State of Stem Cell Pools in Experimental Diabetes Mellitus

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We studied the state of pools of mesenchymal precursor cells of different maturity in the bone marrow and peripheral blood and the dynamics of the content of regional parenchymal stem cells and stromal precursors in the pancreas during experimental diabetes mellitus. Reduced content of organ-specific stem cells and increased content of stromal precursors in the pancreas in the absence of compensatory reaction of deep reserve mechanisms, mesenchymal bone marrow cells, were revealed.

Key Words: *diabetes mellitus; alloxan; stem cells*

Various pathologies of the pancreas are highly prevalent over the world and occupy a considerable place in the structure of mortality and disability of population in our country. Diabetes mellitus, the pathology that unfortunately cannot be radically cured until now, poses a special health hazard and is of primary social importance [5]. This necessitates more comprehensive investigation of the mechanisms of this pathology and the role of adaptation reaction of the organism in the compensation of disturbed functions of the endocrine apparatus of the pancreas. According to current views, tissues of adult organism contain special cell elements, stem cells (SC), which are characterized by high proliferative and differentiation potential and can support physiological and reparative regeneration [2,3]. Morphofunctional recovery of various organs after their alteration is possible due to not only stimulation of regional SC, but also migration of highly plastic cells (mesenchymal SC, MSC) from other tissues, first of all, from the bone marrow [3].

Here we studied the state of bone marrow and circulating pools of mesenchymal precursor cells of different maturity and regional SC of the pancreas during experimental diabetes mellitus.

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MATERIALS AND METHODS

The experiments were carried out on 2-month-old male and female CBA/CaLac mice (n=215, conventional certified mouse strain obtained from the Department of Biomedical Modeling, Institute of Pharmacology, Tomsk Research Center, Siberian Division of Russian Academy of Medical Sciences)

Chronic alloxan-induced diabetes mellitus was modeled by subcutaneous injection of alloxan hydrate (300 mg/kg daily, for consecutive 4 days and then on day 7 after the 4th injection in a volume of 0.2 ml/mouse). On days 8, 11, 15, and 21 of the experiment the blood level of glucose was measured and morphological examination of the pancreas was carried out. We also determined the content of fibroblast colony forming units (CFU-F) in the bone marrow and peripheral blood [4] and the number of CFU-F and regional parenchymal SC in the pancreas (CFU_P). The number of MSC in the bone marrow and peripheral blood was evaluated on day 8 [6,8]. Blood glucose was measured after overnight fast using an Optilite glucometer. For morphological examination, a fragment of the pancreas adjacent to the spleen was fixed in 10% formalin and embedded in paraffin.

Deparaffinized 5-μ sections were stained with hematoxylin and eosin. The area of 10 consecutive

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islets of Langerhans was measured using computed-aided graphic analysis, the total number of cells in these islets and the number of pyknotic cell elements were determined, and the number of cells per islet area unit and the percentage of pyknotic elements were calculated. The number of CFU_P was determined by culturing the suspension of pancreatic cells in 24-well plates in a culture medium containing 80% DMEM, 20% non-inactivated FCS, 10 g/liter glucose, 500 mg/liter L-glutamine, 50 mg/ml gentamicin, 5000 U/liter heparin, 50 mg/liter insulin, 10 ng/ml SC growth factor, 30 ng/ml epidermal growth factor, 10 ng/ml leukemia-inhibiting factor, 10 ng/ml IL-6, and 10 ng/ml basic fibroblast growth factor. The cells were incubated in a CO_2-incubator at 37°C, 5% CO_2, and 100% humidity for 21 days. Then the number of multicellular (>30 cells) acinus-like formations (clonogenic units) was evaluated using an MBS-9 binocular microscope (×56).

The data were processed statistically using Student's *t* test, and nonparametric Mann—Whitney *U* test. The incidence of MSC in the bone marrow and peripheral blood was evaluated using generalized lineary model for Poisson distribution. The correspondence of limiting dilutions to unidimensional Poisson model was evaluated by linear log-log regression. The distribution of theoretic fraction of negative wells μ_i was described by an equation: $\mu_i=\exp(-fx_i)$, where f is the incidence of MSC and x_i is the number of cells seeded to the well [6,8]. Statistica 6.0 software was used.

