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Platelet-Derived Growth Factor Requirement for Cultured Descendants of Rabbit Stromal Bone Marrow Precursors

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Addition of irradiated bone marrow feeder from guinea pigs and rabbits to cultures of passed rabbit bone marrow fibroblasts considerably decreases the efficiency of colony-formation and the total amount of fibroblasts in cultures. The presence of irradiated rabbit blood platelets sharply increases these parameters. The addition of irradiated bone marrow feeder to rabbit bone marrow fibroblasts cultured under conditions promoting cell differentiation also decreases the total content of fibroblasts in cultures. Our results suggest that cultured descendants of rabbit bone marrow stromal precursors preserve both the sensitivity to inhibitory factors produced by bone marrow cells coexisting with stromal precursors in cultures (typical of stromal precursors of these animals) and high sensitivity to growth-stimulating platelet-derived factors (typical of stromal precursors from rabbits and other animal species).

Key Words: *bone marrow stromal cells; growth factors*

Transplantation of stromal cells propagating in monolayer cultures is now widely used in the therapy of various pathologies [7,9,11]. Of particular importance is the development of optimum culturing techniques for this tissue. Populations of stromal precursor cells (ClFU-F) from hemopoietic and lymphoid organs in different animal species are usually cloned in the presence of the same growth factors (GF) under the same culturing conditions. There are data on the existence of considerable interspecies differences in the sensitivity of ClFU-F to factors produced by non-stromal bone marrow cells coexisting with ClFU-F in cultures [2]. For instance, irradiated bone marrow cells from rabbits and guinea pigs (so-called feeder) after increasing cell density to a certain values suppress the formation of rabbit bone marrow ClFU-F colonies [2]. At the same time, bone marrow cells from guinea pigs and rabbits stimulate guinea pigs bone marrow ClFU-F, while rabbit bone marrow cells modulate human ClFU-F [5,7]. Rabbit blood platelets stimulate the growth of rabbit bone marrow ClFU-F. However, combined addition of rabbit bone marrow cells and platelets to these cultures abolish the growth-stimulating effect produced by platelets. These data suggest that populations of bone marrow cells from rabbits and guinea pigs contain cells producing both the inhibitory and stimulating effects on ClFU-F proliferation and that ClFU-F of different animal species have different sensitivity to growth-inhibiting factors [1,2]. Colony-stimulating effects on ClFU-F of the bone marrow feeder are determined by platelets and megakaryocytes [8]. The cells inhibiting the growth of ClFU-F carry macrophage marker F4-80; T cells are not involved in this effect [2]. The stimulating and inhibiting effects are mediated by soluble factors passing through Millipore filter [1,4]. The inhibiting effect of bone marrow cells did not depend on their proliferation and was observed in both hetero- and autologous systems. A necessary condition for manifestation of the inhibiting effect is the presence of fetal bovine serum (FBS). The presence of the autologous serum or the serum from adult animals of other species (other than FBS) considerably reduced the inhibitory effect [2]. It remains unclear, why the inhibitory effect of the bone marrow cells appears only in the presence of FBS. Thus, non-

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stromal bone marrow cells present in cultured together with ClFU-F can synthesize GF producing different (stimulating and inhibiting) effects on ClFU-F. It is still unclear whether the sensitivity to these factors is inherited by cultural offspring of ClFU-F (stromal fibroblasts in passed bone marrow culture). Another question concerns the dependence of stromal cells on GF produced by non-stromal cells in culture: whether the demand for these GF is different for stromal cells at different stages of differentiation. Our previous studies showed that guinea pig stromal bone marrow fibroblasts at different stages of differentiation are characterized by different dependence on GF produced by bone marrow feeder [3].

Here we evaluated whether the sensitivity to the stimulating effect of platelets and inhibiting effect of the bone marrow feeder typical of primary bone marrow ClFU-F is preserved in cultured offspring of rabbit bone marrow ClFU-F and whether this sensitivity of to bone marrow GF varies in rabbit fibroblasts cultured under conditions promoting cell differentiation.

MATERIALS AND METHODS

The experiments were carried out on 1-year-old California or New Zealand rabbits obtained from the Central Breeding Center (Kryukovo). Cell suspension of the bone marrow was prepared with a syringe [8]. The cells were explanted into 25-cm^2 flasks in 5 ml α-MEM medium (Sigma) supplemented with 15% FBS (Paneko) and antibiotics (penicillin and streptomycin, 100 μg/ml). For obtaining diploid fibroblast strains 1-2×10^6 bone marrow cells were grown in 25-cm^2 flasks for 7-10 days, detached with 0.25% trypsin, resuspended in α-MEM and transferred into a new flask. The cells were cultured in complete nutrient medium. Cells after 3-7 passages were used for evaluation of the effect of feeder GF on fibroblast proliferation. For obtaining more differentiated cells dexamethasone (10^{-8} M, Sigma) and L-ascorbate (10^{-4} M, Japan Ascp Wake) was added to the culture medium every other day. The cells were subcultured one time a week after attaining confluence (strains 3 and 4). Then the cells were treated with trypsin and 50-30,000 cells were transferred to 25-cm^2 flasks. Some cultures were grown in the presence of 0.7-1.0×10^7 guinea pig or rabbit bone marrow cells irradiated in a dose of 60 Gy (Co-60, 10 Gy/min) as a feeder or 30×10^7 irradiated rabbit peripheral blood platelets isolated as described previously [8]. All cultures were grown in a CO_2 incubator for 7-9 days. Then the cells were detached with trypsin and the number of fibroblasts in the suspension was determined. In case of small number of fibroblasts seeded (50-1000 cells) the cultures were fixed with ethanol, stained azure and eosin, and cloning efficiency (ECF-F) was determined, *i.e.* the number of colonies containing no less than 50 cells per 100 explanted fibroblasts.

