Cell Transplantation Inhibits Inflammatory Reaction and Stimulates Repair Processes in Burn Wound

M. F. Rasulov, V. T. Vasilenko, V. A. Zaidenov, and N. A. Onishchenko

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We compared the effects of transplantation of fetal fibroblasts and fibroblast-like mesenchymal stem cells of the bone marrow on healing of deep burn wound in rats. It was found that transplantation of fetal fibroblasts and fibroblast-like mesenchymal stem cells on the burn surface reduces cell infiltration, promotes the formation of vessels and granulation tissue, which creates conditions for more rapid healing of the burn wounds.

Key Words: *bone marrow; mesenchymal stem cells; fibroblasts; burn*

Recent studies demonstrated efficiency of individual and combined (bioengineering constructions [6,8,10]) application of cultured auto- and allogenic keratinocytes [4,13] and fibroblasts [5,7,9] for the treatment of burn wound [11,12,14].

We previously showed clinical effectiveness of fetal fibroblasts (FF) and fibroblasts-like mesenchymal stem cells (FMSC) from the bone marrow for acceleration of healing of extensive surface and deep burn wounds [2,3].

Here we present the results of comparative morphological (histological) analysis of biopsy specimens from deep burn wounds treated using application of different cells.

MATERIALS AND METHODS

Deep thermal burn was modeled in 30 male Wistar rats weighing 300-350 g. The burn involving all skin layers was modeled under ether narcosis by 8-sec application of a whole metal cylinder with a semiconcave cross-section heated with circulating hot water (97.7°C). The area of burn was 18-20% of the total skin area.

The animals were divided into 3 groups (10 rats per group). Group 1 comprised rats with deep thermal burns not receiving cell therapy (control group), groups 2 and 3 consisted of animals with deep thermal burns receiving allogenic FF and allogenic FMSC, respectively.

Suspended FF and FMSC (2×10^4 cells) were applied on the surface of burn wound with a pipette on day 2 after burn modeling and removal of necrotic crust. After transplantation the burned surface was covered with gauze soaked with physiological saline containing gentamicin.

FF were isolated from the lungs of 14-17-day-old rat embryos. The lung tissue was minced, the fragments were trypsinized in an incubator for 10-15 min and then suspended by pipetting. The cell suspension was centrifuged at 1500 rpm for 5 min and the supernatant was completely removed. FF sediment was resuspended in DMEM supplemented with FCS.

Bone marrow cells were washed from the femurs of ether-narcotized adult rats with 0.5 ml physiological saline through a 16G needle. The cell suspension was centrifuged at 1500 rpm for 5 min, the sediment was resuspended in lysing solution for 3 min and recentrifuged at 1500 rpm for 3 min. The hemolyzed supernatant was removed and the sediment containing mesenchymal stem cells (MSC) was resuspended in Iskov growth medium (Gibco) containing ECS and supplements.

FF and MSC were preliminary cultured on 90-mm Petri dishes in a CO_2-incubator (37°C, 5% CO_2,

Institute of Transplantology and Artificial Organs. ***Address for correspondence:*** krashen@rambler.ru. V. T. Vasilenko.

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95% humidity). FF were cultured for 4-6 days. MSC attained a monolayer after 14-17 days. Accumulation of MSC during culturing was controlled by cell content of vimentin (100% cells) and CD133 (20% cells). Cells of the hemopoietic and platelet lineages were removed. MSC as the source of FMSC were stored by cryopreservation. For obtaining FMSC MSC were recultured. FMSC monolayer formed from MSC within 4-6 days, which was confirmed by immunochemical staining for types I-III collagen.

Histological control was performed on days 1, 3, 7, and 15 after cell transplantation onto the burned surface. Skin specimens for morphological analysis were obtained at the same terms; 7-μ paraffin sections were stained with hematoxylin and eosin.

RESULTS

In all animal groups we observed activation of regeneration processes in the wounds, but the intensity of these processes was different.

