Transplantation of Human Embryonic Myoblasts and Bone Marrow Stromal Cells into Skeletal Muscle of C57BL/10J-mdx Mice

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We studied expression of dystrophin in skeletal muscles of C57BL/10J-mdx mice after transplantation of human embryonic and fetal myoblasts and bone marrow stromal cells. Dystrophin-positive areas corresponding to the location of transplanted cell were detected in muscles of all recipient mice after transplantation of different cell cultures, but the distribution of dystrophin characteristic of normal muscle fibers was detected only after transplantation of embryonic myoblasts. Dystrophin distribution in muscle fibers after transplantation of fetal myoblasts and bone marrow stromal cells was atypical.

Key Words: *myodystrophy; cell therapy; embryonic myoblasts; bone marrow stromal cells*

Autoregeneration and reparation of tissues in animals is realized at the expense of local and systemic processes. Regional stem cells are involved in local regeneration, while bone marrow stromal cells (BMSC) serve as the source of systemic regeneration. Recent studies showed that BMSC not only directly migrate into foci of injuries, but purposefully differentiate into regional somatic cells without differentiation abnormalities [2,4]. This regularity of somatic cell regeneration and reparation via two sources of stem cells is now used in medicine for the treatment of many hereditary, autoimmune, and degenerative diseases, including human muscular dystrophies. The results of numerous studies evaluating the efficiency of myoblast and BMSC transplantation into skeletal muscles are difficult to interpret, because the studies were carried out with different doses of cells obtained by different methods [1,3,5]. Parallel experiments under similar conditions on the same animal model with cell transplantation by identical method will help to compare the efficiency of myoblast and BMSC transplantation.

MATERIALS AND METHODS

Primary cell cultures obtained from human fetal tissues (9 weeks' gestation, embryonic myoblasts; EM; and 19 weeks' gestation fetal myoblasts; FM and BMSC) were isolated by the standard method and cultured in DMEM/F12 (1:1) supplemented with fetal calf serum, growth additives, and growth factors. Myoblast cultures were transplanted after passages 4-5 (total duration of culturing 15-20 days); transplanted cultures contained up to 95% desmin$^+$ cells, contained no collagen I$^+$ and collagen III$^+$ cells, and formed myotubes during spontaneous differentiation. BMSC cultures for transplantation were taken after passage 1, which corresponded to 18 days of culturing.

Experiments were carried out on 29 male C57BL/10J-mdx (muscular dystrophy x-chromosome-linked) mice aged 4-6 months. The mice were narcotized with intraperitoneal calypsol (0.004 ml/g) and the cells

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(500,000 cells/μl) were injected with a Hamilton syringe to a depth of 2-3 mm into *m. glutaeus superficialis* and *m. glutaeus medius*. Five injections (1 μl) close to each other were made. Controls (n=5) were injected with 0.9% isotonic NaCl in the same volume. Experimental animals were divided into 3 groups (8 mice per group) and injected with: 1) EM, 2) FM, and 3) BMSC.

Cryostat sections (10-15 μ) were made 21 and 37 days after transplantation. Dystrophin was detected by incubating cryosections with mouse monoclonal antibodies to human dystrophin (Abcam, 1:100) for 2 h at 37°C and then with antimurine goat immunoglobulins conjugated with phycoerythrin (PhE, Abcam, 1:100).

RESULTS

Dystrophin-positive muscle fibers (MF) were detected in all mice, but the location of dystrophin in muscle tissue was different.

In group 1 numerous dystrophin$^+$ MF were found at the site of injury 21 and 37 days after transplantation. The fluorescence distribution and intensity did not differ in preparations made 21 and 37 days after transplantation. Dystrophin was primarily located in the cortical area of MF and presented as a continuous fluorescent line along the perimeter of MF (Fig. 1, *a*), which corresponded to its location in mice of normal phenotype. MF fluorescence intensity was weaker in areas distant from the site of embryonic cell injection in all preparations. At the periphery of dystrophin-positive areas in the majority of MF only few fluorescent spots were observed in the cortical area.