RESULTS

Irradiated guinea pig and rabbit bone marrow cells considerably (by 2 and 4.6 times, respectively) decreases ECF-F in the culture of bone marrow fibroblasts (Table 1). At the same time, the presence of irradiated rabbit platelets in cultures considerably increase both the total number of fibroblasts (by 8.2 times) and ECF-F. However, the amount of platelets in the irradiated feeder is sufficient for attaining maximum ECF-F: addition of irradiated platelets to mouse bone marrow cultures increase their ECF-F to the same extent as the addition of the bone marrow feeder [8]. It can be hypothesized that cultural descendants of rabbit bone marrow ClFU-F retain the sensitivity to inhibitory factors produced by bone marrow cells present with ClFU-F in cultures (typical of these cells) and the sensitivity to growth-stimulating factors produced by platelets (typical of ClFU-F from rabbits and other animal species). The population of bone marrow stromal precursor cells is heterogeneous by its dif-

TABLE 1. Growth of Fibroblasts in Rabbit Bone Marrow Cultures in the Presence of Irradiated Feeder Cells of Different Origin*

Number of explanted cells	Feeder	Number of feeder cells, ×10^7	Number of fibroblasts per culture, ×10^3	ECF-F per 100 explanted cells
100-1000	Without feeder	—		2.3±0.3
	Guinea pig bone marrow	0.7		1.1±0.2
	Rabbit bone marrow		0.7	0.5±0.1
	Rabbit blood platelets	30		17.9±2.1
50	Without feeder	—	0.97±0.12	0
	Rabbit blood platelets	30	8.0±1.7	55.2±0.8

Note. *Culturing in the absence of dexamethasone and L-ascorbate.

TABLE 2. Number of Fibroblasts in Rabbit Bone Marrow Cultures Grown under Different Conditions*

Culturing conditions		Number of fibroblasts in culture, $\times 10^5$		Ratio of fibroblast number in cultures grown without feeder/with feeder
		without feeder	with feeder	
Without dexamethasone and L-ascorbate	strain 1	34.7±0.7	16.6±1.4	2.6±0.5
	strain 2	22.5±0.5	7.2±1.0	
With dexamethasone and L-ascorbate	strain 1	42.5±0.9	19.0±1.3	2.5±0.3
	strain 2	31.0±0.9	11.1±0.8	

Note. *A total of 1×10^5 cells were explanted per 25-cm^2 flask. Irradiated guinea pig bone marrow cells were used as a feeder (1×10^7 cells per culture).

ferentiation potential, which is seen from the experiments with re-transplantation of mouse and guinea pig ClFU-F colonies into the body. It was found that only 25% colonies formed both the bone marrow and bone, some colonies formed only the bone tissue without bone marrow, while others formed only reticular tissue [6]. The dependence of guinea pig bone marrow fibroblasts on GF produced by bone marrow feeder considerably varies at different stages of their differentiation [3]. Indeed, addition bone marrow feeder to guinea pig bone marrow stromal fibroblast cultures, where cell differentiation was suppressed by passing the cultures before attaining confluence, did not increase ECF-F. The presence of the feeder in the culture of fibroblasts passed after attaining the confluence and grown in the presence of dexamethasone and L-ascorbate (inductors of osteogenic differentiation of these cells [10]) sharply increased ECF-F and the number of stromal fibroblasts in cultures [3]. However, the route of culturing of passaged rabbit bone marrow fibroblasts did not modulate the dependence of their proliferation on feeder GF (Table 2). Addition of irradiated guinea pig and rabbit bone marrow feeder to rabbit bone marrow fibroblasts cultured in the absence of dexamethasone and L-ascorbate decreases the total number of fibroblasts in cultures (by 2.6 times), while the addition of irradiated bone marrow feeder to rabbit bone marrow fibroblasts grown under conditions promoting cell differentiation (in the presence of dexamethasone and L-ascorbate) decreases the total number of fibroblasts (by 2.5 times).

Thus, there are species-specific peculiarities in the sensitivity of not only initial ClFU-F, but also their cultural descendants to growth-inhibiting factors produced by non-stromal bone marrow cells. In the studied animals species [4,8] both ClFU-F and their cultural descendants are highly sensitive to the growth-stimulating factors produced by platelets.

Our findings suggest that stromal cells should be cultured in the presence of platelets without bone marrow cells (the latter can produce factors inhibiting the growth of both ClFU-F and their cultural descendants). Good results were obtained in experiments with human bone marrow fibroblasts cultured in the presence of platelet-enriched plasma (PRP) [11]. The inhibiting effect of nonstromal bone marrow cells on the growth of ClFU-F usually manifests in the presence of FCS used as a component of the growth medium [1]. Therefore, the population of bone marrow cells should be cultured in the presence of auto- or heterologous sera from adult animals. Otherwise, proliferative potential of some ClFU-F and their cultural descendants can be not realized due to inhibition of their growth.

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