DIFFERENTIATION OF HUMAN BONE MARROW STROMAL PRECURSOR CELLS IN MONOLAYER CULTURE

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KEY WORDS: bone marrow; stromal fibroblasts; cloning; cell differentiation.

The technique of cloning stromal precursor cells of human bone marrow, which has now been developed [1, 4] can be used to study the properties of these cells. It has been shown that cells of colonies of stromal fibroblasts, on cytochemical testing, give a positive reaction for alkaline phosphatase (AP) [2], which is the marker enzyme for osteoblasts. This suggested that an osteogenic type of differentiation of stromal precursor cells is possible in monolayer culture.

The aim of this investigation was to make a further study of the cytochemical properties and features of osteogenic differentiation of human bone marrow stromal precursor cells in monolayer culture.

EXPERIMENTAL METHOD

Bone marrow from the sternum, wrist, and the wing of the ileum, taken from orthopedic operations during reconstructive repair operations for non-neoplastic diseases of the locomotor apparatus, was used. Stromal fibroblasts were cloned by the method described previously, using rabbit feeder [1], in Roux flasks (area of base 62 and 88 cm^2) and in glass Petri dishes (diameter 95 mm).

The density of explanation did not exceed $5 \cdot 10^3$ nucleated human bone marrow cells per square centimeter of the bottom of the culture vessel. Culture took place in medium 199 with 20% human group AB(IV) serum. The colonies which grew were fixed on the 10th-14th day of culture. Activity of magnesium-dependent ATPase (EC 3.6.1.4) in cells of the colonies was determined by the method of Wachstein and Meisel, acid DNase (EC 3.1.4.6) activity by the method of Aronson and Vorbrodt, acid and alkaline RNases (EC 2.7.7.16 and EC 2.7.7.1) by the method of Zagury et al., and AP (EC 3.1.3.1) by Gomor's and Kaplow's methods [3]. Calcium deposition in the colonies was determined by Kossa's method [5]. As the calcification control, cultures were treated with 5% EDTA solution for 2 h at 4°C.

EXPERIMENTAL RESULTS

The growing colonies of stromal fibroblasts were heterogeneous in size and number of cells. On the basis of these features they could be divided into two types: monolayer, loosely arranged, 3.3 ± 0.05 mm in diameter, containing from 50 to 500 cells, and classified, compact, 4.6 ± 0.09 mm in diameter and containing over 500 cells.

Cells in the stratified colonies were densely packed and formed structures oriented spirally around the center of the colony. The ratio between the numbers of stratified and monolayer colonies in the cultures varied and did not depend on the source of bone-marrow cells or the efficiency of cloning (Table 1). Activity of magnesium-dependent ATPase in stromal fibroblasts of the colonies was discovered in the cytoplasm of the cells in the form of fine granules. Activity of acid and alkaline RNases was localized in the perinuclear zone of the cells and coincided with the localization of acid phosphatase. Acid DNase activity was determined in nuclei of stromal fibroblasts.

On determination of AP in the cells by Gomori's method considerable variability of the results was found. The percentage of phosphatase-positive colonies varied from 10 to 95; these fluctuations were observed, moreover, not only between cultures but also between

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TABLE 1. Characteristics of Colonies of Human Bone Marrow Stromal Fibroblasts

Source of cells	Efficiency of colony formation, CFU_f/ 10 cells	Ratio of stratified and monolayer colonies	Percentage of phosphatase-positive colonies in tests by		Percentage of colonies containing calcium
			Gomori's method	Kaplow's method	
Wing of ileum	56,3±16,7	1/2	50	100	34
	63,5±7,1	1/1	25	100	48
	73,3±2,2	1/2	95	100	29
	73,7±1,7	1/4	50	100	20
Sternum	116,8±3,0	1/4	10	100	25
	110,0±3,9	1/2	30	100	30
	130,6±2,8	1/2	90	100	55
Rib	23,0±2,2	1/3	25	100	30
	42,7±2,4	1/1	40	100	36

