Morphological Changes in Burn Wounds after Transplantation of Allogenic Fibroblasts

E. G. Kolokol'chikova*, L. I. Budkevich, A. E. Bobrovnikov, A. K. Badikova, and V. P. Tumanov

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Cultured allogenic fibroblasts labeled with fluorescent acridine orange and transplanted onto burn wound virtually completely disappear from the wound surface within 2 days (are destroyed). Study of wound morphology by light autoradiography and quantitative analysis showed a stimulating effect of transplanted fibroblasts on wound healing. This effect, irrespective of the initial state of the wound, is always directed at accelerated formation of connective tissue similar to the derma and capable of epithelialization.

Key Words: *fibroblast culture; transplantation; wound healing; autoradiography*

Transplantation of allogenic fibroblasts (AFB) to burn wounds, proposed by D. S. Sarkisov [6] and developed at A. V. Vishnevskii Institute of Surgery [1,5], proved to be a highly effective method, which is now used at other clinics as well [7,8]. However, the mechanism of its therapeutic effect and the biological processes developing in the wound after transplantation are not yet clear. We studied the fate of transplanted AFB and morphological changes in the wound after AFV transplantation.

MATERIALS AND METHODS

AFB isolated and cultured as described previously [6] were transplanted on the surface of burn wound. For evaluating the survival of transplanted AFB in the wound, the donor cells were labeled with a fluorescent dye: 24 h before transplantation, 1-2 drops of acridine orange (10^{-5} mg/ml) were added to a dish with fibroblasts, the dish was incubated at 37°C and after 30 min the medium with fluorochrome was discarded and replaced with fresh medium.

Wound morphology was studied in 7 patients (4 children aged 3-8 years and 3 men aged 26-38 years with IIIAB-IV degree burns of up to 70% body surface). Wound replicas and biopsy specimens collected before and 2, 5, 12, and 14 days after AFB transplantation were examined under a microscope.

The replicas were fixed in neutral formalin for 5 min, washed with water, and stained with toluidine blue. The replicas from sites with transplanted AFB were examined under a fluorescence microscope with stimulation (510 nm) and fixation (520 nm) filters.

The biopsy specimens were embedded in paraffin and epoxy resins, semithin and ultrathin sections were prepared. Some preparations were assayed by autoradiography. To this end they were incubated in medium 199 containing ^{3}H-thymidine (20 μCi/ml, specific activity 21.6 Ci/mmol) or ^{3}H-uridine (100 μCi/ml, specific activity 26.0 Ci/mmol) for 1.5 h at 37°C. Radioautographs were prepared as described previously [3] and examined under Leitz (optic) and JEM-100B (electron) microscopes. For improving the contrast of photos, radioautographs in light microscope were taken in transmitted (black silver grains) and reflected light (silver grains seen as white points).

Department of Pathological Anatomy, Department of Thermal Injuries, A. V. Vishnevskii Institute of Surgery, Russian Academy of Medical Sciences; Speranskii Pediatric Clinical Hospital, Moscow. ***Address for correspondence***: 113811 Moscow, B. Serpukhovskaya ul., 27. A. V. Vishnevsky Institute of Surgery, Pathology Department, E. G. Kolokolchikova

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Biopsy specimens were analyzed morphometrically. The cells (except erythrocytes and neutrophils) and vessels were counted, number of destroyed vessels (with poor cell-cell contacts in the vascular wall and degenerative changes in these cells), total count of ^{3}H-thymidine-labeled cells, and number of ^{3}H-thymidine-labeled cells in the vascular wall were evaluated. At least 100 visual fields (3750 μ^2 each) per preparation were estimated.

RESULTS

Incubation with acridine orange provided specific fluorescence of AFB (green nuclei and nucleoli and red-yellow cytoplasm) (Fig. 1, *a*). Fluorochrome incorporation did not impair cell division.

Analysis of replicas from zones containing labeled AFB showed almost complete absence of labeled cells as soon as 2 days after transplantation. Only fragments of pyknotically changes fragments of donor cells with fluorescent label were occasionally seen (Fig. 1, *b*). Therefore, AFB rapidly (within 1-2 days) die in the recipient wound.

Replicas from wounds before AFB transplantation often contained numerous erythrocytes and leukocytes, (primarily, neutrophils). Sometimes phagocytized and free bacteria were seen. Apart from neutrophils, the preparations contained large macrophages with nuclei occupying the greater part of the cell and amorphous or "foamy" (with many vacuoles) cytoplasm. Fibroblasts were elongated and had very long processes forming a net with caught cells. Destroyed or dead fibroblasts were often seen. The picture of depended on the quality of wound cleansing before AFB transplantation.

