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DEPENDING OF STROMAL CFU-f COLONY FORMATION ON STIMULATING EFFECT OF HEMATOPOIETIC CELLS

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CFU-f colonies are formed in cultures of bone marrow cells [7, 9, 13, 21] and consist of fibroblasts, which synthesize collagen of types I and III but do not synthesize Willebrand's factor VIII or angiotensin convertase, and they do not possess Fc- and C-receptors or marker antigens of macrophages [4, 6, 12]. These features distinguish fibroblasts of stromal colonies from other adhesive bone marrow cells: macrophages and endothelial cells. If the serum concentration is sufficient to create a high PDGF concentration (not less than 10 ng/ml) in the culture medium the formation of CFU-f colonies does not require any additional growth-stimulating influences. Meanwhile, if the density of explanation of bone marrow cells is low, the efficiency of colony formation (ECF-f) is low and can be raised if irradiated bone marrow cells are added to the cultures [3, 6, 10]. This suggested that hematopoietic cells stimulate the development of stromal colonies. The investigation described below confirmed that for the formation of CFU-f colonies it is in fact necessary that the cultures contain blood platelets or nonadhesive bone marrow cells. It was found that, under these circumstances, the colony-stimulating activity of these cells is not dependent on their content of PDGF.

EXPERIMENTAL METHOD

Single-cell suspensions of marrow cells were prepared by pipeting bone marrow from the femora of CBA mice through a Pasteur pipet or by trypsinization for 60 min in 0.25% trypsin solution on a magnetic mixer [5, 11]. The cell suspensions thus obtained were filtered through a nylon filter and, after washing twice, the cells were explanted into plastic flasks with an area of 25 cm^2. Testing for CFU-f colonies [6, 9, 10, 13] was carried out in two modifications: by culturing the complete population of bone marrow cells (BMC) or adhesive bone marrow cells (ABMC). ABMC cultures were obtained by changing the medium and removing nonadherent cells 2 h after bone marrow explanation, after which the adherent cells were washed three times and the flasks were filled with fresh culture medium. At this stage, irradiated (feeder) calls or growth factors PDGF, EGF, or IL-3 were added to some of the cultures. Mechanically disaggregated BMC, and spleen, thymus, and lymph node cells from CBA mice and guinea pigs, and also leukocytes and platelets isolated from the blood of guinea

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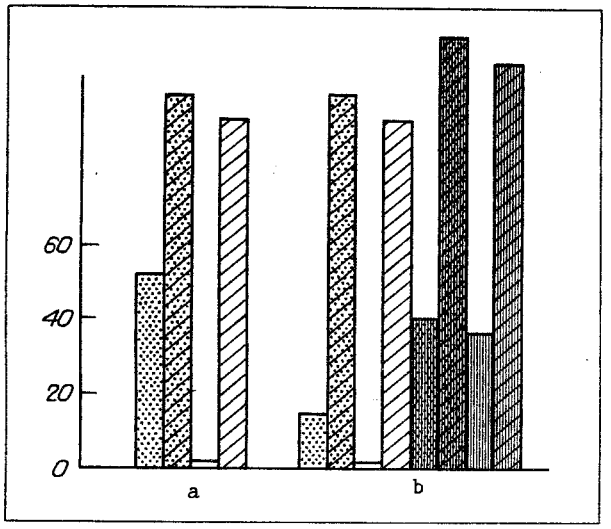

Fig. 1

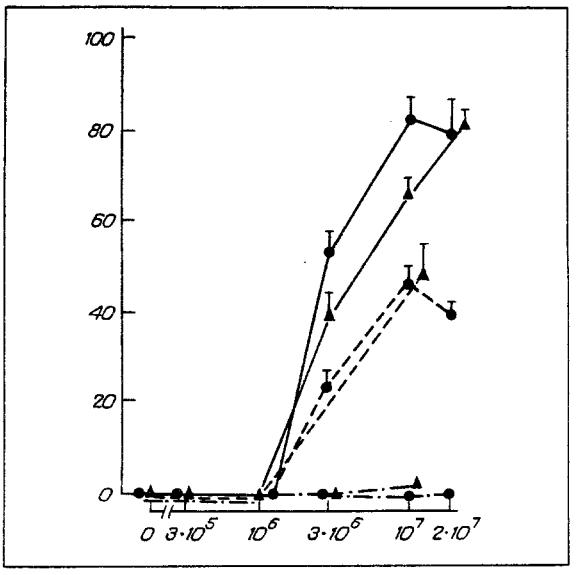

Fig. 2

Fig. 1. Effect of hematopoietic cells on efficiency of colony formation. Cells of mechanically disaggregated bone marrow are explanted into cultures (dotted columns). Nonadherent cells were removed 2 h after explantation (unshaded columns). (10^7) Irradiated guinea pig bone marrow cells were added to some cultures as feeder (obliquely shaded columns). a) Cultures with high initial explantation density ($3 \cdot 10^6$ cells per culture); b) cultures with low explantation density ($3 \cdot 10^5$ cells per culture), some of which were grown in a gaseous phase with 1% O_2 (darkly shaded columns). Ordinate, ECF-f (in percent of ECF-f in BMC cultures grown with the addition of feeder cells in a CO_2 incubator, column obliquely shaded and dotted).

