Mechanism of Mobilization of Mesenchymal Stem Cell under the Effect of Granulocyte Colony-Stimulating Factor

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Translated from *Kletochnye Tehnologii v Biologii i Medicine*, No. 3, pp. 169-172, July, 2007
Original article submitted May 10, 2007

We studied the role of cell components and extracellular matrix of the microenvironment in the mechanisms of mobilization of mesenchymal bone marrow stem cells under the effect of granulocyte colony-stimulating factor *in vitro* and *in vivo* on CBA/CaLac mice. It was found that the mechanisms underlying the dissociation effect of granulocyte colony-stimulating factor do not include its direct effect on the interaction of precursor cells with fibronectin. The major role in this process is played by changes in functional state of stromal precursors and cell component of the microenvironment induced by granulocytic colony-stimulating factor and mediated by regulatory systems of the microorganism.

Key Words: *granulocyte colony-stimulating factor; mobilization; fibronectin; mesenchymal stem cells*

Granulocyte colony-stimulating factor (G-CSF) is one of the best studied stimulators of mobilization of various progenitor cells, in particular, mesenchymal stem cells (MSC) from tissue depots, *e.g.* from the bone marrow, into circulation [1,5]. However, the mechanisms underlying enhanced migration of MSC under the effect of G-CSF are not quite understood. Published reports discuss two pathways of the realization of the effect of cytokine: changes in MSC population phenotype and modulation of the microenvironment [4]. The microenvironment consists of cell elements (macrophages, lymphocytes, fibroblasts, endotheliocytes, adipocytes, mast cells, *etc.* and cytokines produced by these cells) and extracellular matrix (protein fibrillar structures, glycosaminoglycans, proteoglycans) [2,7]. Theoretically, G-CSF can modulate the interaction of MSC with both these components.

Here we studied the role of cell components and extracellular matrix in the mechanisms of mobilization of MSC from the bone marrow under the effect of G-CSF.

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MATERIALS AND METHODS

The experiments were carried out on 2-month-old male and female (n=150) CBA/CaLac mice (conventional mouse strain obtained from the nursery of Institute of Pharmacology, Tomsk Research Center, Siberian Division of Russian Academy of Medical Sciences). Experiments were performed in two stages. In *in vitro* experiments, the capacity of bone marrow stromal precursor cells from intact mice to adhere to plastic, feeder of adherent bone marrow elements, or a substrate consisting of 1% gelatin (hydrolyzed fibronectin, component of extracellular matrix) was evaluated. To this end, nonfractionated myelokaryocytes (5×10^5/ml) were layered onto a substrate and incubated for 90 min in complete nutrient medium without or with 5 ng/ml G-CSF (Vector). The optimum time of cell incubation with G-CSF *in vitro* (sufficient for manifestation of its dissociating effect, but insufficient for induction of cell differentiation) and optimum concentration of the cytokine were determined in a series of preliminary experiments. The medium with nonadherent cells was collected and tested in a test-culture according to the standard method of

0007-4888/07/1441-0151

cloning of fibroblast CFU (CFU-F) [3]. Pure medium incubated with the feeder formed by adherent bone marrow cells was used as the control. On day 7 of the experiment, the number of stromal colonies formed by nonadherent cells or cells detached from the control feeder was counted. The studied parameter was evaluated by the difference between these values (number of bound cells).

In *in vivo* experiments, a wound process was modeled. To this end, the animals were narcotized with ether and after depilation a 10×10-mm skin flap on the back was removed. The crust from the wound was removed every other day throughout the experiment. Experimental animals received subcutaneous injection of 125 mg/kg G-CSF once a day for 5 days starting from the next day after wound modeling. Control mice received physiological saline according to the same scheme. On days 3, 7, and 14 of the experiment, the content of CFU-F in the bone marrow and peripheral blood was determined using cultural methods [3] and the number of regional mesenchymal precursors was counted. In the latter case, the cell material obtained from the wound surface was cultured 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). Fibroblast colonies were counted after 7 days. The capacity of stromal precursors from the bone marrow to bind components of the extracellular matrix was studied as described above during natural wound healing and under the effect of G-CSF treatment.

