In Vitro Formation of Mesenchymal Bone Marrow Islets

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We studied the formation of mesenchymal islets in the culture of bone marrow cells *in vitro*. Islet-like structures detected on day 6 of culturing contained a central epithelioid cell (differing from macrophages and fibroblasts) surrounded with round mesenchymal cells. The number of mesenchymal islets increased on days 12-14 of culturing, but decreased to zero by the 24th day. They appeared not only as individual structures, but also entered the composition of colonies and formed assemblies with surrounding cells of different maturity. Our results show that mesenchymal islets serve as structural and functional units of mesenchymopoiesis.

Key Words: *mesenchymal islets; stem cells; mesenchymopoiesis; bone marrow*

The red bone marrow contains specific structural and functional complexes consisting of the central cell (macrophage) and surrounding erythroid cells [5]. Maturation of red blood cells occurs in these complexes [2]. Central macrophage regulates proliferation and differentiation of surrounding erythroid cells in these complexes. Similar systems were described for myeloid cells [6]. Apart from macrophages, fibroblasts can play a role of the central regulatory cell [1,3].

Hemopoietic islets introduced into the spleen of lethally irradiated mice form colonies including islets of different maturity (undifferentiated cells, mature granulocytes, and erythrocytes), which attest to replication of hemopoietic and stromal cells [4]. It can be hypothesized that hemopoietic islets contain a common precursor for hemopoietic and stromal cells, including mesenchymal stem cells (MSC). Theoretically, MSC can form structural and functional complexes (islets). It is difficult to test this hypothesis at the organism level, since the bone marrow contains extremely low number of MSC [7].

Here we studied whether MSC in high concentrations can *in vitro* form structural and functional complexes.

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MATERIALS AND METHODS

Experiments were performed on 20 male CBA mice weighing 18-20 g. The mice were immobilized by cervical dislocation. The femur was removed under sterile conditions. The bone marrow was washed out with a syringe and placed in flasks. Cellularity and viability of the homogenous suspension were assayed by trypan blue exclusion [1]. The count of cells was brought to $1\text{-}2\times10^6$ cells/ml complete medium. The suspension (20 ml) was placed in 50-ml plastic flasks (Falcon). The complete medium contained 80% DI-MEM medium, 20% fetal calf serum, 50 μg/ml ascorbate diphosphate, 5 μg/ml insulin, 10^{-7} M dexamethasone, 250 mg/liter L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin (Sigma). The cells were

TABLE 1. Dose Dependence between the Count of Bone Marrow Cells from CBA Mice Introduced in the Culture and Number of Mesenchymal Islets Formed on Days 6, 12, and 24 of Culturing (10^4 cells, X, Pu)

Count of introduced cells, 10^6/ml	Number of colonies		
	day 6	day 12	day 24
1.5	0.15±0.03	19.0±0.7	0
3.0	0.79±0.20	33.3±1.3	0.9±0.3
6.0	1.4±0.5	49.0±2.1	1.5±0.5

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cultured in a CO_2 incubator at 37°C, 5% CO_2, and 100% humidity. The supernatant was replaced with a fresh portion of culture medium after 3 days. Culturing was performed for 24 days. The medium was replaced at 6-day intervals.

The samples were taken in various periods of culturing and examined under a phase-contrast microscope. Vital staining involved neutral red. Fixed preparations were stained by the method of Romanovsky—Giemsa. Cytochemical reactions for alkaline phosphatase and α-naphthyl acetate esterase were performed as described elsewhere [1].

The results were analyzed by Wilcoxon test.

RESULTS

Round cells prevailed on day 6 of culturing [7]. They underwent transformation into various cells by the 12th day of culturing. The relative number of cells decreased in the following order: fibroblastoid cells (28.3±3.4%), round cells (21.2±2.2%), chondrocytes (18.9±3.7%), and muscle cells (14.0±2.9%). The ratio of nervous, epithelioid, and undifferentiated cells was 9.7±3.1, 4.1±1.2, and 2.8±0.4%, respectively. Colonies in the culture were revealed on day 6 ($4.3\pm3.3\times10^6$). The number of colonies peaked on days 12-14 ($20.1\pm7.3\times10^6$). In the follow-up period they underwent fu-