Zones of dystrophin$^+$ MF located around the injection track were detected in all group 2 animals on days 21 and 37 postinjection. Peripheral PhE fluorescence corresponded to dystrophin distribution in MF with normal phenotype. However, groups of MF with atypical distribution of dystrophin were detected in the internal zone of dystrophin$^+$ region. In addition to brighter fluorescence of the cortical MF area, pronounced diffuse fluorescence of the entire MF was observed (Fig. 2, *a*, *b*). MF with less intense fluorescence and partial focal fluorescence of the cortical area were seen at the periphery of dystrophin$^+$ area, similarly as in group 1 animals (Fig. 2, *a*). The area of fluorescence distribution was also limited, no dystrophin$^+$ MF were seen beyond this area.

In group 3 animals dystrophin$^+$ areas with atypical distribution of the fluorescent label were observed in muscle tissue around the site of cell injection. Bright

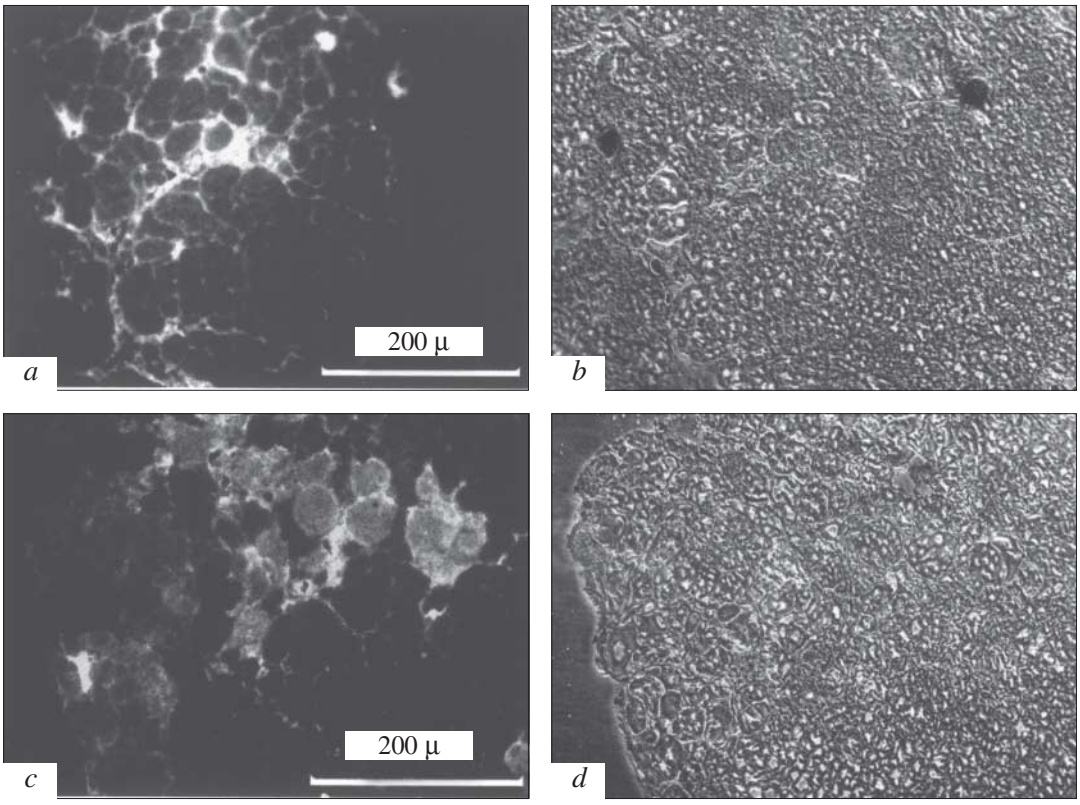

Fig. 1. Distribution of dystrophin$^+$ fibers 37 days after transplantation of human myoblasts in the gluteal muscles of C57BL/10J-mdx mice. *a*) embryonic myoblasts. Dystrophin is present in muscle fibers directly in the zone of injection and occupies a peripheral place in the cortical area, which is characteristic of normal muscle fibers; *c*) fetal myoblasts: atypical distribution of dystrophin, expressed in diffuse fluorescence of the entire fiber; *b*, *d*) phase contrast microphotographs of the same muscle sites.

