Directions of Migration of Bone Marrow Mononuclears after Intracoronary Transventricular Injection

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Directions of migration of mononuclear bone marrow cells after intracoronary transventricular injection procedure developed by us were experimentally studied. After nonselective injection of cells into the right and left coronary arteries in rats, the labeled cells were detected only in the damaged zone of the myocardium. Localization of transplanted mononuclears in the scar attests to their homing into the damaged zone. Numerous cells were found in the red pulp of the spleen and solitary cells were detected in the liver and lungs. In the heart, the labeled transplanted cells were detected only in the scar zone at all terms of the study; they were not incorporated into the vascular walls, but were surrounded by thick bundles of collagen fibers and probably underwent differentiation into fibroblasts. No data on possible differentiation of the transplanted cells into vascular cells or cardiomyocytes were obtained.

Key Words: *mononuclear cells; homing; differentiation*

According to published data, reparation after focal or diffuse death of the myocardium is effected due to hypertrophy of cardiomyocytes (CMC), diffuse or focal sclerosis, and proliferation of resident and exogenous CMC precursors [1]. Resident CMC precursors include immature cells persistent in the heart and capable of differentiating into CMC. Apart from resident cells, exogenous precursor cells originating from the bone marrow can also participate in myocardium regeneration. They include hemopoietic stem cells that can migrate from the bone marrow into the damaged zone and differentiate into endothelial cells and even into CMC or fuse with them [4,16]. Multipotent stromal cells can also be mobilized from the bone marrow into the damaged zone and differentiate into specialized cells of the myocardium and blood vessels [11]. The participation of stem/progenitor bone marrow cells was demonstrated in numerous experiments with transplantation of allogeneic bone marrow and chimerization of the myocardium [3,15].

There is no clear view of the mechanisms of stimulation of reparative processes in the myocardium after transplantation of stem/progenitor cells. Among possible mechanisms of the influence of transplanted cells on global and regional contractility and perfusion of the myocardium, the substitution, induction, and angiogenic effects should be mentioned [7,11]. The substituting effect is realized due to high proliferative activity of stem/progenitor cells and their possible fusion and/or differentiation into specialized myocardial cells replacing the damaged contractile elements of the heart and participating in neoangiogenesis. Regeneration is induced by signal molecules synthesized and released by transplanted cells and regulating proliferation and migration of both resident and exogenous CMC precursors, their differentiation into CMC and blood vessel cells, and production of extracellular matrix proteins.

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Here we studied migration directions, survival, and differentiation of bone marrow mononuclear cells after their intracoronary transventricular injection.

MATERIALS AND METHODS

Experimental model of postinfarction cardiosclerosis. For modeling of postinfarction cardiosclerosis and chronic cardiac failure, transmural infarction of the anterior wall and apex of the left ventricle followed by reperfusion was reproduced as described previously [14] in outbred male CD rats (n=4) 30 days before cell transplantation. To this end, in rats narcotized with ketamine+xylazine (80-100 mg/kg+10 mg/kg, intramuscularly) and ventilated with UGO BASILE Rodent Ventilator thoracotomy was performed and the left descending coronary artery was ligated 3 mm below the auricle of the left atrium. The development of infarction was controlled visually and by ECG.

Cell transplant. The bone marrow was obtained immediately before cell transplantation. The procedure was carried out under narcosis (ketamine+xylazine, 90-120 mg/kg+10 mg/kg, intramuscularly) via puncturing the cancellous tissue of the femur and tibia through the knee joint cavity. Bone marrow mononuclears were obtained routinely in a density gradient [5]. The isolated nucleated cells were resuspended in 1 ml physiological saline to a concentration of 5×10^6 cells/ml. Before transplantation, the cells were labeled with red fluorescent membrane dye PKH26 (Red Fluorescent Cell Linker Mini Kit, Sigma) according to manufacturer's recommendations.

