Participation of Mesenchymal Precursor Cells in Wound Healing on Skin Flap Model

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We studied the state of different pools of mesenchymal precursor cells in the bone marrow, peripheral blood, and wound surface after modeling of tissue damage by skin flap removal. The participation of regional and circulating stromal precursors in the healing of skin defect and the absence of compensatory reaction of the regeneratory process deep reserve, mesenchymal stem cells of the bone marrow, was demonstrated.

Key Words: *skin flap; regeneration; regional stem cells; mesenchymal stem cells*

Wound healing is still an actual problem of modern medicine. The interest to this problem is explained by systematic revision of the concept of the course of wound process. The use of modern methods considerably increased the possibility of studying the mechanisms of tissue regeneration. Recently, principally new data were obtained on the role of inflammation, immunological reactivity of traumatized organism, metabolism, enzyme reactions, and cytological transformation in the dynamics of wound healing [9-11]. The latter aspect seems to be most important, because better understanding of skin repair regularities and the role of the mechanisms of deep adaptation reserve (mesenchymal stem cells, MSC) [2,6] opens new stage in the development of cell technologies for various branches of medicine (first of all, surgery and dermatology) and cosmetology.

Here we evaluated the state of different pools of committed and parental mesenchymal stem precursor cells during experimental wound process.

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MATERIALS AND METHODS

The experiments were carried out on 2-month-old male and female CBA/CaLac mice (n=147, 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. On days 3, 7, and 14 of the experiment, the content of fibroblast CFU (CFU-F) in the bone marrow and peripheral blood was determined using cell culture methods [3], fibronectin-binding capacity of bone marrow CFU-F and the number of regional mesenchymal precursor cells in the wound surface were evaluated; the content of MSC in the bone marrow and peripheral blood was determined by the method of limiting dilutions [7,8].

For evaluation of binding of bone marrow CFU-F to extracellular matrix component, we counted stro-

mal colonies formed by nonfractionated bone marrow cells and CFU-F appeared after 2-h incubation of myelokaryocytes on a plate coated with 1% gelatin (hydrolyzed fibronectin). This parameter was evaluated by the difference between these values (number of unbound cells). The content of committed mesenchymal 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 statistically using Student's *t* test, and nonparametric Mann—Whetney *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 one-dimensional Poisson model was evaluated by linear log-log regression. The distribution of theoretic fraction of

