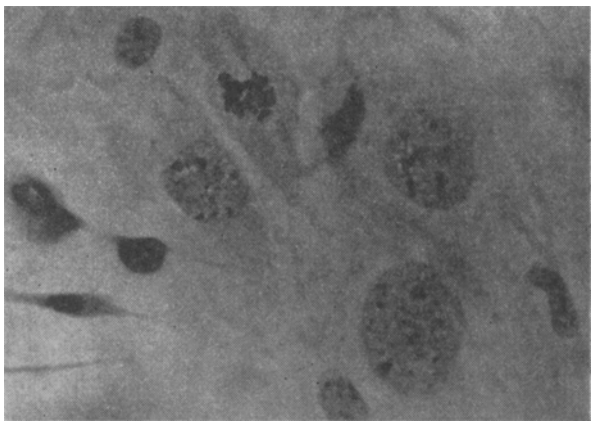

Fig. 2. Culture of spleen cells of a normal mouse. Period of cultivation 12 days. Hematoxylin, 40×. Mitosis in a fibroblast in a colony.

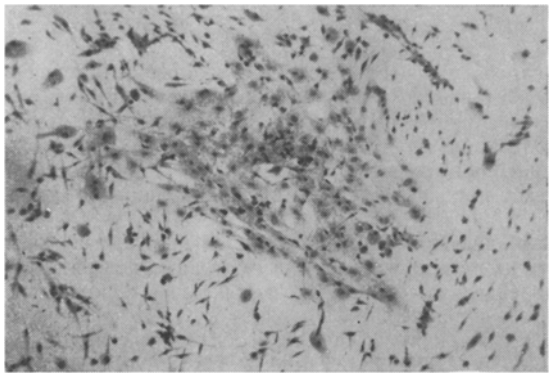

Fig. 3. Culture of spleen cells from a mouse irradiated in a dose of 150 R 10 days before explantation. Period of cultivation 12 days. Hematoxylin, 9×. Focus of fibroblasts among histiocytes.

suspension were stained by the Giemsa method. The cover slips were fixed on the fifth to 20th day in 96° alcohol and stained with hematoxylin or fixed in alcohol–formol and stained by Gomori's method for reticulin fibers and counterstained with hematoxylin.

The flasks were fixed and stained with hematoxylin on the 10th-14th day. The cover slips were used to assess the general morphology of the cultures. The number of colonies of fibroblasts growing on the flasks was counted. Cultures were obtained of spleen cells from normal mice, from mice on the fifth, 10th, 13th, 16th, 20th, and 30th days after irradiation in a dose of 150 R and on the 12th day after irradiation in doses of 300, 600, and 825 R.

EXPERIMENTAL RESULTS

The composition of the cells on the surface of the glass in cultures of spleen cells of normal mice showed regular changes in the course of cultivation. Soon after explantation, cells belonging to practically all categories of nucleated cells present in the spleen attached themselves to the glass. However, by the third to fifth day of cultivation this mixed cell population was replaced by branching histiocytes, oriented regularly over the surface of the glass or forming denser collections, together with macrophages. The cytoplasm of the macrophages contained products of phagocytosis, consisting of remains of cell debris and granules of brown pigment.

Starting with the seventh day groups of fibroblasts joined the histiocytes. These were larger, elongated cells with paler nuclei and with tonofibrils in their cytoplasm.

They differed clearly from the surrounding histiocytes and macrophages in their shape, nuclear structure, presence of tonofibrils, mutual arrangement, and relationship to the collagen fibers.

The fibroblasts were arranged as discrete colonies, in which the number of cells increased gradually. The diameter of the colonies in the 10-day cultures varied from 30 to 3000 μ. These colonies contain several tens or hundreds of cells respectively. The large colonies (2000-3000 μ in diameter) contained several types of fibroblasts, differing chiefly in size. In the center there was a few small cells; these were surrounded by medium-sized fibroblasts constituting the main mass of the focus; at the periphery the largest cells were located, few in number (Figs. 1 and 2).

By the 12th-14th day the colonies of fibroblasts were large enough to be seen with the naked eye and to be easily counted under a loupe. From then until the 20th day the colonies increased in size, but there was no increase in their number after the 10th day.