Intracoronary transventricular cell transplantation. Thirty days after infarction modeling, the cell transplant through a catheter was introduced into the cavity of the left ventricle. To this end, an intracoronary transventricular route of cell delivery was developed allowing cell introduction into coronary vessels of small laboratory animals without X-ray control of catheter localization. The cells were injected into the cavity of the left ventricle with simultaneous short-term (few seconds) clamping of the aorta; under these conditions, many transplanted cells get into the left and right coronary arteries. Clamping of the aorta was

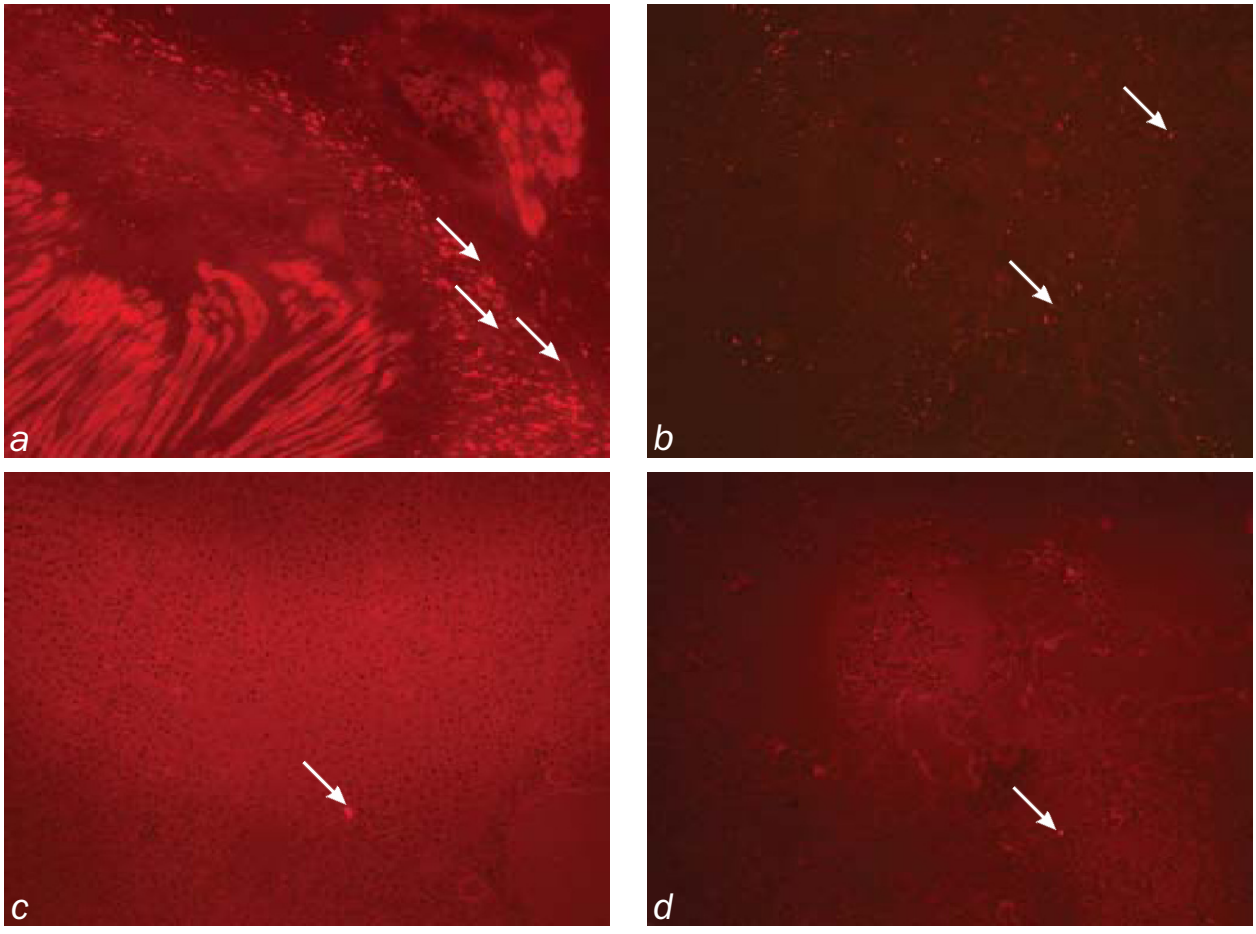

Fig. 1. Mononuclears vitally labeled with PKH26 two weeks after transplantation (×100). *a*) myocardium; *b*) spleen; *c*) liver; *d*) lungs. Arrows: labeled cells.

performed with a subcutaneously introduced metal L-shaped rod. Aorta clamping was controlled by blood pressure drop in the carotid artery. The number of cells for each injection was calculated according to animal body weight (2.5×10^7 cells/kg, concentration 5 mln/ml). Two and four weeks after transplantation the animals were sacrificed and the presence and localization of labeled cells in the heart, spleen, liver, and lungs were studied using a fluorescent microscope.