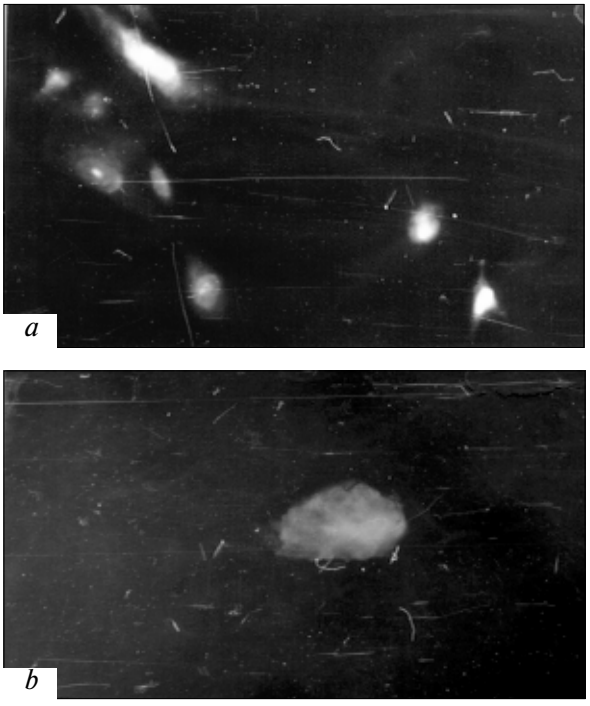

Fig. 1. Fluorochrome labeli in cultured fibroblasts. *a*) specific fluorescence after acridine orange staining, ×200; *b*) fluorescent cell fragment on wound replica 2 days after fibroblast transplantation, ×600.

Two days after AFB transplantation the number of neutrophils in preparations decreased, while that of fibroblasts increased, in some cases essentially. There were very large macrophages containing several phagosomes with neutrophils. This tendency was still observed after 5 days: the number of inflammatory cells (neutrophils and macrophages) decreased, while the number of fibroblasts increased.

Morphometry of biopsy specimens showed that the effect of AFB transplantation depended on the initial state of the wound in the transplantation zone. In zones enriched with cells and vessels (more than 5 cells and 0.3 vessels per visual field) the number of cells and vessels after transplantation decreased and the tissue became more fibrous and looked like cell-rich derma. In cell and vessel-free zones (Fig. 2, *a*), which was observed in only 2 cases, transplantation of AFB culture produced an opposite effect: the number of cells and vessels in the transplantation zone increased (Fig. 2, *b*).

Autoradiography showed suppression proliferation in the first case (with initially abundant cells at the site of transplantation) and stimulation of proliferation, often sharp, in the second (Fig. 3, *a*). Capillary endotheliocytes and pericytes proliferated most often (incorporated ^{3}H-thymidine), fibroblasts adjacent to the vascular walls and, especially, located far from vessels proliferated less intensive. Active RNA synthesis (incorporation of ^{3}H-uridine) was observed in these cells.

Many degrading capillaries (degenerating capillaries and capillaries with poor cell contacts) were seen in the granulation tissue [2,4]. However, our autoradiographic findings indicate that these separating cells of the vascular wall often retain their normal structure and viability, actively synthesize RNA (Fig. 3, *b*), and sometimes can proliferate (synthesize DNA). We assumed that after final degradation of vessels these cells do not disappear, but are transformed into free tissue elements (fibroblasts).

The appearance of young adipocytes intensely labeled with ^{3}H-uridine (Fig. 3, *c*) coincided with histological and clinical (epithelialization) signs of rapid wound healing.

It is now well established that fibroblasts secrete many growth factors, bioactive polypeptide transmitters [9,10]. The most important of them are acid and basic fibroblast growth factors, transforming growth factors-β, keratinocyte growth factor, platelet-derived

