EFFECT OF GROWTH FACTORS ON STROMAL CFU-f COLONY FORMATION IN MOUSE BONE MARROW CELL CULTURES

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The formation of colonies of stromal bone-marrow fibroblasts (CFU-f colonies) takes place through the presence of plasma-dependent growth factor (PDGF) [6] and an as yet unidentified growth factor, which is absent in the serum and produced by megakaryocytes and platelets [2], in the culture medium. The formation of CFU-f colonies in cultures of adhesive bone-marrow cells (A-cultures), i.e., free from nonadherent bone marrow cells, in fact requires the addition of irradiated (feeder) bone marrow cells or platelets [1]. In this connection the analysis of the sensitivity of CFU-f to growth-stimulating and growth-inhibiting factors can usefully be carried out in parallel experiments in A-cultures in which the efficiency of colony formation (ECF-f) is sharply depressed, and in ordinary bone-marrow cultures, i.e., in which ECF-f is high.

EXPERIMENTAL METHOD

Freshly isolated bone marrow cells from adult CBA mice were used for culture. Suspensions of mechanically de-aggregated cells in medium α-MEM were prepared as described in [1, 2] and explanted into 12- and 24-well plates, with wells of an area of 4.5 and 2.5 cm^2, at the rate of (2-10) $\cdot$ 10^5 cells per well. The culture medium consisted of α-MEM with 15% fetal calf serum. The cultures were set up in two modifications: culturing the total population of bone-marrow cells (T-cultures) or adhesive bone-marrow cells (A-cultures), when the nonadherent bone-marrow cells were removed 2 h after explantation [3]. Feeder bone-marrow cells were added to some of the A-cultures.

Human recombinant tissue necrosis factor (TNF-α), from the Institute of Molecular Biology, Academy of Sciences of the USSR, recombinant mouse IL-3 ("Genzyme") and purified mouse IL-1 (N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR) were added to T- and A-cultures 2 h after explantation of the bone-marrow cells. After addition of the growth factors the culture medium remained unchanged throughout the period of culture. To neutralize activity of the TNF-α it was incubated with rabbit antiserum against TNF-α (Institute of Molecular Biology, Academy of Sciences of the USSR) for 1.5 h at 37°C, with the ratio of active units of antiserum to units of TNF-α activity of 1:2.

Culture was carried out in an incubator with 5% CO_2 in air at 37°C. After 7-10 days the cultures were fixed with ethanol and stained with azure-eosin; colonies consisting of not less than 40 fibroblasts were counted. ECF-f was determined on 10^5 explanted bone-marrow cells.

EXPERIMENTAL RESULTS

ECF-f in the presence of growth factors in the culture medium are presented in Figs. 1-3. ECF-f in the control A-cultures was 0.2-0.3, compared with 1.8-2.7 in T-cultures. In the presence of feeder bone-marrow cells ECF-f in the A-cultures was about 10 (see Figs. 2 and 3).

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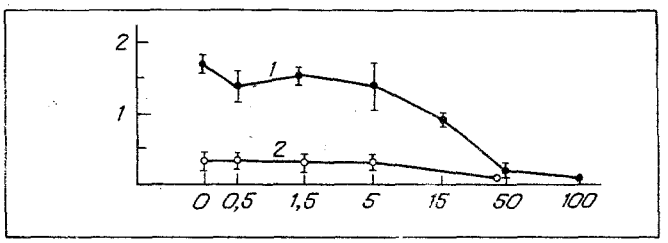

Fig. 1. Colony formation in mouse bone marrow cultures in presence of IL-1. Abscissa, concentration of IL-1 (in U/ml); ordinate, ECF-f in T-cultures (1) and in A-cultures (2); 10^6 bone-marrow cells were explanted per well.

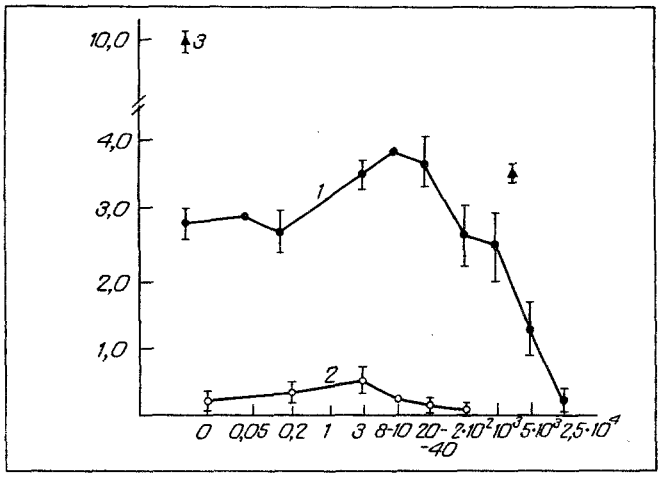

Fig. 2. Colony formation in mouse bone marrow cultures in presence of TNF-α. Abscissa, concentration of TNF-α (in U/ml); ordinate, ECF-f in T-cultures (1) and in A-cultures (2), 10^6 cells explanted per well; ECF-f in A-cultures (3), $2 \cdot 10^5$ bone-marrow cells explanted in the presence of $5 \cdot 10^6$ feeder cells.

