Mechanisms of Therapeutic Effects of Granulocytic Colony-Stimulating Factor in Experimental Diabetes Mellitus

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We studied the mechanisms of therapeutic effects of granulocytic colony-stimulating factor in experimental diabetes mellitus. It was found that this preparation increased the number of mesenchymal precursor cells in the bone marrow and peripheral blood with subsequent increase in the content of progenitor cells in the pancreas. These results attest to mobilization of mesenchymal progenitor cells and their migration into the damaged tissue accompanied by morphofunctional recovery of the insulin-producing apparatus.

Key Words: *diabetes mellitus; alloxan; granulocytic colony-forming factor; progenitor cells*

Diabetes mellitus (DM) is a prevalent severe disease. Unfortunately, there are no radical methods for the treatment of this condition [1,8]. Recent advances in cell technologies open the possibilities for the development of new approaches to the treatment of this pathology [2]. Pharmacological stimulation of the functions of endogenous progenitor elements based on simulation of natural regulatory functional systems is the most physiological method of regenerative medicine [4,5,9]. Granulocytic colony-stimulating factor (G-SCF) is a well-studied and perspective modifier of the function of stem cells for the use in clinical practice in degenerative diseases [5,9].

Here we studied therapeutic effects of G-CSF and mechanisms of their action in modeled DM.

MATERIALS AND METHODS

The experiments were carried out on 2-month-old male and female CBA/CaLac mice (n=180, conventional mouse strain obtained from the nursery of Institute of Pharmacology, Tomsk Research Center, Siberian Division of Russian Academy of Medical Sciences). Chronic alloxan-induced MD was modeled by subcutaneous injections of alloxan monohydrate according to the following scheme: 300 mg/kg for 4 days and then 300 mg/kg on day 7 after the last injection (in a volume of 0.2 ml/mouse). G-CSF (Neutrostim) was injected subcutaneously 5 times in a dose of 125 μg/kg in 0.2 ml vehicle once a day starting from day 5 of the experiment. Controls received equivalent volume of the solvent according to the same scheme.

On days 8, 11, 15, 21, 28, and 40 of the experiment, animal mortality, body weight gain, and glucose content in the peripheral blood were evaluated and morphological study of the pancreas was performed. Blood glucose was measured after overnight fast using 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 equal to 10 Langerhans islets was determined

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by a graphic image computer analysis and the total number of cells, the number of pyknotic cells, the number of cells per islet area unit, and the percent of pyknotic cells were evaluated. On days 8, 11, 15, and 21, the content of fibroblast precursor cells (CFU-F) [7] and mesenchymal stem cells (MSC) [10,12] in the bone marrow and peripheral blood, the number of CFU-F and regional parenchymal precursor cells in the pancreas [3], and the capacity of cell components of bone marrow microenvironment to bind stromal precursors [7] were evaluated.

The data were processed by methods of variation statistics 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 linear model for Poisson distribution [10,12].

RESULTS

Animal mortality was observed in both groups after the 3rd injection of alloxan. By the end of the experiment this parameter attained 23.08 and 15.38% in the control and experimental groups, respectively. DM modeling led to a decrease in weight gain, but in mice receiving G-CSF this parameter significantly surpassed the control.

Injection of alloxan led to pyknosis of many cells of islets of Langerhans, which was observed at all terms of the experiment and peaked on day 8 (417.0% 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 [1,11] (Fig. 1). A regular reflection of pathomorphological changes in the pancreas was the development of sustained glycemia. Considerable increase in glucose content in the peripheral blood was observed throughout the experiment (Fig. 1).

Evaluation of the effect of G-CSF on morphofunctional state of the pancreas during DM modeling showed that administration of G-CSF considerably decreased the number of cells per islet unit area (days 11 and 21) by reducing the degree of infiltration of islets. At the same time, the number of pyknotic cells decreased on days 8 and 11 compared to the corresponding parameters in controls (by 40 and 36%, respectively; Fig. 1). These changes were accompanied by a considerable decrease in glucose content in the peripheral blood (days 15, 21, and 28); baseline values were attained by the end of the experiment (day 40, Fig. 1).