RESULTS

Injection of alloxan induced pyknosis of many cells in islets of Langerhans; these changes were observed at all terms of the experiment and peaked on day 8 (417% from the baseline). Pronounced edema and hyperemia of the endocrine apparatus with abundant lymphocyte-macrophage infiltration of the tissue were seen. This can explain the absence of changes in the number of cells per islet unit area despite the action of the toxic agent on β-cells [1,7] (Fig. 1). A regular reflection of pathomorphological changes in the pancreas was the development of sustained glycemia. We observed a considerable increase in the peripheral blood glucose level throughout the observation period; this parameter peaked on day 8 of the experiment (541.5% from the initial value, Table 1).

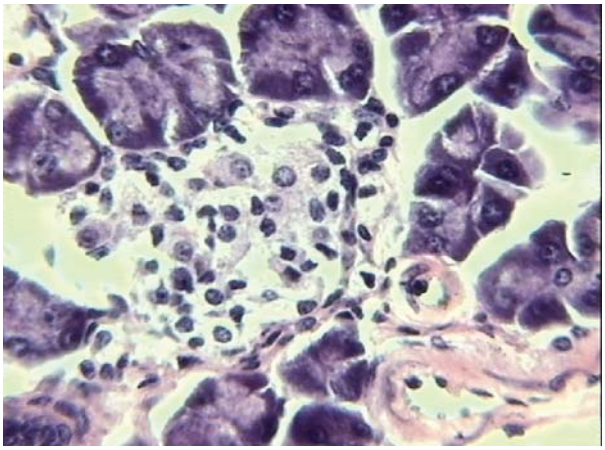

Fig. 1. Fragment of the pancreas containing an islet of Langerhans on day 21 after diabetes mellitus modeling. Hematoxylin and eosin staining, ×600.

The number of CFU-F containing committed precursors of stromal elements and true SC [2] in the bone marrow increased on days 11 and 50 of the experiment. At the same time, the number of primary mesenchymal cells in the hemopoietic tissue did not significantly increase (Fig. 2). These changes in the state of CFU-F pool were nonspecific and were associated with activation of stress-realizing systems of the organism during the development of the alloxan-induced pancreatic disease.

The study of the dynamics of the content of different SC in the peripheral blood revealed no signs of mobilization of committed and primitive SC-precursors into the blood. On day 21 of the ex-

TABLE 1. Dynamics of Peripheral Blood Parameters and Morphological Examination of the Pancreas in CBA/CaLac Mice with Alloxan-Induced Diabetes Mellitus (*X*±*m*)

Parameter	Baseline	Day of experiment			
		8	11	15	21
Number of pyknotic cells in the islet of Langerhans	3.88±0.23	16.18±0.89*	14.35±0.94*	8.80±1.06*	11.80±1.97*
Number of cells per area unit of islet of Langerhans	0.75±0.04	0.75±0.03	0.77±0.03	0.74±0.03	0.90±0.07
Peripheral blood glucose content, mmol/liter	3.16±0.39	17.11±1.15*	12.79±0.96*	13.72±0.59*	13.03±0.90*

Note. *$p \le 0.05$ compared to baseline values.

