Mechanisms Underlying the Effects of Ultralow Doses of Antibodies to Granulocytic Colony-Stimulating Factor on Recovery of Damaged Pancreatic Tissue in Experimental Diabetes Mellitus

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Using the model of alloxan-induced diabetes mellitus on rats we demonstrated the effect of ultralow doses of antibodies to granulocytic colony-stimulating factor on recovery of the pancreas and normalization of blood glucose concentration. The preparation produced antiinflammatory and antisclerotic effects associated with activation of stem cells and their determined homing into the pancreas.

Key Words: *ultralow doses of antibodies; granulocytic colony-stimulating factor; diabetes mellitus; alloxan*

Therapy of diabetes mellitus (DM) is still an urgent problem of modern medicine. The number of patients with DM increases in all countries and according to WHO data reaches 150 mln. [3]. DM has great social importance because it is associated with early disability and high mortality [1,3] and no radical methods of DM treatment were proposed yet [8]. Therefore, the search for drugs restoring the insulin-producing function of the pancreas is of primary importance. A new approach to the therapy of DM can be mobilization of endogenous stem cells with their subsequent homing into the damaged tissues with pharmacological agents, because this will make it possible to retire insulin therapy and to avoid complications related to transplantation of β-cells of Langerhans islets.

Here we studied pancreatoprotective activity of a preparation containing ultralow doses (ULD) of antibodies to granulocytic CSF (G-CSF) in experimental DM. Previous studies demonstrated high efficiency of this preparation in toxic hepatitis [9].

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MATERIALS AND METHODS

The experiments were carried out on 2-month-old male and female CBA/CaLac mice (n=150), 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 diabetes induced by daily injections of 300 mg/kg alloxan for 4 consecutive days and than one more time 7 day after the last injection served as the model of DM. The preparation containing ULD of antibodies to G-CSF in dilutions C12+C30+C200 was administered daily in a dose of 0.2 ml *per os* starting from the first day of the experiment. Controls received distilled water according to the same scheme.

On days 8, 11, 15, 21, 28 and 40 of the experiment, peripheral blood glucose level was measured

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and morphological analysis 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 by standard histological methods. Deparaffinized 5-μ sections were stained with hematoxylin and eosin. On sections, the area of 10 consecutive Langerhans islets was determined by the method of computer assisted graphic analysis and the number of cells per islet area unit and the percent of pyknotic cells were calculated.

Using the method of limiting dilutions in our modification, we evaluated the content of fibroblast precursor cells (CFU-F) in the bone marrow and peripheral blood and the number of CFU-F and regional parenchymatous precursor cells in the pancreas on days 8, 11, 15, and 21 and the number of mesenchymal stem cells (MSC) in the bone marrow and peripheral blood on day 8 of the experiment.

The data were processed 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. The correspondence of the data obtained by the method of limiting dilutions to unidimensional Poisson model was evaluated by linear log-log regression. The 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 [12].

RESULTS

The study of histological preparations of mouse pancreas showed that in animals injected with alloxan cell count per islet area unit did not change over 21 days compared to intact animals despite the death of endocrine cells under the effect of the preparation. This is related to the development of moderate lymphocyte-macrophage infiltration in response to damage. On day 28 of the experiment, the number of cells in islets decreased compared to that in intact animals and fibroblasts appeared, which attested to sclerosis development. On day 40, the cellularity of the endocrine apparatus was restored (Fig. 1, *b*). Alloxan also induced pyknosis of some cells in Langerhans islets. The percent of pyknotic cells in the islet considerably surpassed the background level and remained high until the end of observation, the maximum level 417% was observed on day 8 of the experiment. The pancreatic tissue was characterized by edema and hyperemia at all terms of the study (Fig. 1, *c*).

