CLONING OF PRECURSOR CELLS FOR FIBROBLASTS IN MONOLAYER CELL CULTURES

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UDC 612-085.23

The concentration and total number of precursor cells forming fibroblast clone colonies were determined in monolayer cultures of cells from the bone marrow, thymus, spleen, and peritoneal and pleural cavities of adult guinea pigs. The concentration of these cells was 27.5, 8.4, 1.8, 0.96, and 0.34 per 10^5 cells of the pleural cavity, peritoneal cavity, bone marrow, spleen, and thymus, respectively. The total number of precursor cells for fibroblasts in the bone marrow, thymus, spleen, pleural cavity, and peritoneal cavity was 36×10^3, 2×10^3, 10^3, 8×10^2, and 3×10^2, respectively. Precursor cells for colonies of fibroblasts in cell populations from the hematopoietic organs belong to the category of stromal precursor cells. The origin of the precursor cells for fibroblasts in the pleural and peritoneal cavities is unknown.

In vitro cloning is a method of detecting individual precursor cells responsible for the ability of hematopoietic and lymphoid tissues to give rise to fibroblast development.

As the writers have described previously, fibroblast clone-colonies are formed in monolayer cultures of guinea pig [7, 9], rabbit [5], mouse [2], and human [6] bone marrow, guinea pig [9] and mouse [2] spleen, and guinea pig peritoneal macrophages [3].

Cloning in monolayer cultures has enabled light to be shed on some properties of fibroblast colony forming cells (FCFC). The radiosensitivity of guinea pig bone marrow FCFC is expressed by the parameters $D_0 = 178 \pm 14$ and $n = 1.44$ [1]. These cells are highly adhesive [8]. The number of FCFC in the mouse spleen after total irradiation in a dose of 150 R [2] and in the guinea pig bone marrow after blood loss undergoes characteristic changes. It has also been shown that FCFC circulate in the blood stream [4].

This paper gives details of the comparative efficiency of fibroblast colony formation (EFCF). They provide a basis for estimating the content of FCFC in the bone marrow, spleen, thymus, and peritoneal and pleural cavities of adult guinea pigs.

EXPERIMENTAL METHOD

Cells from guinea pigs weighing 180-200 g were explanted. Preparation of the cell suspensions and the explantation technique were described previously [6, 9]. The medium was changed for the first time after 24-48 h and later as it became acid. On the 10th-14th day the cultures were fixed with 96° ethanol and stained with azureeosin. The colonies were counted and their structure studied under the dissection microscope.

EXPERIMENTAL RESULTS

After the first change of medium, cells which were not adherent to the surface of the slide were removed, leaving in the cultures histiocyte-macrophages, monocytes, and degenerating lymphocytes; in the course of cultivation the number of these cells diminished progressively. After the 3rd day groups of fibroblasts appeared in the cultures; these subsequently increased in size to form discrete colonies visible with

Laboratory of Immunomorphology, N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR, O. V. Baroyan.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 76, No. 10, pp. 90-94, October, 1973. Original article submitted February 12, 1973.

TABLE 1. EFCF in Cultures of Cells from Bone Marrow, Spleen, Thymus, and Peritoneal and Pleural Cavities

Source of cells	No. of explanted cells per flask* (10^6)	No. of colonies per flask	EFCF (per 10^5 cells)
Bone marrow	5,4 1,8	78; 80 17; 20; 21; 31	1,5 1,2
Spleen	30,0 10,0	176; 318 64; 66; 61; 87	0,8 0,7
Thymus	52,0 14,0	147; 143 47; 43; 46; 65	0,3 0,3
Peritoneal cavity	1,3† 0,45†	184; 167; 153 56; 66; 60	13,0 13,5
Pleural cavity	1,2†	495; 667	48,4

* Flasks with a base area of 42 cm^2.
† Cultivation with the addition of 10^7 bone marrow cells irradiated in a dose of 4000 R as feeder.