At the next stage, we studied the mechanisms of the therapeutic effects of G-CSF related to progenitor elements of various classes.

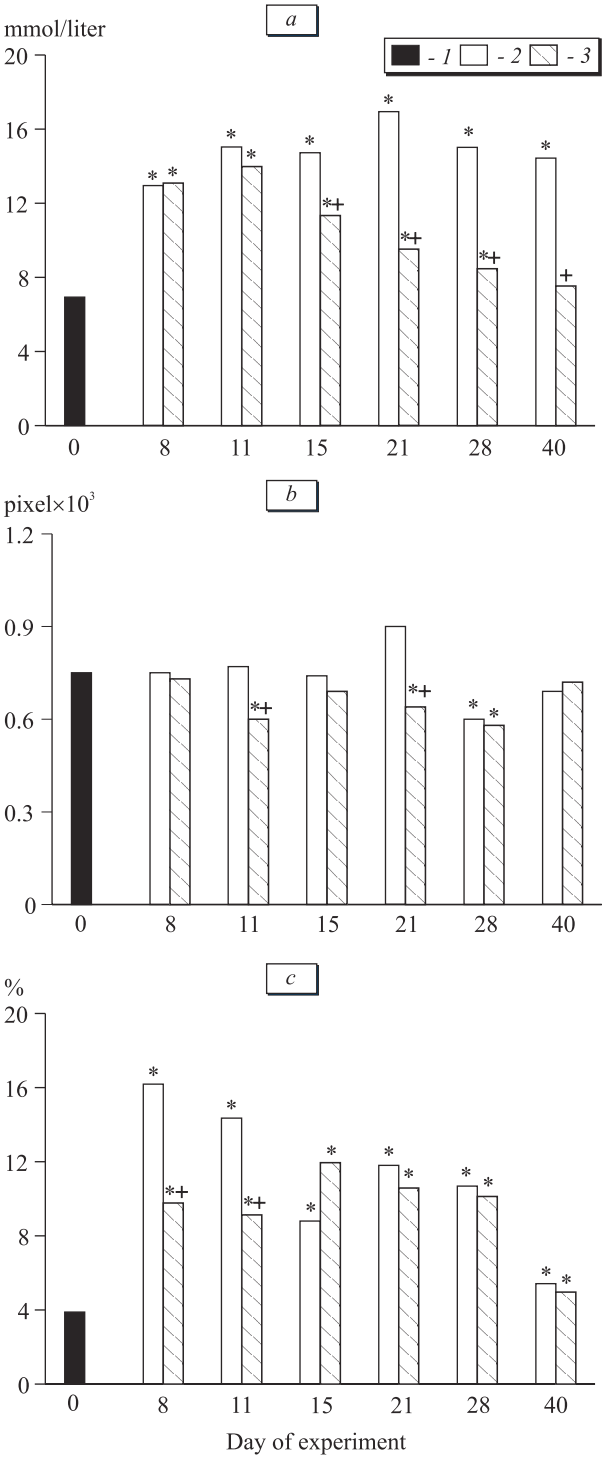

Fig. 1. Dynamics of glucose content in the peripheral blood (*a*), total number of cells pre unit area (*b*) and number of pyknotic cells in Langerhans islet (*c*) in CBA/CaLac mice with experimental DM treated and not treated with G-CSF. *1*) baseline; *2*) experimental DM; *3*) treatment with G-CSF. Abscissa: day of experiment. **p*<0.05 compared to: *baseline, +control.

TABLE 1. Dynamics of the Content of Progenitor Cells in CBA/CaLac Mice after Course Treatment with the Preparation against the Background of Experimental Diabetes Mellitus ($X \pm m$)

Day of experiment		CFU-F, per 2.5×10^5 nuclears			Parenchymal precursor cells in the pancreas
		bone marrow	peripheral blood	pancreas	
Baseline		2.00±0.37	14.33±1.98	0	15.17±1.3
Day 8	control	7.67±0.76*	17.17±0.79	0	8.83±0.54*
	G-CSF	4.67±1.17	22.83±1.87*+	0	8.00±0.52*
Day 11	control	5.67±1.05*	16.83±0.65	0	6.17±0.87*
	G-CSF	4.83±0.60*	17.33±1.45	0	9.5±0.76*+
Day 15	control	5.50±0.67*	11.50±0.85	5.33±0.42*	8.17±1.25*
	G-CSF	3.67±0.56*	12.67±0.99	7.67±1.02*+	3.83±0.31*+
Day 21	control	3.50±0.67	6.83±1.40*	1.50±0.34*	6.50±0.76*
	G-CSF	0.50±0.22*+	11.33±1.50	0.67±0.21*	6.17±0.31*

Note. *$p \leq 0.05$ compared to: *baseline, +control.

