Effect of Platelet Growth Factors on Proliferation of Guinea Pig Bone Marrow and Splenic Stromal Precursor Cells and Proliferation of Cultural Descendants from Bone Marrow Precursor Cells

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Elimination of platelets from guinea pig splenocyte suspension (laking megakaryocytes) with EDTA considerable reduces the efficiency cloning of splenic stromal precursor cells. It means that platelet-derived growth factors are necessary for stromal precursor cells from different organs (bone marrow, thymus, and spleen). The dependence on the platelet growth factors can vary within a wide range in descendants from cultured bone marrow precursor cells (passaged bone marrow fibroblasts at different stages of differentiation.

Key Words: *bone marrow and splenic stromal precursor cells; growth factors*

Cloning of stromal precursor cells (CFC-F) from different organs is usually carried out in the presence of the same growth factors under the same culturing conditions. However, it remains unclear, whether CFC-F from different organs require the same growth factors (*e.g.* platelet-derived factors). It was shown that the efficiency of formation of CFC-F colonies (ECF-F) in guinea pig splenocyte cultures grown on a feeder (irradiated bone marrow cells containing platelet-derived growth factors [3]) little differed from ECF-F in the same cultures grown without feeder. By contrast, ECF-F in guinea pig bone marrow cultures without feeder decreased almost 10-fold [1]. A possible explanations is that splenic CFC-F need less platelet-derived growth factors; another hypothesis is that CFC-F growth-stimulating activity is maintained by platelets present in the suspensions, because platelets can adhere to monocytes [3]. The aim of the present study was to elucidate which of these hypotheses is true. Another question concerning the relationship between proliferation of stromal cell and platelet growth factors is whether the need for these factors varies at different stages of stromal cells differentiation. We compared ECF-F in guinea pig bone marrow fibroblast cultures grown under conditions preventing or favoring cell differentiation in the presence and absence of feeder growth factors [4].

METHODS

Guinea pigs of both sexes aged 4-5 months (Kryukovo Breeding Center) were used for the experiments. Bone marrow and splenic cell suspensions were prepared as described previously [3]. Platelets were removed with 0.77 mM EDTA (Sigma) [3]. Splenocytes ($1\text{-}3\times10^6$) were transferred into 25-cm^2 flasks with 5 ml α-MEM culture medium (Sigma) supplemented with 5% ETC (Paneko). After 2 h the medium with nonadherent cells was discarded, the cultures were washed twice with α-MEM, and complete culture medium containing α-MEM, 20% ETC, and antibiotics (penicillin and streptomycin, 100 μg/ml each) were added. Irradiated (60 Gy, ^{60}Co, 10 Gy/min) guinea pig bone marrow cells (1.5×10^7) were added to some cultures as the feeder. After 12-day incubation in a CO_2 incubator the cultures were fixed with ethanol, stained with azur-eosin,

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and colonies containing at least 50 fibroblasts were counted. ECF-F was estimated as the number of colonies formed by 10^6 explanted cells.

In order to obtain diploid fibroblast strains, 1-2× 10^6 guinea pig bone marrow cells were cultured in 25-cm^2 flasks for 7-10 days. The cells were then removed with 0.25% trypsin, resuspended in α-MEM, and transferred into another flask with complete culture medium.

The effect of platelet growth factors of the feeder on the proliferation of fibroblasts was studied after 3-7 passages. For inhibition of differentiation the cells were subcultured twice a week before attaining confluence (strains 1 and 2). To obtain more differentiated cells dexamethasone (10^{-8} M, Sigma) and L-ascorbate (10^{-4} M, Japan AscP Wake) were added to the culture medium every other day and the cells were subcultured once a week after attaining confluence (strains 3 and 4). Cells of both groups were removed with trypsin and transferred to 25-cm^2 flasks (200 cells/flask). Irradiated bone marrow (10^7 cells) was added to some cultures. The cultures were fixed with ethanol on days 10-11, stained with azur-eosin, and colonies containing at least 50 fibroblasts were counted. ECF-F was estimated as the number of colonies formed by 100 explanted cells.

RESULTS

After EDTA treatment, ECF-F decreased several times in cultures from which hemopoietic cells were removed by double washing. After addition of irradiated feeder this value notably increased, but did not reach the control level (cultures treated similarly, but without EDTA), which can be explained by death of some CFC-F during treatment (Table 1). Hence, platelet growth factors are required for *in vitro* proliferation of splenic CFC-F, that is, the need for these growth-stimulating factors is almost the same for CFC-F derived from different organs (bone marrow, thymus, and spleen).

TABLE 1. ECF-F (Number of Colonies per 10^6 Explanted Cells) in Guinea Pig Splenocyte Culture (*M*±*m*)

Culturing conditions	Control	EDTA treatment
Without feeder	11.5±1.0	3.2±0.7
With feeder	32.2±2.2	24.9±4.9

TABLE 2. ECF-F (Number of Colonies per 100 Explanted Cells) in Guinea Pig Bone Marrow Culture (*M*±*m*)

Culturing conditions	Without feeder	With feeder
Without dexamethasone and L-ascorbate		
strain 1	0	0.5±0.2
strain 2	0	0.25±0.00
With dexamethasone and L-ascorbate		
strain 3	0	15.0±2.5
strain 4	0	25.0±3.0

The population structure of CFC-F is very intricate. This is confirmed by experiments with re-transplantation of mouse and guinea pig CFC-F colonies into the body. Only 25% colonies gave rise to bone with the bone marrow. Some colonies formed only the bone tissue without bone marrow cavity and others formed only the reticular tissue [2]. Hence, the population of CFC-F consists of cells of different lines and differentiation degree. It was interesting to find out whether stromal cells at different stages of differentiation similarly depended on growth factors, specifically on feeder factors. In our experiments, cultured guinea pig bone marrow fibroblasts required feeder growth factors depending on the method of cell culturing (Table 2). In fibroblast cultures subcultured before attaining confluence, the addition of the feeder did not increase ECF-F. By contrast, the presence of the feeder in fibroblast cultures grown in the presence of dexamethasone and L-ascorbate (which is known to induce osteogenic differentiation of these cells [4]) and subcultured after attaining confluence drasticly increased ECF-F. These data indicate that the dependence of fibroblasts on the platelet growth factors varies within a wide range at different stages of differentiation. Highly differentiated cells require more platelet growth factors.

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