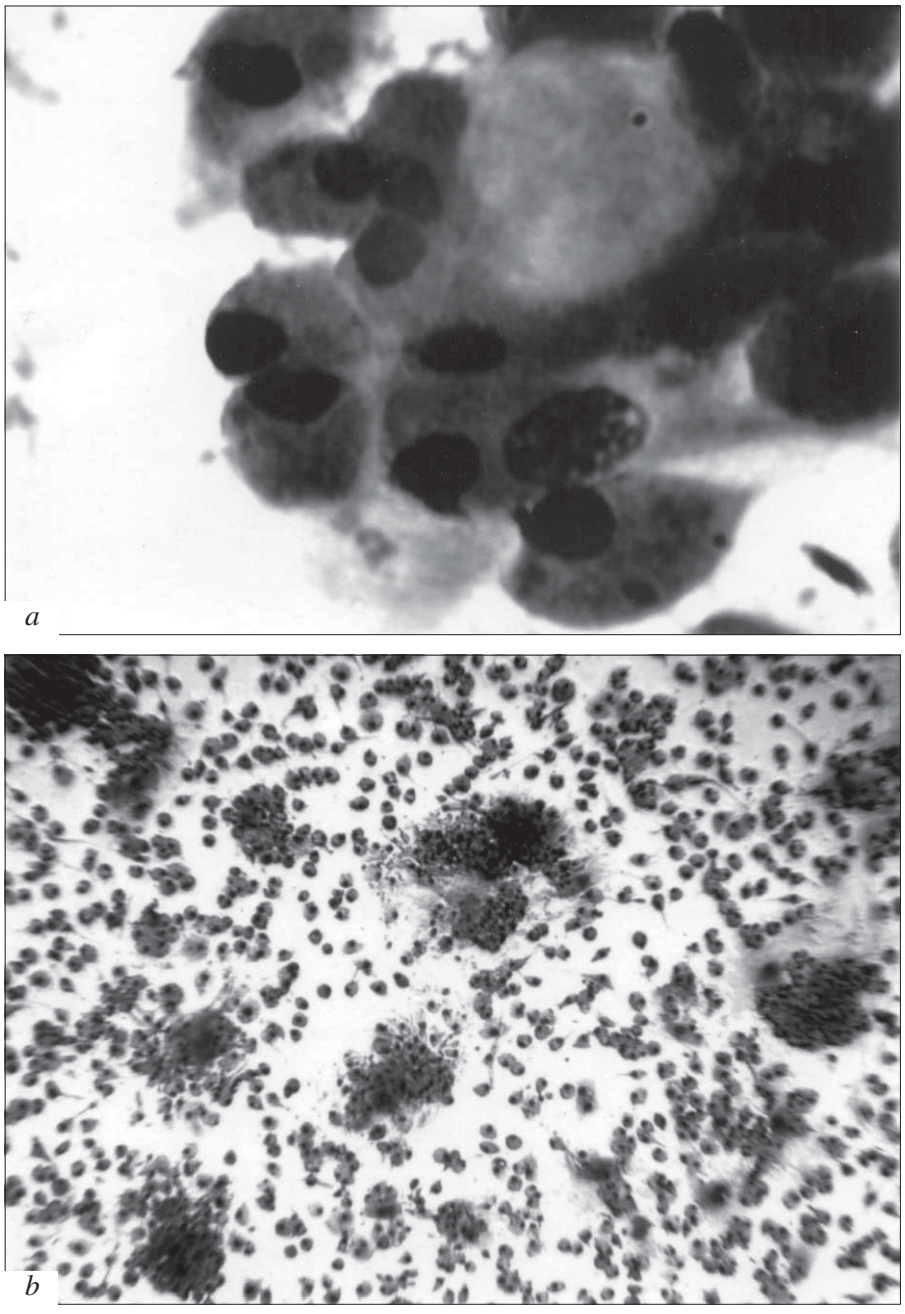

Fig. 1. Individual islet (*a*) and group of mesenchymal islets grown from adherent bone marrow cells of CBA mice on day 12 of culturing *in vitro*. Romanovsky—Giemsa staining (×400, *a*; ×900, *b*).

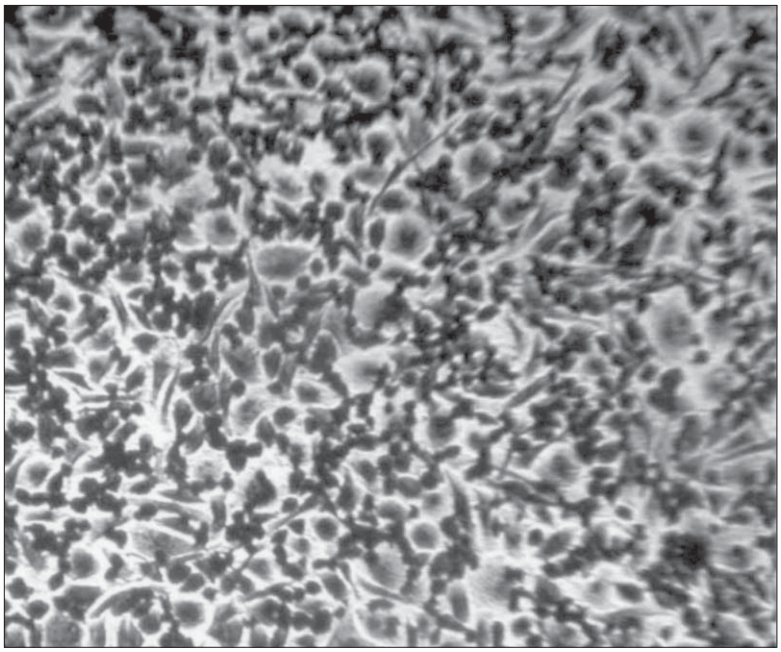

Fig. 2. Mesenchymal colony consisting of numerous islets of different maturity and grown from adherent bone marrow cells of CBA mice on day 14 of culturing. Romanovsky—Giemsa staining, ×400.

sion and formed a monolayer, which is consistent with published data [7].

Specific structures consisting of a large central cell and surrounding round cells (crown) appeared in the culture on day 8 (Fig. 1). The number of structures progressively increased and peaked on the 14th day of culturing, after that their involution and complete disappearance by the 24th day of culturing were observed (Table 1). In contrast to erythroid and hemopoietic islets, the central cell in these complexes differed from macrophages and fibroblasts and had epithelioid structure (Fig. 1). A dose dependence was revealed between the count of passaged cells and number of these structures, which attested to their clonogenic nature. Moreover, large colonies contained numerous mesenchymal islets of different maturity that formed cell assemblies (Fig. 2). It can be hypothesized that the detected structures are mesenchymal structural and functional units of the mesenchymopoiesis that serve as a site of cell development and maturation.

The data show that mesenchymal islets can be formed *in vitro*. They consist of a central epithelioid cell surrounded by round karyocytes. The count of karyocytes varies from 3-6 to 18-20. Apart from round cells, colonies include stromal and other cells of different maturity. These islets exist no longer than 14 days, which is close to the mean period of the existence of mesenchymal colonies. This period is sufficient for the formation of more differentiated cells. Further investigations are required for evaluation whether these structures exist in living organisms during various periods under normal and pathological conditions.

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