The means and standard deviations for the absolute and relative (%) parameters were calculated. Comparison was performed using one-way dispersion Kruskal–Wallis rank test. Statistical processing of the results was performed using SigmaPlot 11.0 software. The differences were significant at $p\leq0.05$.

RESULTS

Two weeks after transplantation, accumulations of labeled mononuclears were detected in the heart and spleen, whereas in the liver and lungs only solitary cells were visualized (Fig. 1). In the heart, labeled cells were found only in the scar zone (55.5±17.7 per field of view at ×400). In the spleen, the number of cell per field of view was 52.3±9.5; in the liver and lungs only solitary cells were found (2.9±1.9 and 1.4±1.7, respectively). In the myocardium and spleen, the number of labeled cells per field of view considerably surpassed the number of labeled mononuclears in the liver and lungs.

Thus, mononuclears transplanted by the methods developed by us migrated primarily into the myocardium and spleen. The scar zone of the heart and the spleen contained 49.5 and 46.7% detected cells, respectively; the liver and the lungs contained 2.6 and 1.2% cells, respectively. These findings suggest that the method of transventricular intracoronary cell transplantation is effective and ensures cell delivery to the damaged zone.

On histological preparations of the heart, three clear-cut zones can be distinguished: infarction zone, scar, and undamaged myocardium. The transplanted cells were detected only in the scar tissue: they were not located in the walls of blood vessels, were surrounded by thick bundles of collagen fibers, and prob-