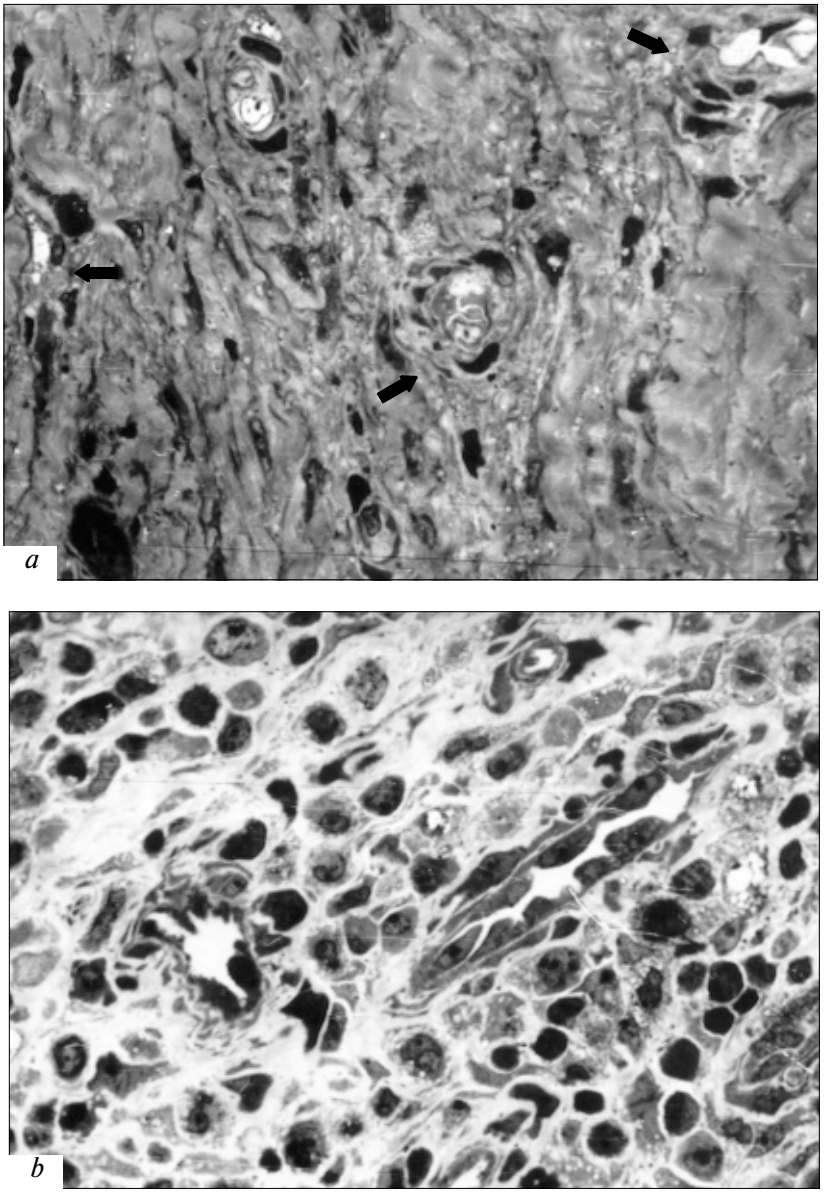

Fig. 2. Wound tissue from patient V. Semithin sections, toluidine blue staining, ×630. *a*) before fibroblast transplantation: few cells and capillaries with signs of destruction (arrows) among abundant fibrous structures; *b*) 3 days after transplantation: granulation tissue with numerous cells and well-developed capillaries.

growth factors, *etc.* During wound healing these factors affect wound cell growth and functions and are actively involved in angiogenesis. They also play an important role in the realization of intracellular connections, successive involvement of various cells in the process, which eventually ensures wound healing. Growth factors are believed to be released via the autocrine mechanism, and no additional mechanisms except cell death (apoptosis) was described [11].

We offer the following explanation of our results. AFB transplanted onto the wound are destroyed and release growth factors by the autocrine mechanism. Growth factors react with recipient cells and stimulate wound healing. This stimulation manifests itself in accelerated formation of connective tissue and epithelialization. It is known that the epidermis does not grow on young granulation tissue enriched with in cells and vessels, nor on subcutaneous fat, nor on coarse fibrous tissue depleted of cells and vessels. This explains why AFB transplantation can stimulate or inhibit cell and vessel proliferation, depending on the structure of wound surface in the transplantation zone, but in any case it accelerates wound epithelialization.