Fig. 2. Effect of concentration of serum and feeder cells on formation of CFU-f colonies. $5 \cdot 10^5$ mechanically disaggregated BMC were explanted into cultures in medium with 5% fetal calf serum (FCS). After 2 h, nonadherent cells were removed and fresh medium with 1% (line of dots and dashes), 5% (broken line), or 20% (continuous line) FCS (circles) or human serum (triangles) was poured into the flasks. Abscissa, number of feeder irradiated guinea pig bone marrow cells per culture; ordinate, number of CFU-f colonies.

pigs and rabbits were used as feeder cells [8, 15]. All feeder cells were irradiated in a dose of 60 Gy (^{60}Co) and some of the feeder cells were treated with EDTA to remove the culture medium consisted of α-MEM with 1, 5, or 20% fetal calf serm or inactivated human serum. Culture was carried out at 37°C with a gaseous phase consisting either of air with 5% CO_2 (in a CO_2 incubator) or of nitrogen with 5% CO_2 and 1% O_2. After 10-12 days the cultures were fixed with 10% formalin in phosphate buffer and stained by the Giemsa method. The number of CFU-f colonies consisting of not less than 50 fibroblasts was counted under a dissection microscope. ECF-f was determined as the number of CFU-f colonies per 10^4 primarily explanted bone marrow cells.

EXPERIMENTAL RESULTS

ECF-f was much lower in ABMC cultures than in BMC cultures (Fig. 1). On the addition of feeder bone marrow cells to the ABMC cultures ECF-f increased sharply: this was also found in the case of BMC cultures explanted with low initial density. Stimulation of colony formation depended on the number of feeder cells (Fig. 2) and was maximal if their concentration was of the order of $5 \cdot 10^5/cm^2$. Under these circumstances ECF-f remained stable in ABMC cultures, differing in explantation density by hundreds of times (Table 1). According to the result of 20 experiments, ECF-f in the presence of feeder bone marrow cells was 1.7 ± 0.2 for mechanically disaggregated and 14.6 ± 4.1 for trypsinized bone marrow cells. Besides feeder cells, ECF-f also was affected by the serum concentration in the medium. The colony-stimulating action of the feeder cells was maximal if the serum concentration was high, and no action

TABLE 1. Dependence of ECF-f on Number of Bone Marrow Cells Explanted in the Presence of Feeder Cells

Number of explanted* cells $\times 10^4$	0,3	1,0	3,0	10,0	30,0	30,0
Number of feeder** cells, $\times 10^4$	30	30	30	30	30	—
Number of colonies	3; 3; 3	10; 11; 11	21; 33; 37	101; 105; 118	298; 338	0; 0; 1
ECF-f	10,0	10,7	10	10,8	10,5	0,01

Note. *) Cells of trypsinized bone marrow were explanted into a flask with surface area of 42 cm^2. Here and in Table 2, irradiated guinea pig BMC were used as feeder cells.

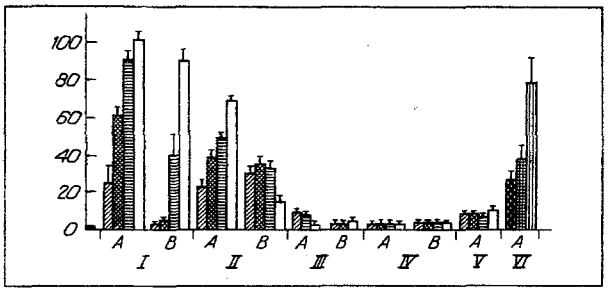

Fig. 3. Colony-stimulating activity of irradiated cells from hematopoietic and lymphoid organs. Abscissa, source of irradiated guinea pig (A) or mouse (B) feeder cells added to cultures, into which were explanted $(1\text{-}5)\cdot 10^5$ mechanically disaggregated bone marrow cells; ordinate, cloning efficiency in cultures differing in density of feeder cells (black columns – 0; obliquely shaded – 10^5; cross-hatched – $2.5\cdot 10^5$; horizontally shaded – $5\cdot 10^5$; unshaded – 10^6) and of platelets (chequer board column – $4\cdot 10^5$; squared – $1.2\cdot 10^6$, vertically shaded – $4\cdot 10^6$) to 1 cm^2 of culture surface, in percent, and ECF-f in cultures with density of irradiated guinea pig bone marrow cells $10^6/cm^2$. I) Bone marrow; II) spleen; III) lymph nodes; IV) thymus; V) blood lukocytes; VI) platelets.