The data were processed by methods of variation statistics by Student's *t* test and nonparametric Mann—Whitney *U* test using Statistica 6.0 software.

RESULTS

In *in vitro* experiments, the number of stromal CFU adhering to plastic in a sublayer of adherent bone marrow cells in pure medium (without G-CSF) was taken as 100%, because no colonies grew in the test cultures. Addition of mobilization factor little affected binding, but led to a release of 0.67% cells from the sublayer (Fig. 1). Binding of bone marrow cells incubated on a feeder of adherent elements in pure culture medium was 84.67%. G-CSF decreased this parameter to 70.67%. Binding of cells from nonfractionated bone marrow to gelatin did not depend on the presence of mobilization factor in the culture medium and in both cases was ~20%. G-CSF added to the medium reduced adherence of nonfractionated bone marrow cells to plastic: the number of adherent cells in pure culture medium was 3-fold higher than in a medium containing mobilization factor (Fig. 1).

These findings suggest that the mechanisms underlying the dissociation effect of G-CSF do not include its direct effect on the interaction of precursor cells with fibronectin. The major role in mobilization of mesenchymal precursors from the bone marrow under the effect of G-CSF is played by the loss of their direct contacts with cell elements in the microenvironment. The changes involve both stromal cells and precursors [6].

In vivo experiments showed than in both groups of animals with experimental wounds, the number of stromal precursors in the bone marrow increased on days 3 and 7 compared to baseline values. Moreover, on day 3 this parameter in mice receiving G-CSF considerably surpassed the corresponding value in the control group (Fig. 2, *a*). These changes were accompanied by a pronounced decrease in CFU-F binding to components of the extracellular matrix compared to that in healthy animals. Moreover, on day 3 this parameter in mice receiving G-CSF considerably surpassed the corresponding value in the control group (Fig. 2, *b*). On days 7 and 14 of the experiment, G-CSF had no appreciable effects on cell binding: there were no differences between the control and experimental groups.

The content of CFU-F in the peripheral blood of control mice did not differ from the control, while in the experimental group this parameter increased by 2 times on days 3 and 14 of the experiment (Fig. 2, *c*). The number of regional mesenchymal precursor cells responsible for regeneration of the damaged tissue increased under the effect of mo-

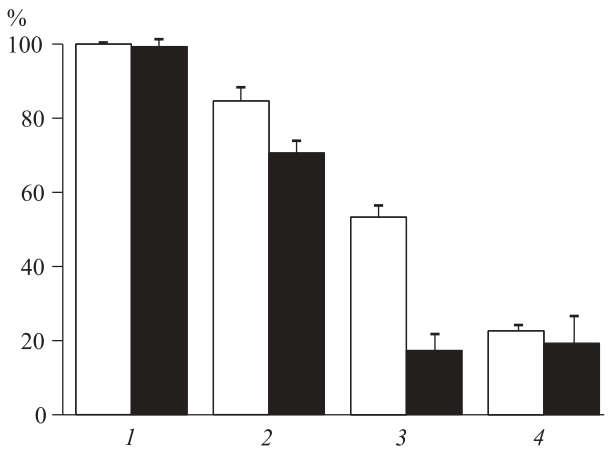

Fig. 1. Number of stromal precursors bound to the substrate after 90-min incubation without (light bars) or with G-CSF (dark bars). *1*) control; *2*) feeder consisting of adherent elements; *3*) bone marrow cells on plastic; *4*) bone marrow cells on gelatin. Here and on Figs. 2: confidence intervals at $p<0.05$.