Thus, on day 3 after burn modeling necrotized dermal tissue with intensive edema and inflammatory cell infiltration was seen in histological preparations from group 1 animals (controls, Fig. 1). Necrotized zones, cell debris, and intensive lymphocytic and leukocytic infiltration of the granulation tissue were seen on paraffin sections. Erythrocyte stasis was noted in sharply dilated capillaries.

In group 2 animals, extensive areas of erythrocyte stasis and diapedesis, moderate interstitial edema, and intensive inflammatory cell infiltration were observed in the upper layers of tissue sections on day 1 after transplantation of allogenic FF (day 3 after burn modeling). The cells were usually arranged parallel to the wound surface. In some fields of view we observed newly formed vessels and areas coated with fibrin. However, the inflammatory cell infiltration of the granulation tissue was moderate and had signs of fibroblast activation (Fig. 2, *a*).

In group 3 on the first day after FMSC transplantation the surface of the burn wound was coated with a fibrin film; plasma impregnation of the upper layers of the burn wound was noted. Pronounced inflammatory cell infiltration consisting of leukocytes, lymphocytes, and macrophages was seen in wound specimens. Somewhere we observed focal hemorrhages and the formation of new granulation tissue with capillaries (Fig. 3, *a*).

On day 9 after burn modeling (day 7 after cell transplantation in the experimental groups) granulation tissue with few newly formed capillaries was seen in wound specimens from controls. Inflammatory cell infiltration in these zones was insufficient. Areas of erythrocyte hemorrhages were somewhere noted. The inflammatory cell infiltration and hemorrhages were more pronounced in view fields containing no newly formed vessels. Despite abundant lympholeukocytic infiltration, weak signs of activation of connective tissue cells were observed in deep layers of the granulation tissue.

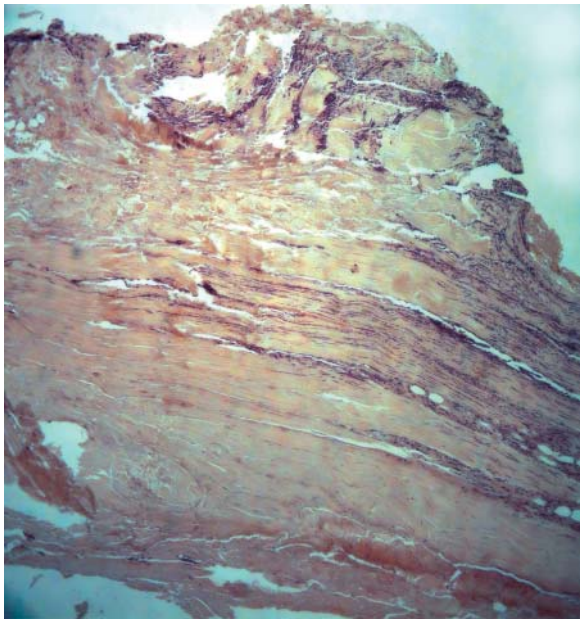

Fig. 1. Burn wound in rats receiving no cell therapy; necrosis of all skin layers with pronounced edema and inflammatory cell infiltration. Day 3 after burn modeling. Here and on Fig. 2: hematoxylin and eosin staining, ×200.

In tissue samples from burned wounds obtained from group 2 animals on day 7 after transplantation of FF, the surface of the granulation tissue was somewhere covered with blood clots consisting of erythrocytes and leukocytes. Abundant vascular network with newly formed capillaries containing blood cells was seen under these clots, which attested to recovery of circulation and trophics in the studied tissues. Moderate cell infiltration (primarily, leukocytes) was somewhere seen around newly formed vessels (Fig. 2, *b*).

In group 3 animals newly formed vessels with less pronounced cell infiltration were found on day 7 after FMSC transplantation (Fig. 3, *b*).

On day 17 of the experiment (day 15 after cell transplantation in the experimental groups) tissue samples obtained from controls were characterized by less pronounced cell infiltration and increased number of newly formed vessels. However, these signs of the reparative phase of the wound process were less pronounced compared to those in groups 2 and 3 animals demonstrating the absence of inflammatory cell infiltration at these terms (Fig. 2, *c*; Fig. 3, *c*).