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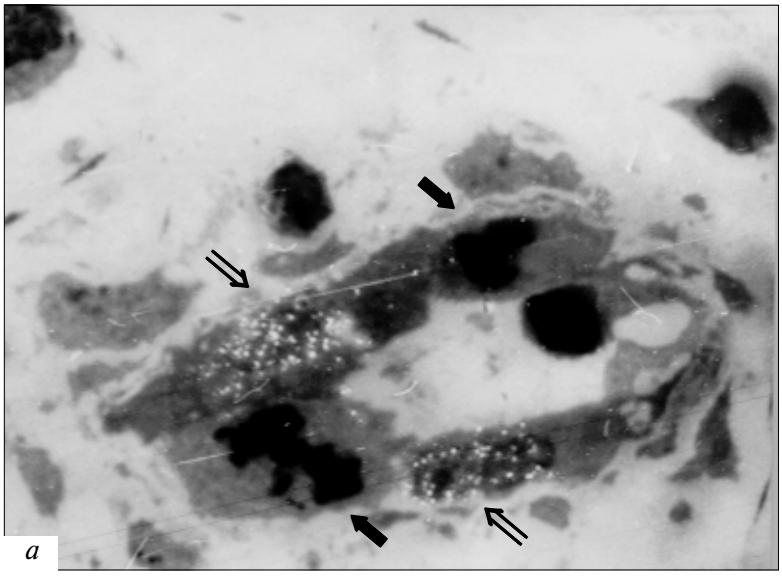

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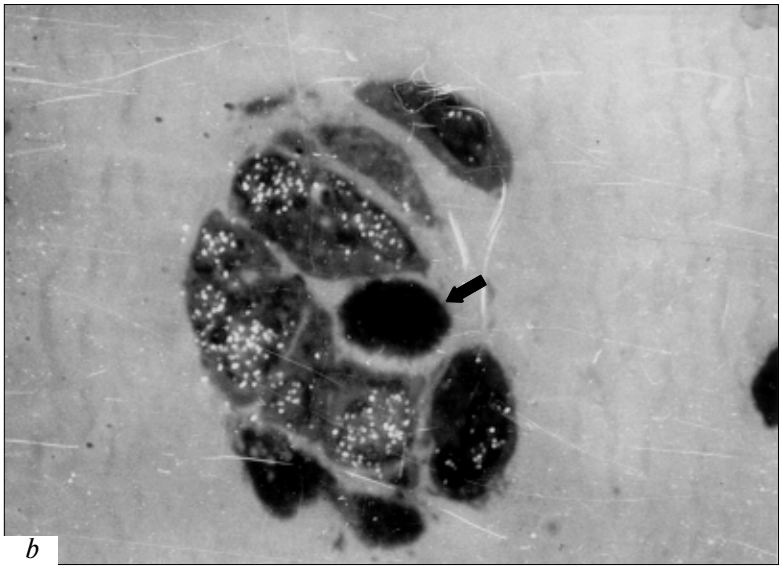

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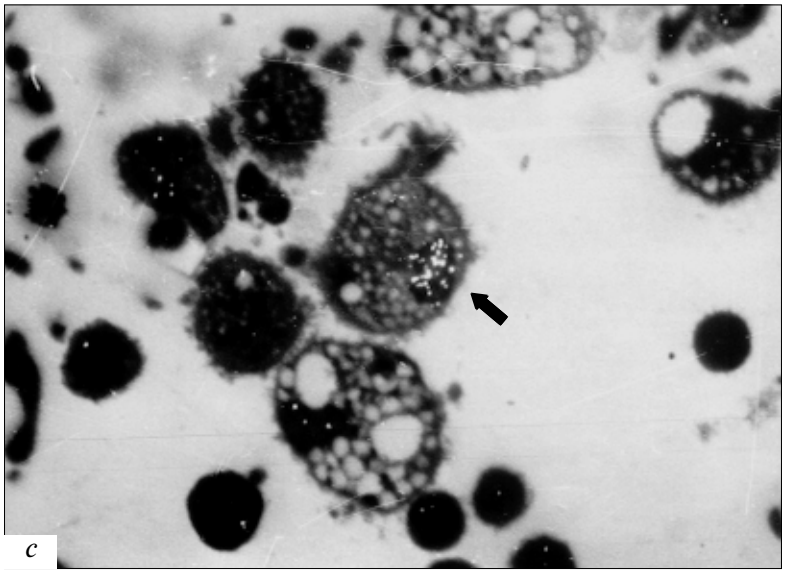

c

Fig. 3. Autoradiography of wound cells after transplantation of cultured fibroblasts. Semithin sections. Toluidine blue staining, ×1000. *a*) two mitoses (arrows) and two ^{3}H-thymidine grains (white silver grains, double arrows) in cells of vascular wall; *b*) degenerating vessel, whose vascular walls intensely synthesize RNA (white silver grains). Arrow shows a leukocyte in vascular lumen; *c*) incorporation of ^{3}H-uridine (white silver grains) in the nucleus of a young adipocyte (arrow).

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