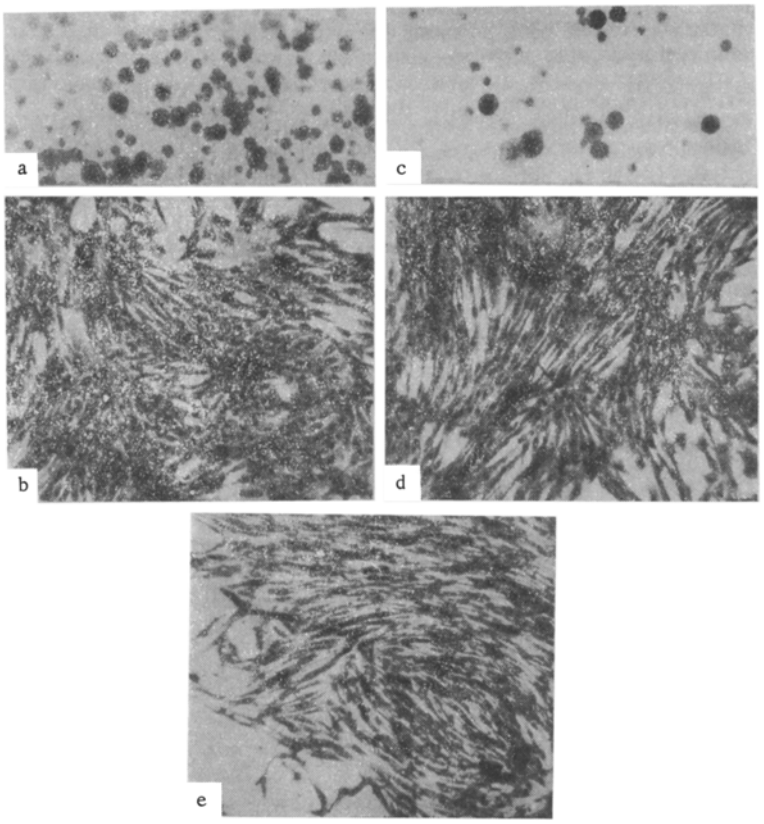

Fig. 1. Colonies of fibroblasts in 12-day cultures of guinea pig bone marrow (a, b), thymus (c, d), and pleural cells (e).

the unaided eye on the 8th-10th day (Fig. 1). When the counting was done, fibroblast foci containing no fewer than 50 cells were regarded as colonies. The cells in the colonies had the morphology of typical fibroblasts with tonofibrils in their cytoplasm, a pale nucleus, and a large nucleolus. Outside the colonies there were no fibroblasts. The exceptions were cultures of peritoneal macrophages and cells from the pleural cavity: besides colonies, these also contained isolated fibroblasts shaped like tiles with a pycnotic nucleus.

No significant differences were found in the morphology of the fibroblasts in cultures of different origin. However, differences were observed in the structure of the colonies, and these will be described specially.

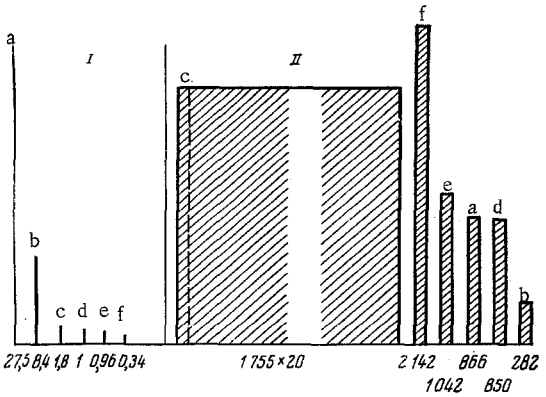

Fig. 2. Number of colony-forming cells in bone marrow, thymus, spleen, blood, and peritoneal and pleural cavities of guinea pigs. Data for blood taken from the literature before 1972: I) concentration of colony-forming cells; II) number of colony-forming cells in whole population. a) Pleural cavity; b) peritoneal cavity; c) bone marrow; d) blood; e) spleen; f) thymus. The part of column c bounded by the broken line represents the number of colony-forming cells in one femur (1755); the whole of column c represents the number of colony-forming cells in the whole bone marrow (1755 × 20).

Results of experiments to determine EFCF are given in Table 1. The differences between EFCF for the different cell populations were revealed very clearly. The corresponding results (combined results of 43 experiments) are illustrated in Fig. 2.