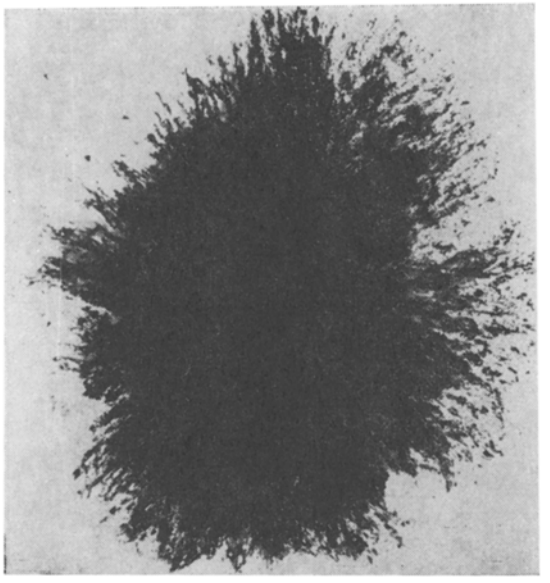

Fig. 1. Calcium deposits in colony of human bone marrow stromal fibroblasts. Kossa's stain. 70 ×.

parallel flasks of the same culture. By Kaplow's method of cytochemical determination of AP activity all colonies were shown to contain cells which reacted positively for AP [2]. Within the culture the percentage of phosphatase-positive cells in the colonies was fairly constant, but there were marked differences between cultures. The limits of variation were from 5 to 93%. The percentage of phosphatase-positive cells in both stratified and monolayer colonies was the same. In large stratified colonies phosphatase-positive cells formed islets.

Calcium deposits were observed in stratified colonies only. The more layers of cells the colony contained, the greater the intensity of calcium deposition. Some colonies were completely calcified (Fig. 1); they were opaque in transmitted light and had the appearance of white spots on the bottom of the culture flask. The intensity of calcification was greater in the center of the colony, and typical fibroblasts could be seen at the periphery.

In monolayer colonies, despite the presence of cells containing AP, no calcium deposits were observed. This fact can be explained on the grounds that primitive conditions are present in stratified colonies for three-dimensional orientation of the cells, and for that reason later stages of cell differentiation are observed.

These results agree with those of Sudo et al. [6], who also observed calcium deposition in stratified islets in a culture of mouse fibroblasts. The histogenetic process was similar to that of ossification centers in membrane bones *in vivo* as described by Ham [6].

The results of this investigation suggests that processes of differentiation of osteogenic precursor cells are observed in monolayer cultures of human bone marrow stromal fibroblasts, although further investigations are necessary in order to obtain more convincing data.

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CHRONOBIOLOGICAL STUDY OF THE COURSE OF EXOGENOUS GLUCOSE RELEASE INTO THE BLOOD STREAM

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The study of circadian biological rhythms (BR) can provide a deeper understanding of the principles governing the course of biological processes and can lead to greater therapeutic effectiveness of drugs [1, 5]. An urgent problem in chronobiology is the study of BR of carbohydrate metabolism in health and disease. This is particularly true of tissues whose activity depends on insulin.

The aim of this investigation was to study BR of release of exogenous glucose (G) into the blood stream, and the effect of an electromagnetic field and of latent insulin deficiency on it.

EXPERIMENTAL METHOD

Altogether 360 noninbred male albino mice weighing 20-25 g were used. The animals were first deprived of food for 24 h. There were three series of experiments. Mice of series I were given an intramuscular injection of 0.1 ml of 40% G solution. The animals were decapitated every 5 min after the beginning of the injection, blood was collected, and the G level determined in the plasma obtained from it. Mice of series II were divided into three groups: group 1) intact mice, group 2) mice with latent insulin deficiency, and group 3) healthy mice exposed to an electromagnetic field. At 9.30 a.m. all mice of the experiments of series II were given an intramuscular injection of 0.1 ml of 40% G solution, and all the manipulations described for series I were carried out, except that these animals were decapitated 30 min after the beginning of the injection. The experiment was repeated 12 times at 2-hourly intervals in the course of the 24-h period. In series III (control) intact mice received an injection of 0.1 ml of 40% sucrose solution. Latent insulin deficiency was induced by the method described previously [2], and the plasma glucose level (PGL) was determined by the glucose oxidase method [9]. The animals were exposed to an electromagnetic field by placing them between two parallel magnet coils, to which square pulses were applied. The pulse frequency was 15 Hz and the intensity of the electromagnetic field was 5 Oe. Exposure lasted 90 min before injection of G and 30 min thereafter.

EXPERIMENTAL RESULTS

The experiments of series I showed that the PGL of the mice reached a maximum 25-30 min after injection of G (Fig. 1). This time was chosen for taking samples of series II and III.

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