Administration of ULD of antibodies to G-CSF to mice with alloxan-induced DM significantly decreased the number of cells per islet area unit on days 11-21 due to a decrease in islet infiltration (Fig. 1, *b*). Moreover, the number of pyknotic cells in the islet on days

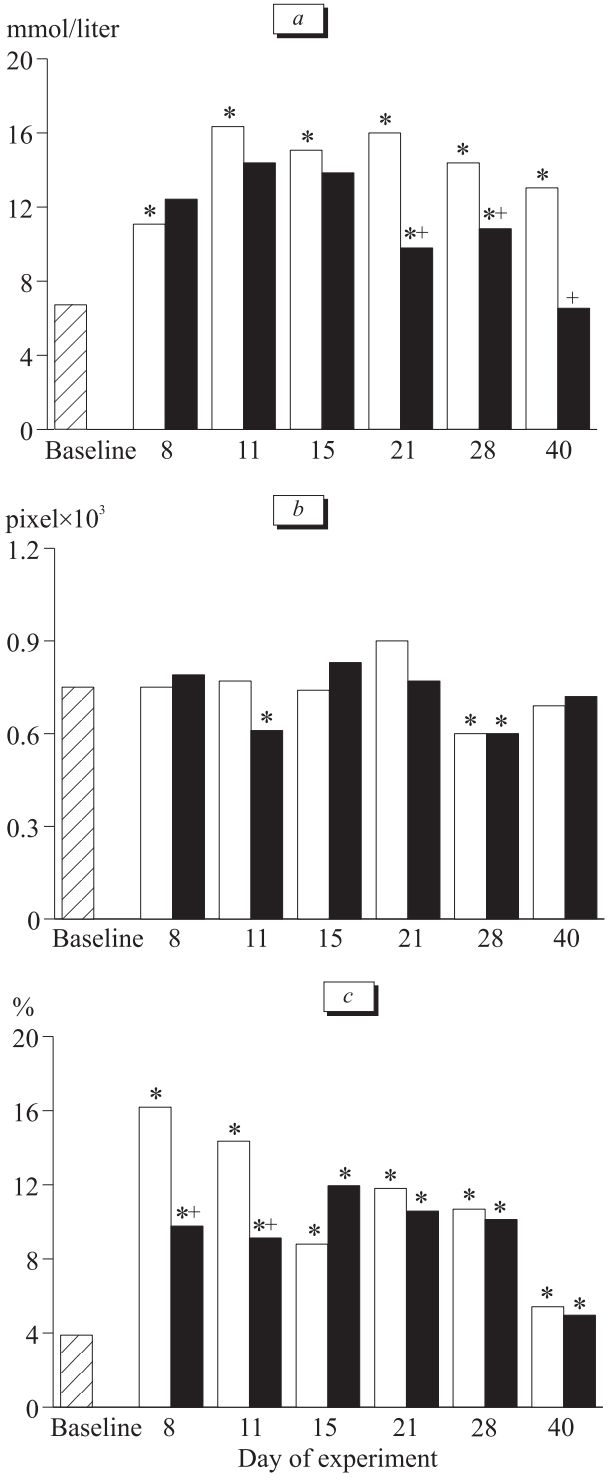

Fig. 1. Dynamics of blood glucose content (*a*), cells per Langerhans islet area unit (*b*), and pyknotic cells in Langerhans islet (*c*) in CBA/CaLac mice with experimental DM (open bars) and therapy with ULD of antibodies to G-CSF (dark bars). Here and on Fig. 2: $p<0.05$ compared to: *baseline, +control.

8 and 11 decreased compared to that in controls (by 22 and 41%, respectively, Fig. 1, *c*).

In our study, sustained glycemia was the main criterion reflecting dysfunction of the pancreas. The content of glucose in the peripheral blood in control animals considerably increased throughout the experimental period and attained the maximum (243% from the initial value) on day 11 of the study. At the same time, in mice receiving ULD of antibodies to G-CSF blood glucose concentration on days 21 and 28 was significantly lower than in the control and on day 40 returned to the baseline values (Fig. 1, *a*).