To maintain stable EFCF and to maintain a linear relationship between the number of explanted cells and the number of growing colonies, the initial density of explantation of cells from the pleural and peritoneal cavities, bone marrow, and spleen must not be below 0.5×10^5 cells/cm^2, and the number of thymus cells must not be below 5×10^5 cells/cm^2. In the presence of standard feeder (irradiated bone marrow cells) the EFCF characteristic of each of the cell populations analysed is reproduced [1]. Hence it follows that EFCF reflects the concentration of colony-forming cells. It was highest for cells of the pleural cavity, followed by peritoneal macrophages and cells of the bone marrow, spleen, and thymus. Meanwhile, first place for the total number of FCFC was occupied by the bone marrow (assuming that, as in mice, about 1/20 of the whole bone marrow in guinea pigs is accounted for by one femur): it contained about 36×10^3 FCFC, compared with about 2×10^3 for the thymus and 10^3 for the spleen. The corresponding numbers of FCFC in the pleural and peritoneal cavities were 8×10^2 and 3×10^2.

Although fibroblasts forming colonies in cultures of different origin differed morphologically from each other very little, there is no doubt that they differed in their properties. When retransplanted in vivo, fibroblasts from bone marrow cultures form bone tissue [9], which is populated by hematopoietic cells carrying out myeloid hematopoiesis on the territory of the graft, while under the same conditions fibroblasts from spleen cultures form reticular tissue [9], on the territory of which lymphopoiesis takes place. Fibroblasts from the cultures thus create a specific microenvironment which is characteristic of the corresponding hematopoietic organ. This shows that the FCFC of bone marrow and spleen can be categorized as stromal precursor cells. In agar cultures of cells from the pleural cavity mixed with venous blood, colonies of fibroblast-like cells, described as a "new type of colony-forming cells," were found in 1972 [10]. Agar cultures are evidently not suitable for cloning fibroblasts. In fact, if cells of the peritoneal cavity, thymus, lymph glands, spleen, and bone marrow of mice were explanted into agar cultures, only random colonies appeared, on the basis of which it was incorrectly postulated that these cell populations do not in general contain FCFC. Meanwhile, as the data given above show, bone marrow, thymus, and spleen of guinea pigs are rich in FCFC in which their concentration is 1-2, 0.3, and 0.8 per 10^5 cells, respectively. So far as mice are concerned, the EFCF of their bone marrow and spleen is the same as in guinea pigs. It is not surprising that during explantation of 3×10^5 cells of each of these populations into agar cultures colony formation with a stable level of efficiency could not be obtained [10].

LITERATURE CITED

1. G. N. Kuz'menko, A. F. Panasyuk, A. Ya. Fridenshtein, et al., Byull. Éksperim. Biol. i Med., No. 10, 94 (1972).
2. G. N. Kuz'menko and A. Ya. Fridenshtein, Byull. Éksperim. Biol. i Med., No. 1, 74 (1971).
3. E. A. Luria, A. F. Panasyuk, G. N. Kuz'menko, et al., Cell. Immunol., 3, 133 (1972).
4. E. A. Luria, A. F. Panasyuk, and A. Ya. Fridenshtein, Transfusion (Philadelphia), 11, 345 (1971).
5. É. D. Miskarova, K. S. Lalykina, I. N. Kokorin, et al., Byull. Éksperim. Biol. i Med., No. 9, 78 (1970).
6. A. F. Panasyuk, E. A. Luria, A. Ya. Fridenshtein, et al., Probl. Gematol., No. 8, 34 (1972).
7. R. K. Chailakhyan, A. Ya. Fridenshtein, and A. V. Vasil'ev, Byull. Éksperim. Biol. i Med., No. 2, 94 (1970).
8. A. Ya. Fridenshtein, in: Hard Tissue Growth, Repair, and Remineralization. Ciba Foundation Symposium, Amsterdam (1973), p. 169.

9. A. Ya. Fridenshtein, R. K. Chailakhyan, and K. S. Lalykina, Cell Tissue Kinet., 3, 393 (1970).
10. D. Metcalf, Nature, 238, 253 (1972).