# EFFECT OF BONE MARROW CELLS ON COLONY-FORMING GUINEA PIG STROMAL CELLS AND ON PROLIFERATION OF THEIR DESCENDANTS IN CULTURE

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Nonadhesive hematopoietic bone marrow cells stimulate the formation of colonies of fibroblasts by clonogenic stromal cells (CFU-F) in cultures of mouse bone marrow. If the culture medium contains 20% of serum the efficiency of colony formation (ECF-F) and the size of the colonies are sharply increased with the addition of irradiated bone marrow cells to the cultures, i.e., proliferation of the early descendants of the CFU-F is stimulated and the mouse bone marrow fibroblasts are difficult to maintain by passage. It is a different state of affairs with descendants of guinea pig bone marrow CFU-F in culture: During passage they yield strains of diploid bone marrow fibroblasts which can be maintained for a long time.

In this investigation the degree of dependence of colony formation by CFU-F isolated originally from guinea pigs on hematopoietic cells and proliferation of their in vitro descendants (bone marrow fibroblasts of passage strains) were compared.

## EXPERIMENTAL METHOD

Experiments were carried out on adult guinea pigs weighing 180-250 g. Bone marrow cell suspensions were prepared as described in [3, 4]. To determine CFU-F in the primary cultures $10^5$-$3 \cdot 10^5$ bone marrow cells were explanted in flasks (area of bottom 25 $cm^2$), using complete α-MEM culture medium with 5% embryonic calf serum (ECS).

After 1-1.5 h the medium with the nonadherent cells was removed, the flasks were rinsed 3-5 times with medium, and complete culture medium was added. To some cultures $10^7$ bone marrow cells irradiated in a dose of 60 Gy on a cobalt source, with dose rate of 10 Gy/min (feeder cells) were added. To obtain diploid strains of fibroblasts, $(3\text{-}5) \cdot 10^7$ guinea pig bone marrow cells were explanted into flasks (area of bottom 50 $cm^2$) and 10 days later the cells were removed with trypsin, resuspended in α-MEM medium, and transferred into fresh flasks. Culture was carried out in complete culture medium. Subculture was repeated until the cultures reached the state of confluence. To determine the effect of the irradiated bone marrow cells on proliferation of the subcultured fibroblasts, cells were used after 3-10 passages. These cells were transferred at the rate of $(1\text{-}2) \cdot 10^5$ into flasks with a bottom having an area of 25 $cm^2$ and were cultured as follows: 1) to maintain the cells in a state of proliferation ("stimulated cultures") the medium was changed every 3 days for 3-6 days; the cells were removed with trypsin 1 day after the last change of medium and subcultured at the rate of 150-200 fibroblasts per flask; under these circumstances $10^7$ irradiated bone marrow cells were added to some cultures; to stop cell proliferation ("inhibited cultures") the cells were cultured for 8-14 days without a change of medium, after which the cells were removed with trypsin and subcultured with or without the addition of irradiated bone marrow cells just as in the previous group. All cultures were fixed on the 10th-11th day with 96° alcohol, stained with azure-eosin, and colonies containing no fewer than 50 fibroblasts or the total number of fibroblasts in the cultures was counted. ECF-F, i.e., the number of colonies formed by $10^4$ explanted cells, was determined from the number of colonies.

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TABLE 1. ECF-F of Guinea Pig Bone Marrow (14 experiments)

| ECF-F per $10^4$ explanted cells | |
|---|---|
| without addition of feeder | in presence of $10^7$ feeder cells |
| 0,3 | 2,2 |
| 0,1 | 4,9 |
| 0,1 | 2,6 |
| 0,2 | 2,4 |
| 0,1 | 1,8 |
| 1,2 | 3,3 |
| 1,3 | 3,5 |
| 1,3 | 3,3 |
| 1,5 | 3,6 |
| 1,0 | 5,5 |
| 0,9 | 2,6 |
| 1,1 | 2,6 |
| 0,4 | 3,4 |
| 1,9 | 8,2 |
| 0,8±0,2 | 3,6±0,4 |

TABLE 2. ECF-F in Stimulated (with change of medium) Strains of Guinea Pig Bone Marrow (four experiments)

| Number of explanted fibroblasts | Number of irradiated bone marrow cells | Number of fibroblasts in culture | Mean number of colonies | Mean values of ECF-F per $10^4$ explanted cells |
|---|---|---|---|---|
| 150 | — | | 20, 30 | 25,0,17 |
| 150 | $10^7$ | | 83, 87 | 85,0,57 |
| 200 | — | $16 \cdot 10^3$, $10 \cdot 10^3$ | $13 \cdot 10^3$ 32, 33, 46 | 37,0,19 |
| 200 | $10^7$ | $12 \cdot 10^3$, $18 \cdot 10^3$ | $15 \cdot 10^3$ 39, 43, 44 | 42,0,21 |
| 200 | — | 42, 123 | 83 0,0 | 0,0 |
| 200 | $10^7$ | 204, 220 | 212 0,0 | 0,0 |
| 150 | — | $2,6 \cdot 10^3$, $2,6 \cdot 10^3$ | $2,6 \cdot 10^3$ 3,6 | 4,5,0,03 |
| 150 | $10^7$ | $3,0 \cdot 10^3$, $4,0 \cdot 10^3$ | $3,5 \cdot 10^3$ 7,4 | 5,5,0,04 |