The cell composition of the cultures of spleen cells of irradiated mice was indistinguishable from that described above (Fig. 3). However, the number of cells in the culture and the number of growing foci of fibroblasts showed substantial changes.

The results of counting the colonies in cultures of spleen cells of normal mice and also of mice irradiated in the early periods after explantation, in doses of 150, 300, 600, and 800 R, are given in Table 1.

The results of experiments in which explants of the same cell suspension were made in different concentrations are given in Table 2. Two features will be noted: there was a linear relationship between the

TABLE 1. Effectiveness of Colony Formation (ECF) by Spleen Cells of Normal and Irradiated Mice

Dose of irradiation (in R)	Days after irradiation	Number of colonies per flask	ECF	
			per 10^6 explanted cells	per spleen
—	—	27	0,3	19
150	5	110	1,1	70
150	10	200	2,0	140
150	16	108	1,0	190
150	20	156	1,5	160
150	30	50	0,4	40
300	12	27	0,3	13
600	12	3	0,06	0,5
825	12	0	—	—

Note. The number of colonies is given per 10^6 explanted cells. In each experiment a mixture of cells from seven to ten mice was used; mean numbers of foci from two to three flasks are given, except for 10 days after irradiation with 150 R, when one flask was used.

TABLE 2. Relationship between Number of Colonies and Number of Explanted Cells

Number of explanted cells per flask	Number of foci per flask	ECF per 10^6 explanted cells	Mean ECF
$2 \cdot 10^8$	228	1,0	
10^8	113	1,0	1,0
10^8	99	1,0	

Note. Spleen cells from mice 16 days after irradiation in a dose of 150 R were used for explantation.

number of explanted cells and the number of colonies formed in individual experiments; many more colonies appeared in cultures of mouse spleen cells 5 days or more after irradiation in a dose of 150 R than in cultures of spleen cells of normal mice. The effectiveness of colony formation in cultures of spleen cells of normal animals is 0.3 colony per 10^6 cells, and there are about 19 foci to a whole spleen.

On the fifth, 10th, 16th, and 20th days after irradiation the effectiveness of colony formation per 10^6 cells increased by two to six times, and the effectiveness per spleen by two to 10 times.

Although the morphology of the fibroblastic foci in cultures of spleen cells from irradiated animals was unchanged, a more rapid rate of development of the colonies was observed, and their mean diameter was increased.

The number of colonies in cultures of cells taken from animals irradiated in a dose of 300 R corresponded to the number of colonies formed in cultures of spleen cells of normal animals (Table 1). Irradiation of the animals in a dose of 600 R led to a sharp decrease in the number of colonies formed in the cultures. Cultivation of spleen cells taken on the 12th day after irradiation of the animals in a dose of 825 R did not lead to colony formation; neither histiocytes nor fibroblasts grew in these cultures.

Cultivation of mouse spleen cells in monolayers thus yields colonies consisting of fibroblasts. It can be assumed that the colonies formed in cultures of the hematopoietic tissue of mice, like those formed in cultures of spleen and bone marrow cells of guinea pigs, are clones. This hypothesis is confirmed by the similarity between the morphology of the fibroblastic colonies in cultures from mice and guinea pigs, the similar dynamics of their development, and the linear relationship between the number of colonies and the number of explanted cells. The number of colonies formed in the cultures reflects the concentration of fibroblast precursor cells in the original cell suspensions. The effectiveness of colony formation for the spleen cells of normal mice was $0.3 \cdot 10^6$ cells or about 20 colonies per spleen.

Regeneration of hematopoietic tissue taking place as a sequel to irradiation of the animals in a dose of 150 R led to an increase in the number of colonies formed in the cultures and, consequently, to an increase in the number of precursor cells. The concentration of these cells in the suspension 5-20 days after irradiation was on the average four times greater, and in the whole spleen six times greater, than normal.

The true concentration of fibroblast precursor cells in the spleen is probably higher than that discovered in this investigation, because of the low effectiveness of cloning of these cells. The morphology of the precursor cells and their relationship with hemopoietic stem cells and immunocompetent cells, on the one hand, and with the stromal cells on the other, still remain unknown.

LITERATURE CITED

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