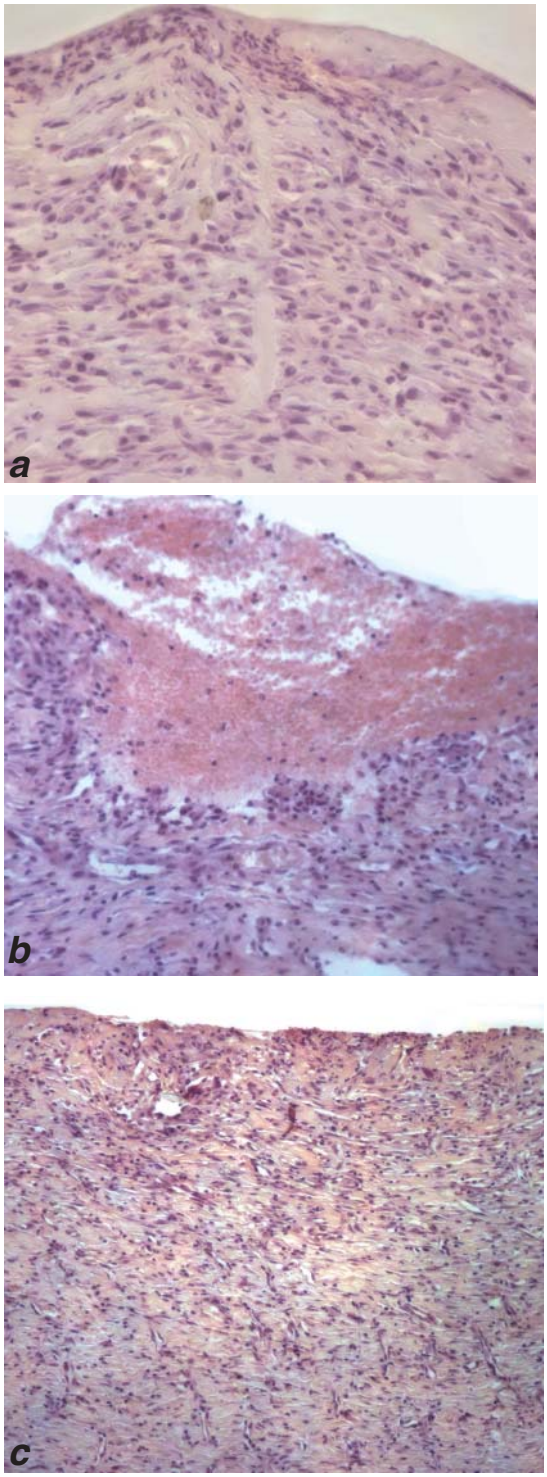

Fig. 2. Histological characteristics of burn wound in the dynamics of reparative process after transplantation of allogenic fibroblasts. *a*) day 1 after cell transplantation: moderate inflammatory cell infiltration of the derma; signs of fibroblast activation; *b*) day 7 after cell transplantation: the wound is covered with a blood clot, inflammatory cell infiltration and development of young granulation tissue with newly formed capillaries are observed; *c*) day 15 after cell transplantation: burn wound contained well-developed young granulation tissue with numerous newly formed capillaries, inflammatory cell infiltration is practically absent.