TABLE 2. Effect of Growth Factors on Formation of CFU-f Colonies in Cultures of Adhesive Cells of Mechanically Disaggregated Bone Marrow

Number of explanted cells	Growth factor	Conc. of growth factor in 1 ml medium	Number of feeder cells	Number of colonies
$3\cdot 10^5$ (35 mm dish)	—	—	—	0; 1; 2
	PDGF	10 ng	—	0; 0
	The same	50 ng	—	0; 0
	—	—	$2\cdot 10^6$	42; 45
	—	—	$4\cdot 10^6$	43; 48
			$6\cdot 10^6$	58; 61
$2\cdot 10^5$ (flask, 25 cm^2)	—	—	—	0, 0, 0, 1
	IL-3	20 ng	—	0,0
	The same	20 ng	10^7	60
	—	—	10^7	44, 60
$2\cdot 10^5$ (35 mm dish)	—	—	—	0; 0; 0; 1
	IL-3	3 ng	—	0;0
	The same	10 ng	—	0; 0
	»	30 ng	—	0; 0
	»	70 ng	—	0; 0
	»	100ng	—	0
	—	—	$3\cdot 10^6$	22; 36
$3\cdot 10^6$ (flask, 25 cm^2)	—	—	—	1; 8
	EGF	5 ng	—	1; 8
	The same	20ng	—	7; 7
	»	100 ng	—	4; 7

was exhibited in the presence of 1% of serum; in other words, feeder cells did not replace the action of serum growth factors (Fig. 2). In the absence of feeder cells a decrease in the oxygen concentration increased ECF-f in ABMC cultures, but it remained lower than in cultures with feeder cells, in the presence of which oxygen concentration had virtually no effect on ECF-f (Fig. 1).

Bone marrow and spleen cells from mice and guinea pigs and platelets from guinea pigs and rabbits had a colony-stimulating action: 10^8 platelets, moreover, had a stimulating action equal to that of $5\cdot 10^6$ bone marrow feeder cells (Fig. 3). Cell populations containing megakaryocytes preserved their colony-stimulating activity after removal of their platelets. Conversely, after removal of the platelets, guinea pig spleen cells, among which there were no megakaryocytes, lost their activity. Thymocytes, lymph node cells, and blood leukocytes did not stimulate the formation of CFU-f colonies.

Addition of PDGF, IL-3, and EGF to the cultures did not lead to the formation of CFU-f colonies, i.e., these growth factors did not replace the colony-stimulating action of the feeder cells (Table 2).

Thus the development of CFU-f colonies requires the colony-stimulating action of platelets and also, evidently, of megakaryocytes. This was revealed in ABMC cultures, and has so far remained unnoticed in BMC cultures, where CFU-f are accompanied by natural feeder colony-stimulating cells. The colony-stimulating action of feeder cells is dose-dependent, species-nonspecific, and cannot be replaced by serum growth factors, including PDGC, EGF, and IL-3.

Platelets and megakaryocytes contain PDGE, which is the most active growth-stimulating factor for fibroblasts [19, 20]. However, it follows from the data given above the colony-stimulating action of megakaryocytes and platelets in relation to CFU-f colonies is not due to PDGF. In fact, addition of PDGF to ABMC cultures did not enable CFU-f colonies to be formed, although in BMC cultures with a low serum concentration (i.e., with deficiency of serum growth factor but in the presence of natural feeder cells) addition of PDGF stimulates the formation of CFU-f colonies [15, 17]. PDGF likewise is essential [15-17, 21] for the formation of CFU-f colonies as for the proliferation of all kinds of fibroblasts. However, for CFU-f to embark on proliferation, the additional colony-stimulating action unconnected with PDGF is necessary. This may perhaps depend on the fact that primarily explained CFU-f in the body are in the G_0-period of the cell cycle [1, 2, 13].

The mechanism of the colony-stimulating action of platelets on CFU-f has not been explained, and the growth stimulating factors responsible for it have not yet been identified. Feeder cells stimulate the formation of CFU-f colonies in the presence of both a high and a low O_2 concentration, i.e., their colony-stimulating action evidently cannot be reduced to a general improvement of the conditions for colony formation that are provided by lowering the O_2 concentration [12, 14].

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