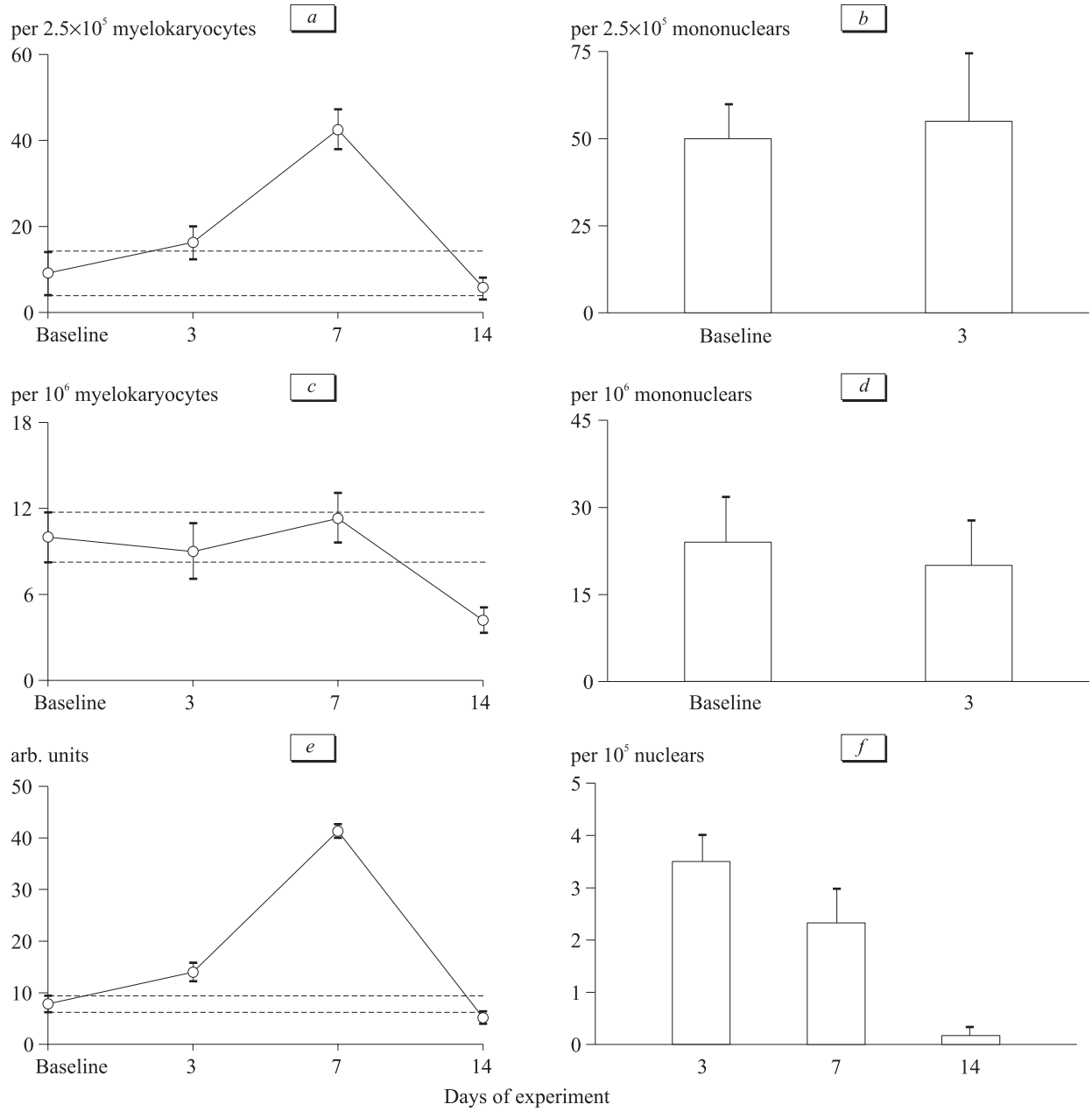

Fig. 1. Content of CFU-F (*a*) and MSC (*b*) in the bone marrow and CFU-F (*c*) and MSC (*d*) in the peripheral blood, binding of CFU-F to extracellular matrix components (*e*) and number of stromal precursors in the wound surface (*f*) of CBA/CaLac mice after wound modeling by skin flap removal. Dotted lines show the confidence area for the test parameter in intact mice at $p<0.05$.

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]. Statistica 6.0 software was used.

RESULTS

Natural dynamics of wound healing was observed during the experiment. Complete regeneration of the experimental skin defect was attained on day 18 of the experiment. The content of mesenchymal precursor cells in the wound surface gradually decreased from 3.50±0.25 CFU-F on day 3 to 0.17± 0.17 CFU-F (per 10^5 nuclears) on day 14 of the experiment (Fig. 1). These changes in the state of regional precursors were probably determined by stimulation of differentiation of these elements into specialized cell types [6] finally replacing the removed skin flap by secondary intention.

We studied the possible role of deep reserve regeneration mechanisms, *i.e.* bone marrow mesenchymal precursor cells [1,2], in the processes of skin healing. We observed an increase in the number of stromal precursor cells in the hemopoietic tissue (to 178.1 and 463.5% from the baseline on days 3 and 7, respectively). This cell pool probably included, apart from committed mesenchymal precursor cells, true (mesenchymal) stem cells [1]. However, the content of MSC in the bone marrow did not change after skin flap removal. The number of these cell elements did not differ from that in intact animals. However, accumulation of CFU-F was accompanied by considerable impairment of their binding to extracellular matrix components on days 3 and 7 of the experiment (Fig. 1). These changes in the population of stromal precursors largely coincided with changes observed in other extreme exposures [2,5], which attests to their nonspecific nature and obvious dependence on functional activity of the stress-realizing systems of the organism.

At the same time, despite impaired binding of precursor cells to extracellular matrix components, skin wound modeling had practically no effect on the dynamics of CFU-F content and the number MSC in the peripheral blood. The only exception was the number of clonogenic stromal elements in the peripheral blood on day 14 of the experiment (Fig. 1), which was probably related to their homing in the damaged zone and involvement in processes of skin regeneration. The absence of correlations between qualitative and quantitative parameters of CFU-F pool suggests the existence of primary cell mobilization mechanisms not related to their adhesion to fibronectin.

Thus, skin wound did not induce MSC mobilization phenomenon. At the same, regional and circulating the peripheral blood stromal precursors actively participate in tissue regeneration by secondary intension.

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