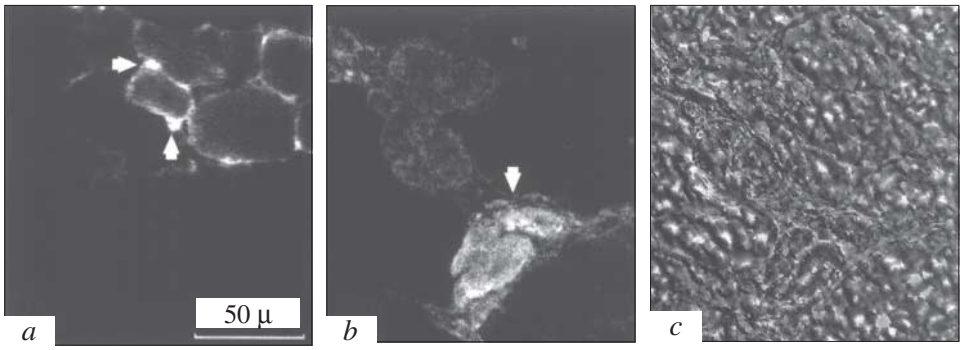

Fig. 2. Atypical distribution of dystrophin in muscle tissue of C57BL/10J-mdx mice 37 days after transplantation of fetal myoblasts. *a*) weak diffuse fluorescence of the cytoplasm in addition to brighter fluorescence of the cortical area of muscle fibers; *b*) even diffuse fluorescence of muscle fibers; *c*) phase contrast microphotograph of the same site of the muscle. *a*, *b*: arrows show small dystrophin$^+$ cells adjacent to regenerating muscle fibers.