IL-1 (Fig. 1) had no effect on ECF-f if used in concentrations below 1.5 U/ml in the culture medium; higher doses of IL-1 inhibited colony formation, although we know that IL-1 behaves as a mitogen for cells of nonstromal diploid passage strains [6].

It will be clear from Fig 2 that TNF-α caused some increase in ECF-f, which was not significant compared with the colony-stimulating activity of the feeder bone-marrow cells, but in large does TNF-α inhibited the formation of CFU-f colonies, including in the presence of feeder cells. Meanwhile, TNF-α, like IL-1, has a marked stimulating action on proliferation of cells of diploid strains of nonstromal fibroblasts [5, 6]. Table 1 shows that in the presence of a mixture of TNF-α and antiserum against TNF-α the inhibitory action of TNF-α was largely abolished.

IL-3 (Fig. 3) in a concentration of between 0.8 and 50 U/ml caused an increase of 1.6 times in ECF-f in the T-cultures, whereas in cultures of bone-marrow cells with the same original explantation density, the feeder bone-marrow cells increased ECF-f 50-fold. In higher concentrations IL-3 inhibited colony formation (see Fig. 3). In A-cultures explanted with an initial density of 10^6 bone-marrow cells per well a stimulating effect of IL-3 also took place, but its inhibitory action in the presence of high concentrations was not observed. With an explantation density of $2 \cdot 10^5$ cells per well IL-3 had neither inhibitory nor stimulating action in A-cultures A weak colony-stimulating action of IL-3 also was observed when total bone marrow cell cultures were used [7]. It must be recalled that on the addition of IL-3 to T-cultures, and also to A-cultures explanted with a high initial density of bone marrow cells, appreciable stimulation of hematopoiesis took

TABLE 1. Colony Formation in Cultures of Mouse Bone Marrow Cells in Presence of TNF-α and Antiserum to TNF-α

Concentration of antiserum to TNF-α, U/ml	Number of colonies of fibroblasts per well. Concentration of explanted cells, ×10⁶		Concentration of TNF-α, U/ml	Number of colonies of fibroblasts per well. Concentration of explanted cells, ×10⁶		Concentration of TNF-α + antiserum to TNF-α, U/ml	Number of colonies of fibroblasts per well. Concentration of explanted cells, ×10⁶	
	3,0	1,0		3,0	1,0		3,0	1,0
—	76±14	5±1	—	78±9	4±1	—	95±2	7±1
20	73	5	10	35±5	5±2	10+20	93±7	7±1
$2\cdot10^2$	70	5	10^2	31±1	4±1	$10^2+2\cdot10^2$	67±7	7±1
$2\cdot10^3$	66	5	10^3	10±1	3	$10^3+2\cdot10^3$	64±2	7±2
$2\cdot10^4$	70	5	10^4	2	1	$10^4+2\cdot10^4$	60±5	7±2

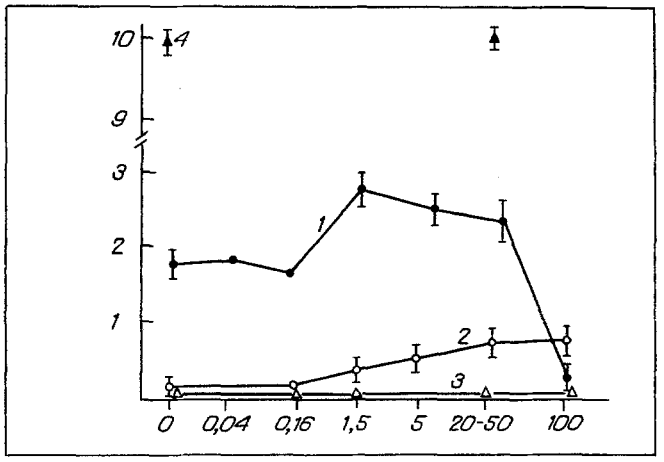

Fig. 3. Colony formation in mouse bone marrow cultures in presence of IL-3. Abscissa, IL-3 concentration (in U/ml); ordinate, ECF-f in T-cultures (1) or in A-cultures (2), 10^6 bone-marrow cells were explanted per well; ECF-f in A-cultures (3), $2 \cdot 10^5$ bone-marrow cells were explanted per well; ECF-f in A-cultures (4), $2 \cdot 10^5$ bone-marrow cells were explanted per well in the presence of 10^7 feeder cells.

place. Not all nonadherent bone marrow cells could be removed from these A-cultures by washing, and the effect of stimulation of colony formation could depend, therefore, not on the action of IL-3 itself, but on the effect of a certain proportion of proliferating hematopoietic cells.

On the whole, none of the growth factors tested had a growth-stimulating effect on colony formation, taking place in the presence of feeder cells. It is therefore unlikely that their action is connected with the growth factors tested in this investigation. It is also important to point out that IL-1 and TNF-α have no growth-stimulating action on colonies consisting of medullary fibroblasts, such as they have on diploid strains of nonstromal fibroblasts.

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