TABLE 3. ECF-F in Inhibited Strains (without change of medium) of Guinea Pig Bone Marrow (four experiments)

| Number of explanted fibroblasts | Number of irradiated bone marrow cells | Number of fibroblasts in culture | Mean number of colonies | Mean values of ECF-F per $10^4$ explanted cells |
|---|---|---|---|---|
| 150 | — | | 1,1 | 1, 0,01 |
| 150 | $10^7$ | | 19,21 | 20, 0,14 |
| 150 | — | | 9,11 | 10, 0,07 |
| 150 | $10^7$ | | 95,131 | 113, 0,75 |
| 150 | — | $0,4 \cdot 10^3$, $0,7 \cdot 10^3$ | $0,6 \cdot 10^3$ 0,4 | 2, 0,01 |
| 150 | $10^7$ | $5,1 \cdot 10^3$ | $5,1 \cdot 10^3$ 17 | 17, 0,12 |
| 150 | — | | 0,2 | 1, 0,01 |
| 150 | $10^7$ | | 22,22 | 22, 0,15 |

## EXPERIMENTAL RESULTS

It will be clear from Table 1 that ECF-F of the primarily explanted guinea pig bone marrow cells, in the absence of feeder cells, was $(0.8 \pm 0.2) \cdot 10^{-4}$. CFU-F of guinea pig bone marrow thus differ from CFU-F of mouse bone marrow, which form virtually no stromal colonies if the explantation density of the bone marrow cells is low [2]. Irradiated bone marrow cells increased ECF-F of the guinea pig bone marrow cells to $3.6 \cdot 10^{-4}$ and equalized the scatter of values of ECF-F for individual donors. During culture in the absence of irradiated bone marrow cells the scatter amounted to $(0.1\text{-}1.9) \cdot 10^{-4}$, compared with $(1.8\text{-}8.2) \cdot 10^{-4}$ in the presence of irradiated bone marrow cells. The sensitivity of the fibroblasts in subculture to the growth-stimulating action of bone marrow cells was tested by comparing how the irradiated bone marrow cells affected proliferation of the subcultured fibroblasts from "inhibited" and "stimulated" cultures. During passage with a low density of cells of "stimulated cultures" without the addition of irradiated bone marrow cells, many small colonies consisting of squamous cells grew. The diameter of the remaining colonies reached 0.1-0.2 cm and the cells were widely scattered in them. On the addition of irradiated bone marrow cells the number of squamous colonies was reduced. ECF-F and the diameter of the colonies in most cases were not significantly increased and the total number of fibroblasts rose not more than threefold compared with cultures to which no irradiated cells were added. On the whole, the feeder had no significant action under these conditions. During passage of cells of "inhibited" cultures without the additon of irradiated bone marrow cells the diameter of the colonies was 0.1-0.15 cm. Addition of irradiated bone marrow cells caused a sharp increase both in ECF-F and in the dimensions of the colonies (diameter 0.2-0.45 cm). The total number of fibroblasts was correspondingly increased more than tenfold.

Thus the action of the irradiated bone marrow cells on multiplication of cells of the subcultured stains of bone marrow fibroblasts depends on the state of the strain during passage. If cells of the strain are in a state of proliferation, addition of feeder after their passage has a weak growth-stimulating action; if, however, most cells are in the resting state,

i.e., outside the proliferative cycle [6], addition of irradiated bone marrow cells will have a marked stimulating effect on proliferation of the subcultured bone marrow fibroblasts. The impression is created that the growth-stimulating action of bone marrow cells in the presence of 20% serum in the culture medium depends on the stimulating effect on cells in the $G_0$ phase of the cell cycle. This stimulating effect on cells in the $G_0$ phase of the cell cycle. This stimulating effect may perhaps be provided by the presence of megakaryocytes among the bone marrow cells [5]; these cells contain platelet growth factors and may secrete them into the cultures. Dependence of proliferation of CFU-F and of their descendants in culture on additional growth-stimulating influences besides those provided by the presence of high concentrations of embryonic serum (20%) in the culture medium, is essential for growth of other types of fibroblasts in the cultures. In fact, for diploid strains of fibroblasts of different origin in culture, a sufficient quantity of growth-stimulating factors for maximal proliferation is present in medium with a high concentration of embryonic serum (20%). Accordingly, to demonstrate the growth-stimulating action of the various additional factors, besides those present in serum, it is necessary to use a cultural system which contains far lower concentrations of serum (usually 1-5%). The growth-stimulating action of the bone marrow cells on CFU-F is thus evidence that bone marrow CFU-F may have an unusual requirement for growth-stimulating factors compared with other fibroblasts. We know that CFU-F of bone marrow in situ are in the $G_0$ phase [1]. Evidently both CFU-F isolated from the body and bone marrow fibroblasts undergoing passage and withdrawn from the cell cycle ("cultural $G_0$") are necessary for entry into the $G_1$ period in higher concentrations of growth factors compared with stimulated fibroblasts in the course of passage, and located in the proliferative pool.

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# PROLIFERATIVE POTENTIAL OF HEMATOPOIETIC STEM CELLS (CFU-S) DURING SERIAL PASSAGE IN IRRADIATED MICE IN FOCI OF ECTOPIC HEMATOPOIESIS


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According to the established view, hematopoiesis is maintained by hematopoietic stem cells (HSC), which are capable of self-maintenance [2, 3], i.e., by cells the number of divisions of which is not restricted to any finite value. Nevertheless, it is impossible to prove this by serial passages of hematopoietic cells: After 3-5 transfers the donor's hematopoietic cells are exhausted and the recipient mice either die or revert to the recipient type of hematopoiesis [4]. However, such results do not necessarily prove that the proliferative potential of

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