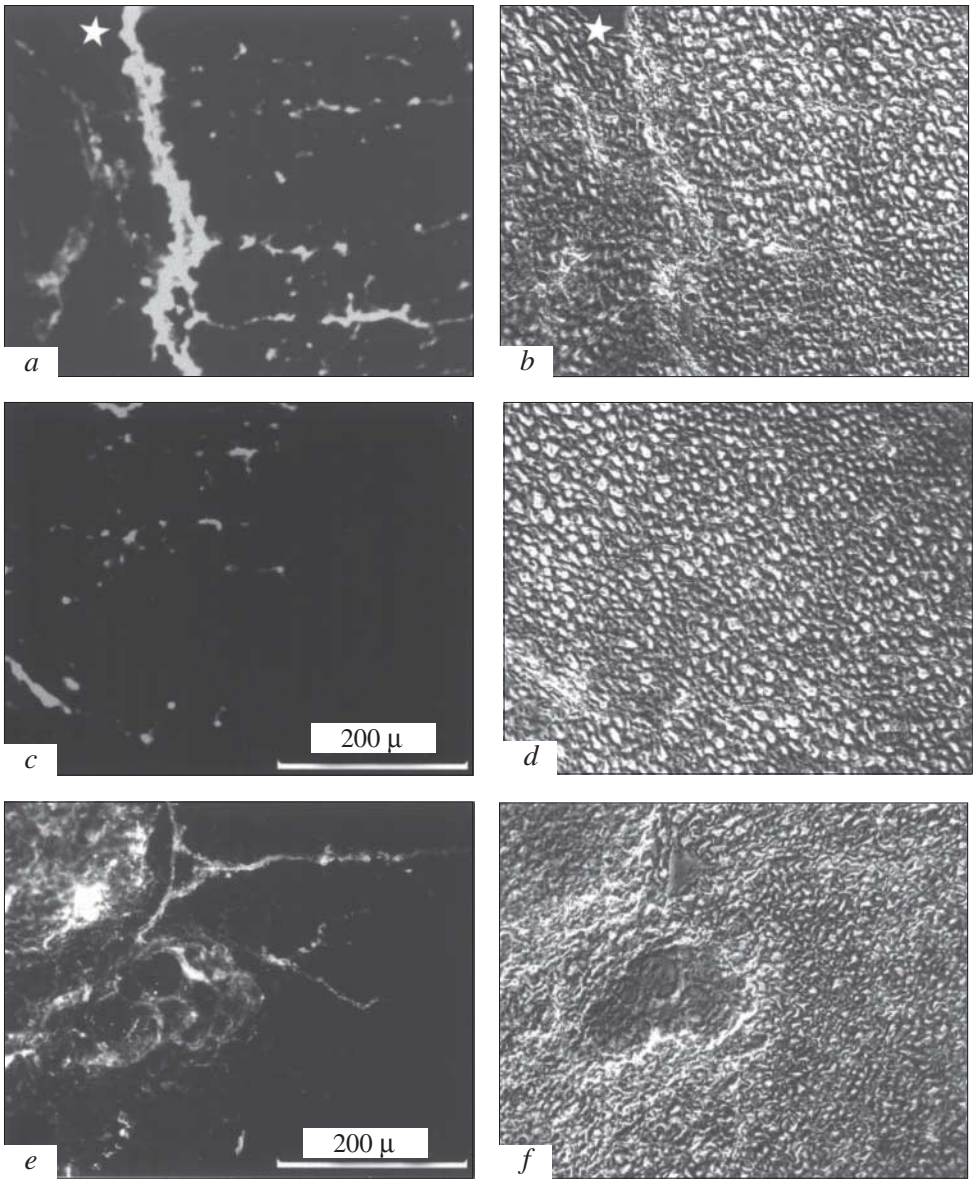

Fig. 3. Dystrophin$^+$ cells 37 days after transplantation of embryonic bone marrow stromal cells into gluteal muscles of C57BL/10J-mdx mice. Cells located mainly in the perimisium and epimisium areas. *a*, *b*) area directly adjacent to the injection track (asterisk); *c*, *d*) periphery of the dystrophin distribution area; *e*, *f*) solitary muscle fibers with normal distribution of dystrophin.

fluorescence was observed around large connective tissue muscle membranes, in the epimysium and mainly in the perimysium (Fig. 3, *a*). Connective tissue membranes of MF directly around the injection track fluoresced brighter (Fig. 3, *a*, *b*), while those at the periphery of the injection zone fluoresced less brightly (Fig. 3, *c*). PhE fluorescence along the connective tissue membrane was often uneven and presented as discrete bright foci corresponding to the location of individual dystrophin$^+$ cells (Fig. 3, *a*, *c*). The zones of cell distribution were similar at various terms postinjection (21 and 37 days), but after injection of BMSC the fluorescence of FM was observed in sites more distant from the injection track compared to myoblast transplantation. Solitary MF with normal dystrophin distribution in the cortical layer were very rare among MF with such atypical distribution of dystrophin in this experimental group (Fig. 3, *e*), but the diameter of these MF was greater in comparison with MF of recipient animals, and the morphology of these fibers differed from that of the adjacent muscle tissue (Fig. 3, *f*).

In all experimental groups injected with different cell cultures the dystrophin$^+$ areas corresponding to the location of transplanted cell occupied limited spaces in muscle tissue around the injection tracks.

In our experiments characteristic distribution of dystrophin was observed after transplantation of myoblasts from earlier embryos, *i. e.* a population enriched with poorly differentiated cells, while after transplantation of BMSC dystrophin distributed atypically (along the connective tissue membranes). Hence, for effective transplantation of BMSC for MF repair, BMSC should be rendered the muscle phenotype at the stage of culturing.

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