Role of Mesenchymal Precursor Cells in the Stimulation of Wound Healing under the Effect of Ultralow Doses of Antibodies to Granulocytic Colony-Stimulating Factor

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On the model of skin flap we studied the possibility of stimulating the processes of wound healing with a preparation containing ultralow doses of antibodies to granulocytic colony-stimulating factor. The preparation accelerated tissue regeneration against the background of mobilization of bone marrow mesenchymal precursor cells into circulation accompanied by an increase in the number of stromal precursor cells in the area of lesion.

Key Words: *skin flap; regeneration; mesenchymal precursor cells; ultralow doses of antibodies; granulocytic colony-stimulating factor*

Acceleration of wound healing is an important problem of modern medicine. The development of cell technologies considerably extended the possibilities of studying the mechanisms of tissue regeneration associated with progenitor elements. Regional and circulating in the peripheral blood stromal precursor cells actively participate in the process of wound healing vie secondary intention [2]. The population of mesenchymal stem cells (MSC) of the bone marrow, *i.e.* elements of "deep" reparation reserve [1,3], practically does not respond to skin damage [2]. At the same time, recent theoretical data gave way to a new trend in the treatment of some diseases, cell therapy, applying, among other things, pharmacological modification of functions of endogenous stem cells [1,3,5]. For realization of this strategy of regeneratory medicine, granulocytic colony-stimulating factor (G-CSF) is most often used [1,3].

According to modern concept, ultralow doses of antibodies (ULD) to various regulators of physiological processes can produce effects similar to the effects produced by the corresponding bioactive substances [6]. It was experimentally demonstrated that this is also true for modulation of the functional state of stem cells by ULD of antibodies to G-CSF [5].

Here we studied the possibility of stimulating regeneration of surface tissues with ULD of antibodies to G-CSF in dilutions C12+C30+C200 due to modulation of activity of endogenous stem cells.

MATERIALS AND METHODS

The experiments were carried out on 2-month-old male and female CBA/CaLac mice (n=268), conventional mouse strain obtained from the nursery of Institute of Pharmacology, Tomsk Research Center, Siberian Division of Russian Academy of Medical Sciences. Skin wound was modeled under ether narcosis by removal of a 10×10-mm skin flap on the back after depilation. For prolonging wound healing, the crust was removed every other day. The regeneration process was evaluated by the dynamics of the mean diameter of the wound surface, which was measured every other day until complete healing.

The preparation containing ULD of antibodies to G-CSF (Materia Medica Holding) was daily administered *per os* in a single dose of 0.2 ml throughout the

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experimental period. Controls received the same volume of distilled water according to the same scheme.

Using cell culture methods, the content of fibroblast CFU (CFU-F) [4] and MSC [7,8] in the bone marrow and peripheral blood and the content of regional mesenchymal precursor cells in the wound surface [2] were evaluated on days 3, 7, and 14 of the experiment. The content of committed stromal precursors in the zone of damage was determined by culturing of cell material obtained from the wound surface over 7 days in complete nutrient medium supplemented with 30 mg/liter insulin, 10 ng/ml stem cell growth factor, 30 ng/ml epidermal growth factor, 10 ng/ml IL-6, and 10 ng/ml basic fibroblast growth factor (all growth factors were from Sigma).

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 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 [7,8].

RESULTS

After removal of the skin flap we observed a natural dynamics of wound healing. Regeneration of the modeled defect was completed by day 18 of the experiment. Course administration of ULD of antibodies to G-CSF significantly reduced the mean diameter of the wound compared to the control on days 4, 6, and 14 of the experiment (by 9.3, 12.3, and 38.7%, respectively), the wound surface was completely replaced by the connective tissue by day 16.

The content of MSC in the wound surface significantly differed between the groups. In both cases, the content of regional CFU-F gradually decreased probably due to stimulation of their differentiation into specialized cell types [2] finally replacing the removed