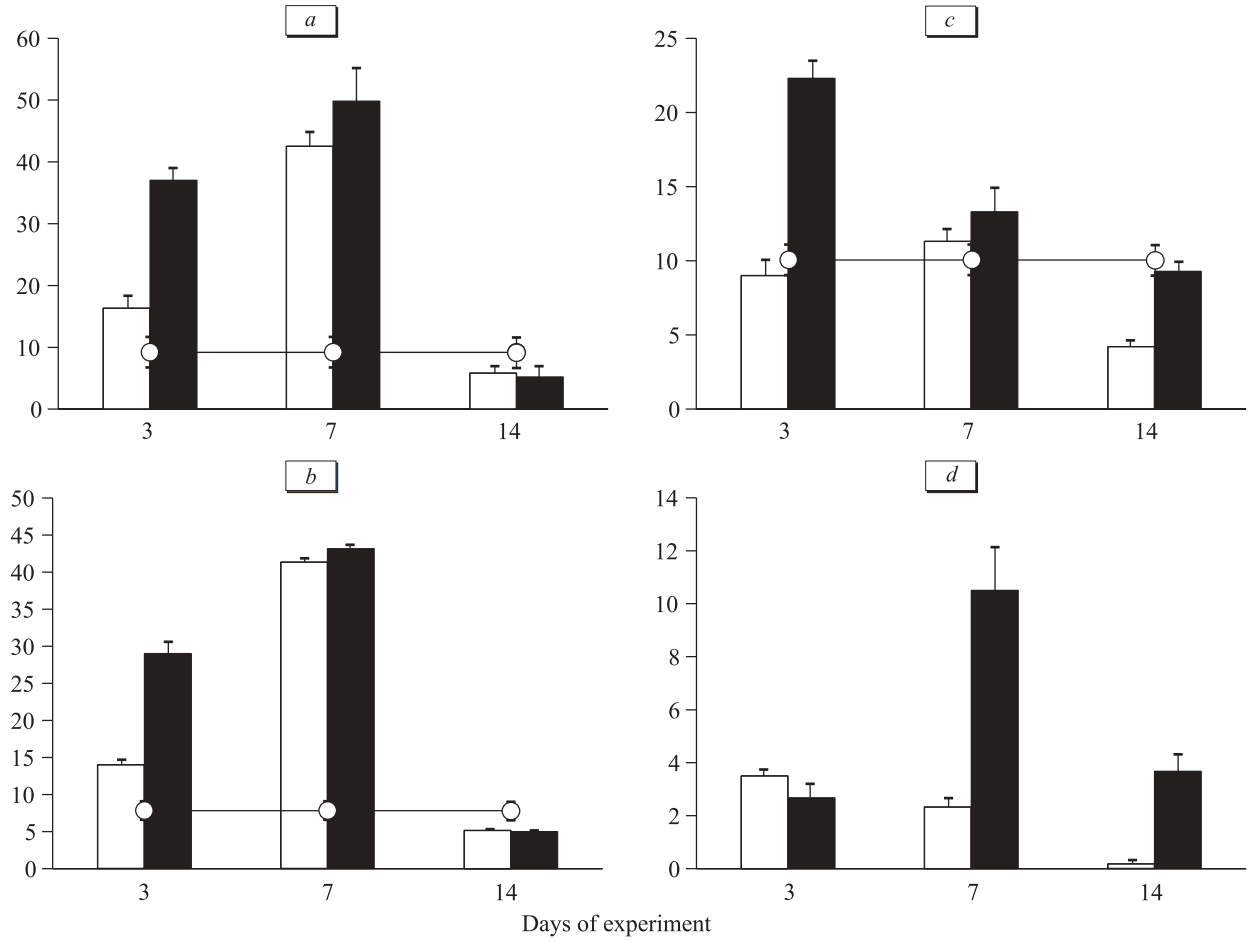

Fig. 2. Effect of G-CSF on CFU-F content in the bone marrow (*a*), peripheral blood (*c*), wound surface (*d*), and number of precursor cells not binding to fibronectin (*b*) in CBA/CaLac mice. Light bars: control; dark bars: experiment; horizontal line shows baseline value. Abscissa: parameter per 250,000 cells.

bilization factor on days 7-14 of the experiment (Fig. 2, *d*).

The absence of a correlation between qualitative and quantitative parameters of CFU-F pool suggests that the mechanisms of mobilization of progenitor cells upon G-CSF treatment are not related to their tropism to fibronectin. The major role in this process is played by G-CSF-induced changes in the functional state of stromal precursors mediated by regulatory systems of the microorganism. The increase in the content of precursor cells in the peripheral blood and in the wound in this case can be explained by not only direct effect of the mobilizing factor, but also by changes in microcirculation and neurohumoral profile. Our findings agree with the data obtained in other extreme exposures, which attests to nonspecific nature of changes and their relationship with the function of general stress-limiting systems of the organism [4].

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