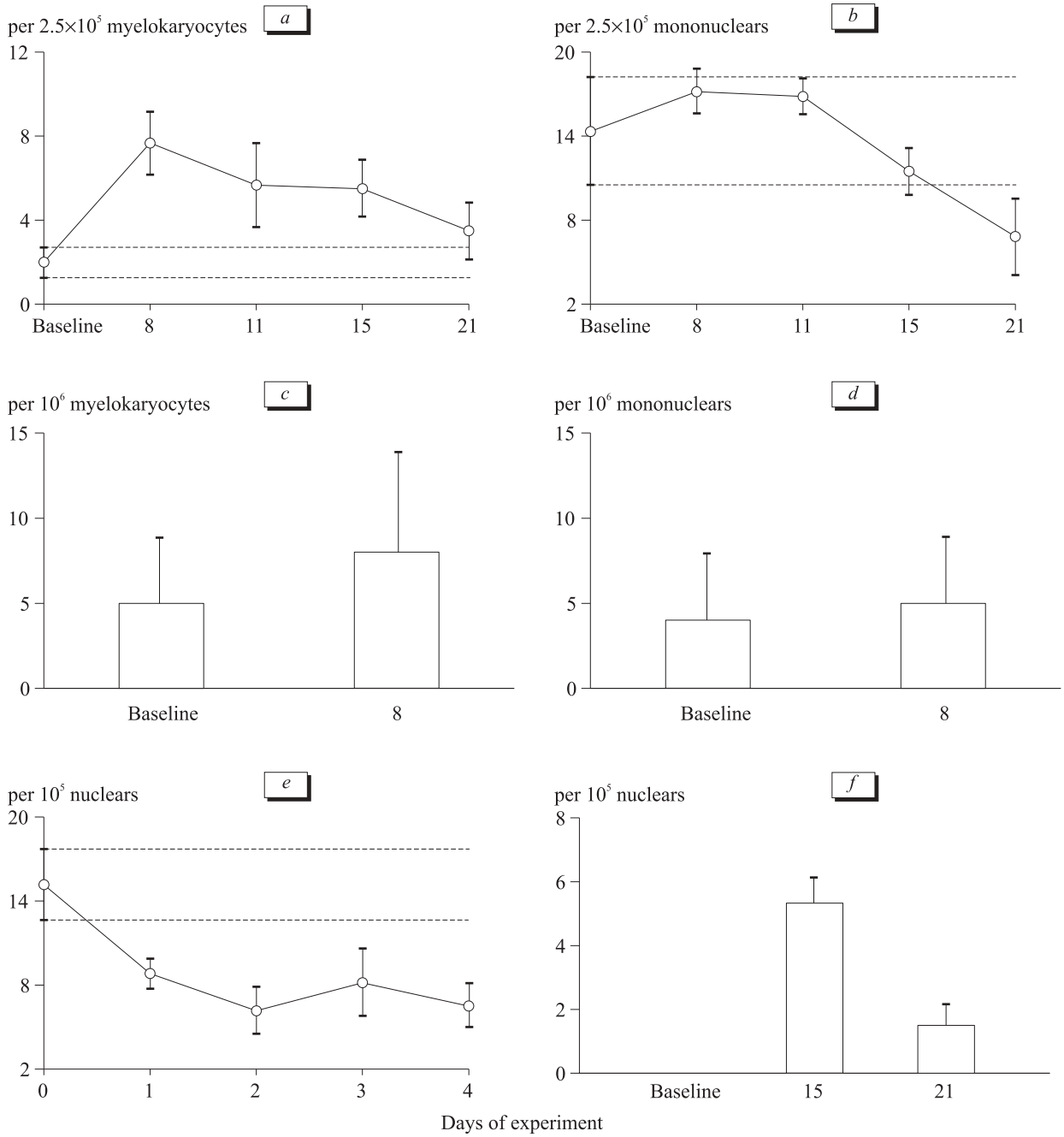

Fig. 2. Content of CFU-F (*a*) and MSC (*c*) in the bone marrow and CFU-F (*b*) and MSC (*d*) in the peripheral blood, number of CFU_P (*e*) and CFU-F (*f*) in the pancreas of CBA/CaLac mice with experimental diabetes mellitus. Dotted lines show confidence interval for the test parameter in intact mice at $p<0.05$.

periment the content of CFU-F significantly decreased (compared to baseline values), which was probably related to settling of SC of different maturity under conditions of this experimental model. Nevertheless, even in the absence of homing of these cell elements against the background of the absence of mobilization of CFU-F and MSC from the bone marrow, the content of regional precursor cells in the pancreas was not replenished. The content of CFU_P in the pancreas decreased throughout the experiment (minimum values, 40.7%, were recorded on day 11), which was probably determined by the action of the toxic agent on these elements characterized by high proliferative potential [2,3] and their high mitotic activity under conditions of this experimental model. At the same time, mesenchymal precursor cells (CFU-F) appeared in the pancreas at later terms (days 15 and 21, Fig. 2), but

their biological role cannot be determined on the basis of our results. This phenomenon can be related to activation of sclerotic processes or intensification of restitution processes. Regeneration of the tissue microenvironment, which was also damaged after alloxan treatment, can also take place. However, we know that microenvironment in its turn considerably modulates functional activity of organ-specific cells *in situ*.

Thus, experimental diabetes mellitus was associated with exhaustion of the pool of parenchymal SC of the pancreas, increase in the content of stromal precursor cells in the organ against the background of the absence of targeted compensatory reaction of deep reserve adaptation mechanisms, bone marrow MSC.

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