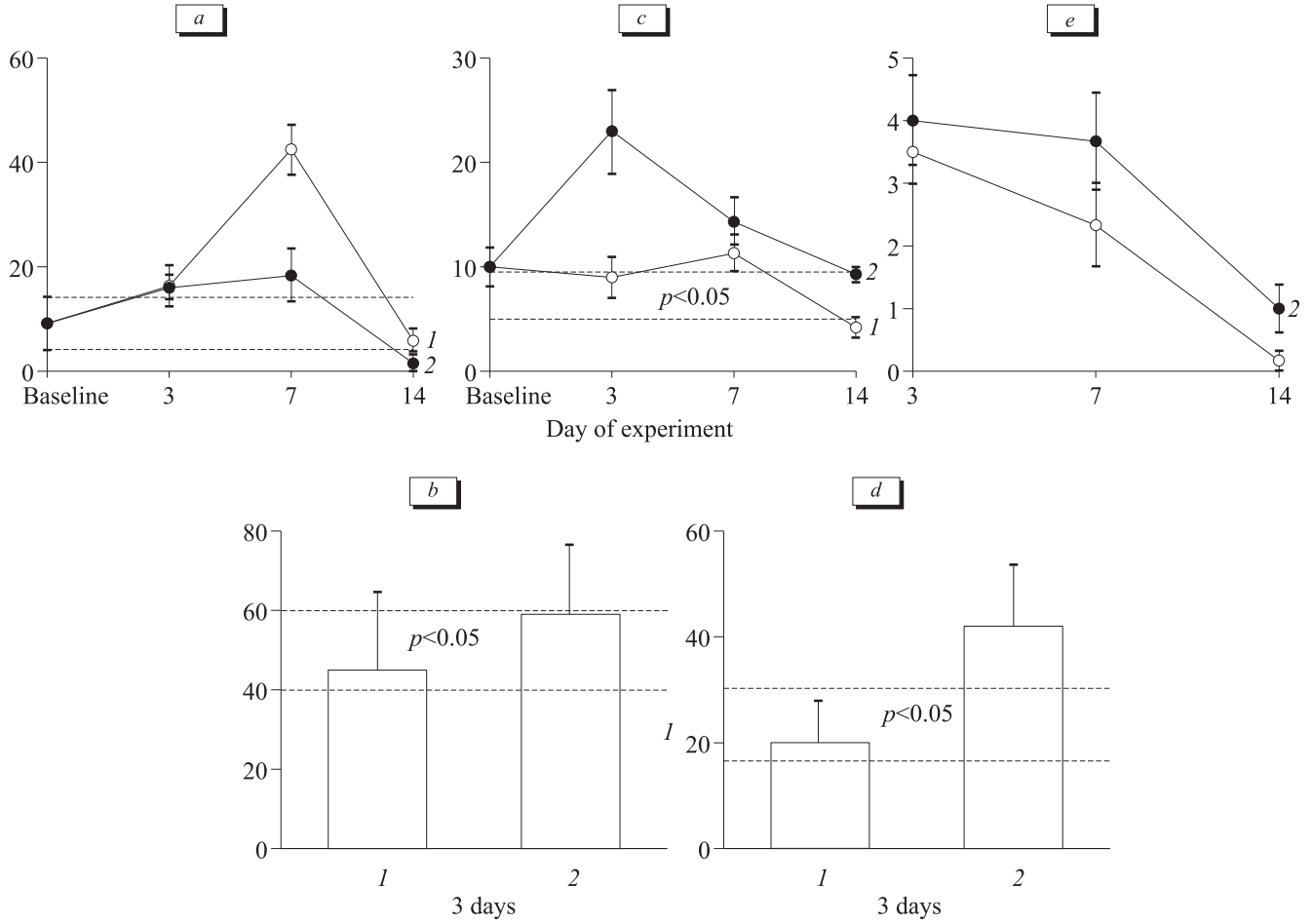

Fig. 1. Content of CFU-F (*a*) and MSC (*b*) in the bone marrow and CFU-F (*c*) and MSC (*d*) in the peripheral blood and number of stromal precursors in the wound area (*e*) in CBA/CaLac mice after removal of skin fragment (*1*) and administration of ULD of antibodies to G-CSF after skin wound modeling (*2*). Ordinate: parameter: per 2.5×10^5 myelokaryocytes (*a*, *c*); per 10^6 myelokaryocytes (*b*); per 10^6 mononuclears (*d*); per 10^5 nuclears (*e*).

skin fragment by the secondary intention mechanism. In animals receiving antibodies to G-CSF, the number of stromal precursor cells in the damaged area considerably surpassed the control value throughout the experimental period (the maximum increase by 488.4% was observed on day 14).

At the next stage we evaluated the participation of "deep" reserve mechanisms involving bone marrow MSC [1,3] in the processes of skin regeneration under the effect of the preparation containing ULD of antibodies to G-CSF. In the control group, minor accumulation of CFU-F was observed, which was probably a result of activation of stress-limiting systems of the organism. These changes were accompanied by a drop in the content of clonogenic stromal elements in the peripheral blood, which was probably associated with their homing into the damaged zone and participation in skin regeneration processes (Fig. 1). MSC did not participate in the regeneration of the tissue defect after removal of the skin fragment, which completely agrees with our previous data [2].

In mice receiving ULD of antibodies to G-CSF, the content of CFU-F in the hemopoietic tissue decreased on days 7 and 14, while their content in the peripheral blood significantly increased on days 3 and 14 of the experiment (by 155.6% and 121.4%, respectively). According to modern views, CFU-F apart from committed MSC can include true MSC [5]. However, our experiments showed that ULD of antibodies to G-CSF had no effect on the state of the pool of bone marrow MSC. At the same time, the number of circulating MSC considerably increased in mice receiving ULD of antibodies to G-CSF (by 90% compared to the control on day 3 of the experiment), *i.e.* we observed their recruitment into the peripheral blood. Taking this fact into account we can assume that the absence of changes in the number of MSC in the hemopoietic tissue is related to extremely high self-regenerating capacity of these progenitor elements [3] and realization of the proliferative potential of these regeneratory-competent cells.

Thus, course administration of ULD of antibodies to G-CSF to mice with removed skin fragment leads to considerable activation of "deep" reparation reserve of stem cells [1,3], which manifests in mobilization of mesenchymal precursor cells of different maturity into the blood accompanied by accumulation of stromal precursors in the damaged area and finally results in accelerated wound healing. The principal mechanism underlying the effect of preparations containing ULD of antibodies to regulators of physiological processes is stimulation of the production of the corresponding endogenous bioactive substances [6].

Our experiments demonstrated principal possibility of stimulating regeneration of surface tissues and some internal organs of the organism [1,3,5] by pharmacological modification of functions of stem cells based on the principle of simulation of the activity of natural regulatory systems.

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