Using the cell culture methods we showed that the content of CFU-F including both committed stromal cell precursors and MSC in the bone marrow increased on days 8, 11, and 15 of the experiment. At the same time, in mice receiving ULD of antibodies to G-CSF no significant changes in CFU-F content in the bone marrow were observed; on days 8 and 15 this parameter was below the control (Fig. 2, *a*, Table 1). The content of MSC in the bone marrow of control and experimental mice remained unchanged (8±3 and 10±3 per 10^6 myelokaryocytes, respectively, *vs.* baseline value of 5±2 per 10^6 myelokaryocytes).

Evaluation of the dynamics of the content of various stem cells in the peripheral blood against the background of DM revealed no signs of their mobilization into circulation. On the contrary, the number of circulating CFU-F significantly decreased on day 21 of the experiment compared to baseline values (Fig. 2, *b*). Moreover, the content of parenchymatous precursor cells of the pancreas decreased throughout the observation period (maximum drop by 60% was observed on day 11), which was most likely related to the toxic effect of the agent (Fig. 2, *d*). On days 15 and 21, CFU-F appeared in the pancreas (Fig. 2, *c*). This phenomenon probably underlies the development of fibrous tissue in the pancreas during the proliferative phase of alloxan-induced inflammation.

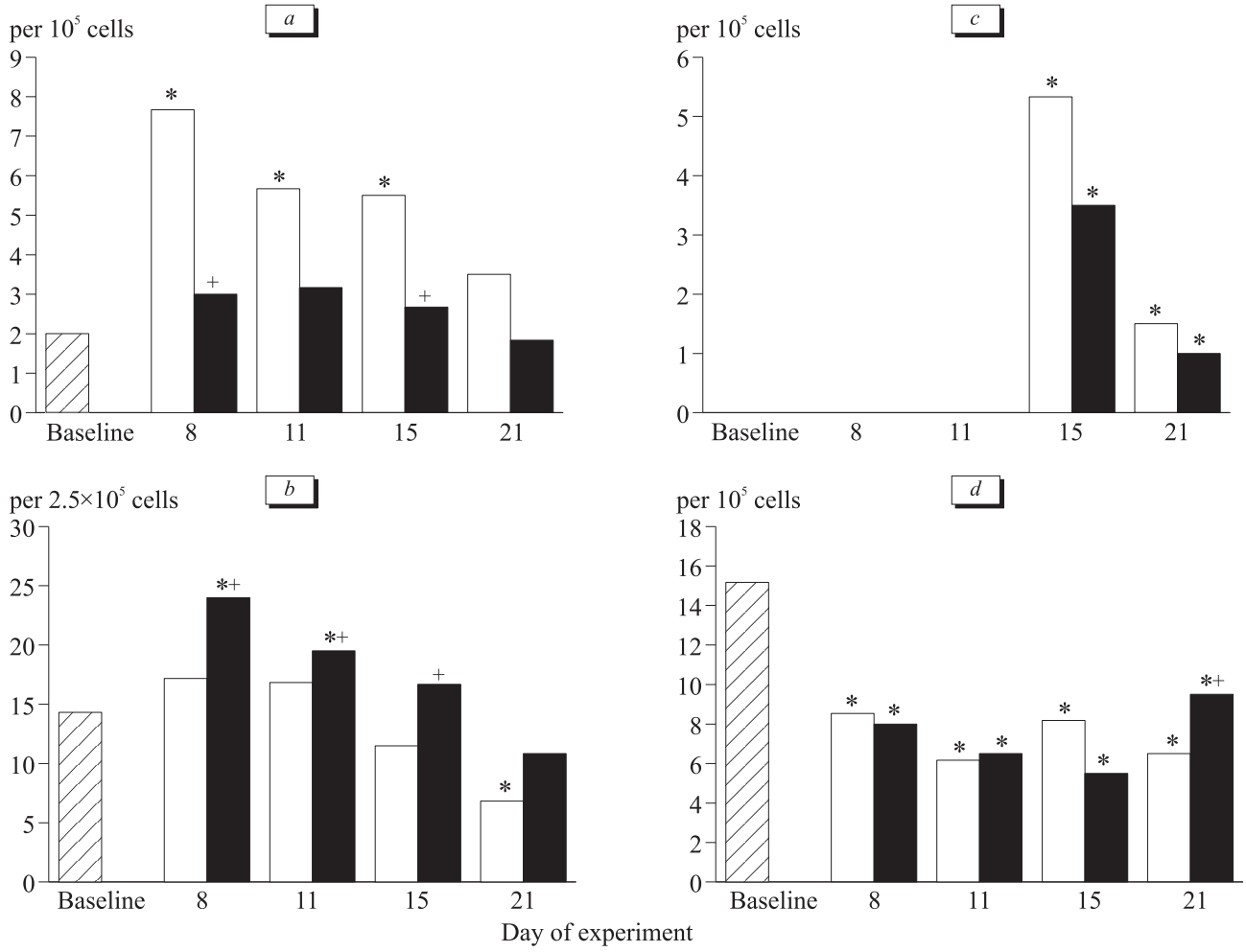

Fig. 2. Content of CFU-F in the bone marrow (*a*), peripheral blood (*b*), and pancreas (*c*) and the count of parenchymatous precursor cells in the pancreas (*d*) of CBA/CaLac mice with DM (open bars) and during therapy with ULD of antibodies to G-CSF (dark bars).

TABLE 1. Dynamics of Various Pools of CFU-F and Content of Parenchymatous Precursor Cells in the Pancreas (per 10^5 Nuclears) in CBA/CaLac Mice with DM after Course Treatment with ULD of Antibodies to G-CSF (*M*±*m*)

Day of experiment	Group	Content of CFU-F in			Content of parenchymal precursor cells in the pancreas
		bone marrow	peripheral blood	pancreas	
Baseline		2.00±0.37	14.33±0.98	0	15.17±1.30
8	Control	7.67±0.76*	17.17±0.79	0	8.83±0.54*
	ULD of antibodies to G-CSF	3.00±0.37+	24.00±1.03*+	0	8.00±0.37*
11	Control	5.67±1.05*	16.83±0.65	0	6.17±0.87*