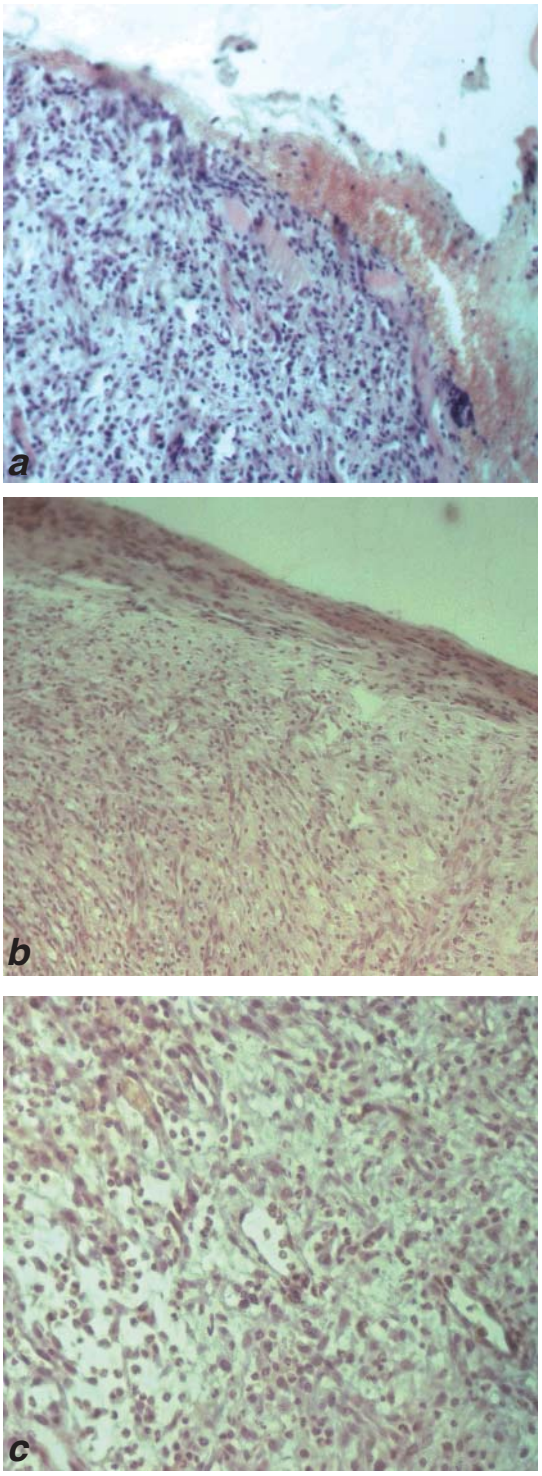

Fig. 3. Histological characteristics of burn wound in the dynamics of reparation process after transplantation of autogenous FMSC. *a*) day 1 after cell transplantation: the wound is covered with a fibrin film, pronounced inflammatory cell infiltration and signs of fibroblast activation are seen in the derma; *b*) day 3 after cell transplantation: moderate interstitial edema, inflammatory cell infiltration of different severity are seen in the derma, young granulation tissue with newly formed capillaries; *c*) day 3 after cell transplantation: young granulation tissue with numerous newly formed capillaries containing erythrocytes. Hematoxylin and eosin staining, ×200 (*a*, *b*), ×400 (*c*).

Despite cell therapy, healing of infected burn wounds was accompanied by pronounced lympholeukocytic infiltration even at late terms of the wound process. The granulation tissue in samples was poorly developed and the formation and growth of new capillaries were rare.

Thus, according to the data of morphological analysis, healing of burn wounds in animals receiving no cell therapy was characterized by predominance of destructive processes with extensive necrotization of tissues, hemorrhages, and pronounced inflammatory cell infiltration and edema up to day 3 of the experiment. The signs of healing, *i.e.* initial formation of granulation tissue, were noted only on day 7 after burn.

The use of FF for promoting burn wound healing considerably reduced tissue destruction, inflammatory cell infiltration and accelerated fibroblast activation (on day 3). The formation of granulation tissue with newly formed and functioning capillaries in these animals was noted on day 7.

The most pronounced positive results were observed after transplantation of allogeneic FMSC. The initial signs of activation of reparative processes appeared 24 h after application of these cells on the wound surface (Fig. 3, *a*), while on day 3 granulation tissue with numerous newly formed capillaries containing blood cells appeared in the wound (Fig. 3, *b*, *c*).

Thus, our assumption that FF as well as auto- and allogenic MSC from the bone marrow producing bioactive substances (growth-stimulating factors, tissue-specific peptides, *etc.*) promote wound regeneration [3] was confirmed by the results of histological studies. Cell therapy reduced the intensity of inflammatory cell infiltration and activates vessel formation. Lymphocytic infiltration was observed in some cases in the perivascular zones, which agrees with modern concept on the role of lymphocytes in tissue morphogenesis [1]. Thus, cells therapy promotes normal interaction between cell assemblies during regeneration of burn wounds, which prevents the formation of cicatrix deformation of tissues.

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