Cell culture study revealed an increase in the number of CFU-F in the bone marrow on days 11 and 15 of the experiment, the number of MSC remained at the baseline level (Fig. 2). These changes in the state of CFU-F pool were nonspecific and were associated with activation stress-realizing systems of the organism during the development of the alloxan-induced pancreatic disease. Evaluation of the dynamics of the content of progenitor cells in the peripheral blood reveled a sharp decrease in the number of circulating CFU-F (day 21). These changes were recorded against the background of reduced CFU-F-binding capacity of bone marrow microenvironment cell. Moreover, the number of parenchymal precursor cells in the pancreas was decreased throughout the experiment, probably due to toxic action of alloxan [1,11]. At the same time, mesenchymal precursor cells (CFU-F) appeared in the pancreas at later terms (days 15 and 21, Table 1); the biological role and function of these cells cannot be determined from the obtained results.

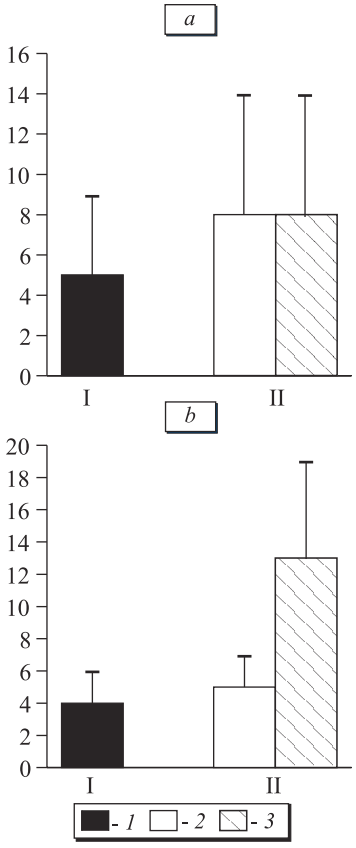

Fig. 2. Content of MSC in the bone marrow (*a*) and peripheral blood (*b*) in CBA/CaLac mice with experimental DM treated and not treated with G-CSF. I: baseline; II: day 8 of the experiment. *1*) baseline; *2*) experimental DM; *3*) treatment with G-CSF. Abscissa: *a*) per 10^6 myelokaryocytes; *b*) per 10^6 mononuclears. Confidence intervals at $p<0.05$.

The study of mechanisms of regeneration of "deep" reserve associated with stem cells [2,4,9] under the effect of G-CSF revealed the following phenomena. Course treatment with the preparation led to considerable mobilization of parental cells accompanied by a decrease in the number of committed mesenchymal precursors in the bone marrow (day 21; Table). The number of CFU-F and MSC in the peripheral blood considerably increased on day 8 of the experiment. These shifts were not related to changed interaction between adherent cells of the hemopoietic tissue and progenitor elements. Mobilization of the parental cells was accompanied by considerable changes in the pool of regional progenitor elements of the pancreas. We observed a considerable increase in the number of parenchymal stem cells (to 154.0% compared to the control on day 11) and stromal precursors (to

143.0% on day 15) in the pancreas (Table 1). Taking into account the results of histological study demonstrating the absence of sclerotic processes after treatment with G-CSF, the increase in the content of CFU-F in the target organ can be considered as a result of intensification of restitution processes, in particular, recovery of the microenvironment regulating functional activity of organ-specific cells *in situ* [2,4].

On the whole, these results attest to pronounced therapeutic effects of G-CSF in experimental DM. The mechanisms of the effects of G-CSF are probably based on stimulation of the progenitor elements of various classes.

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