	ULD of antibodies to G-CSF	3.17±0.48	19.50±0.99*+	0	6.50±1.31*
15	Control	5.50±0.67*	11.50±0.85	5.33±0.42*	8.17±1.25*
	ULD of antibodies to G-CSF	2.67±0.56+	16.67±1.33+	3.50±0.72*	5.50±0.34*
21	Control	3.50±0.67	6.83±1.40*	1.50±0.34*	6.50±0.76*
	ULD of antibodies to G-CSF	1.83±0.60	10.83±1.85	1.00±0.26*	9.50±0.56*+

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

The culture of pancreatic cells obtained by the method of cloning used in our experiments was capable of insulin secretion; the concentration of insulin in the medium increased *in vitro* after adding calcium salts to the medium, which induced hormone release from the producer cell.

Administration of ULD of antibodies to G-CSF induced more pronounced release of MSC (13±3 and 5±2 per 10^6 mononuclears, respectively, *vs*. baseline value of 4±2 per 10^6 mononuclears) and CFU-F (on days 8-15, Fig. 2, *b*) into the peripheral blood, compared to the control. Changes in the state of stem cell pool on whole attest to mobilization and migration of MSC, their possible homing into damaged pancreatic tissue, and their further differentiation into tissue-specific precursors, which manifested in increased content of parenchymatous precursor cells in the pancreas on day 21 (146% compared to the control, Fig. 2, *d*).

These findings suggest that administration of ULD of antibodies to G-CSF to animals with experimental DM induced pronounced changes in the functional state of MSC aimed at stimulation of recovery processes in the pancreas. This activity of the preparation is probably determined by general properties of preparations containing ULD of antibodies to endogenous regulators, *i.e.* stimulation of the production of substances to which they are directed [10]. It is known that G-CSF stimulates the release of hemopoietic and stromal precursors into the blood and increases their functional activity [5].

Thus, the pancreatoprotective effects of the preparation containing ULD of antibodies to G-CSF are determined by stimulation, mobilization, migration, and determined homing of MSC into the pancreas followed by their differentiation into parenchymatous cells and elements of stromal microenvironment.

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