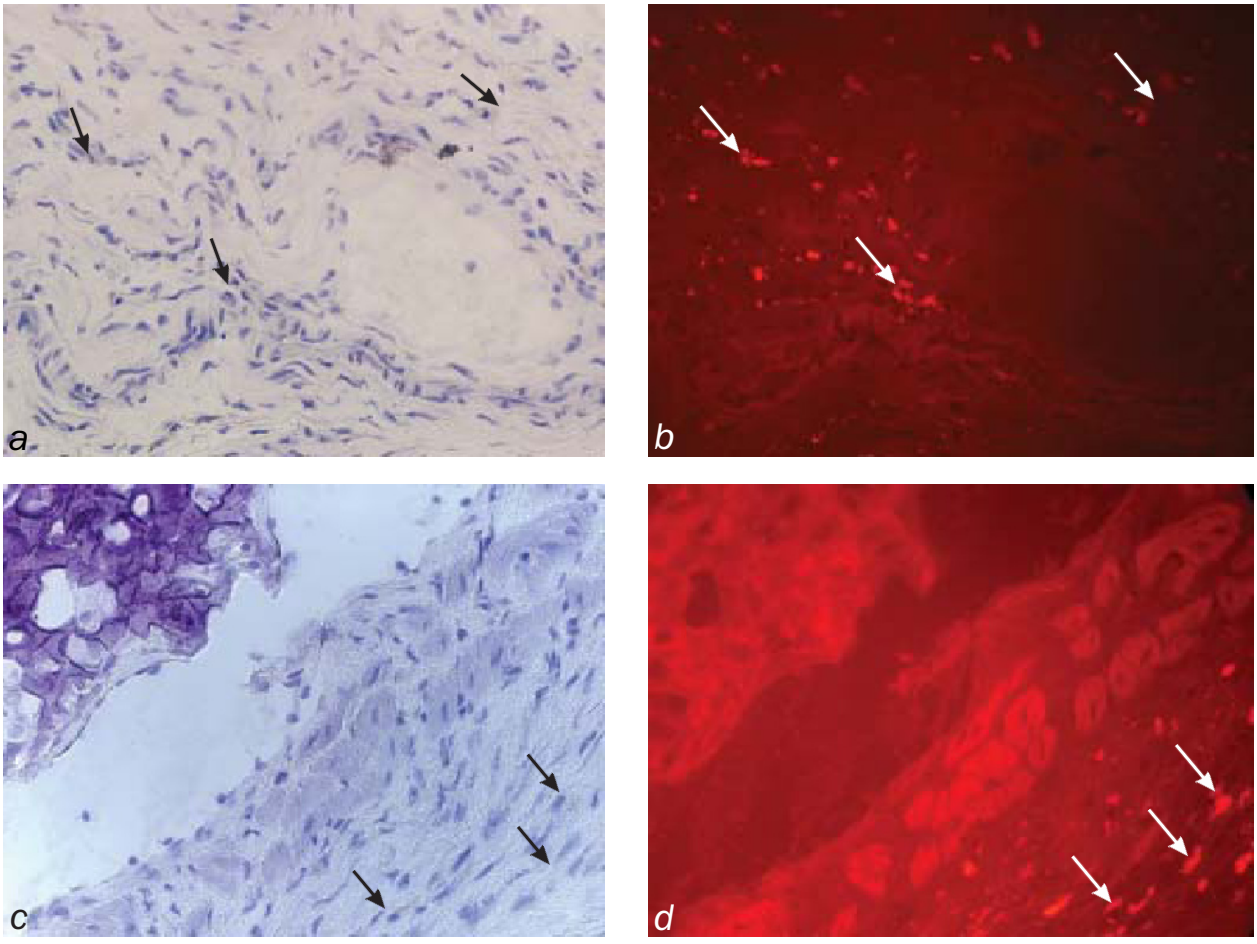

Fig. 2. Localization of labeled mononuclears in the heart (×400). *a*, *c*) light microscopy; *b*, *d*) fluorescent microscopy. Arrows show labeled mononuclears located in the scar tissue.

ably underwent differentiation into fibroblasts. No labeled cells were found in other zones (infarction zone and undamaged myocardium (Fig. 2). In the spleen, the transplanted cells were primarily detected in the red pulp (Fig. 3).

After 4 weeks, the labeled cells were also seen only in the scar zone (34.5±13.4 per field of view). Great number of labeled cells were also seen in the spleen (43.8±13.1 cells per field of view), the liver and the lungs contained 7.6±3.1 and 3.1±4.0 cells per

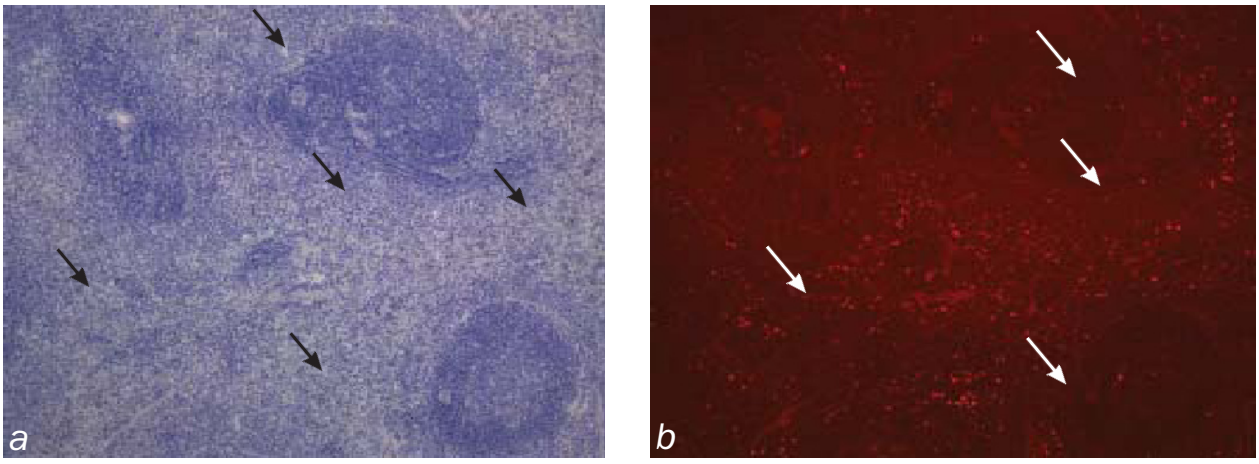

Fig. 3. Localization of labeled mononuclears in the spleen (×100). *a*) light microscopy; *b*) fluorescent microscopy. Arrows show labeled mononuclears located in the red pulp.

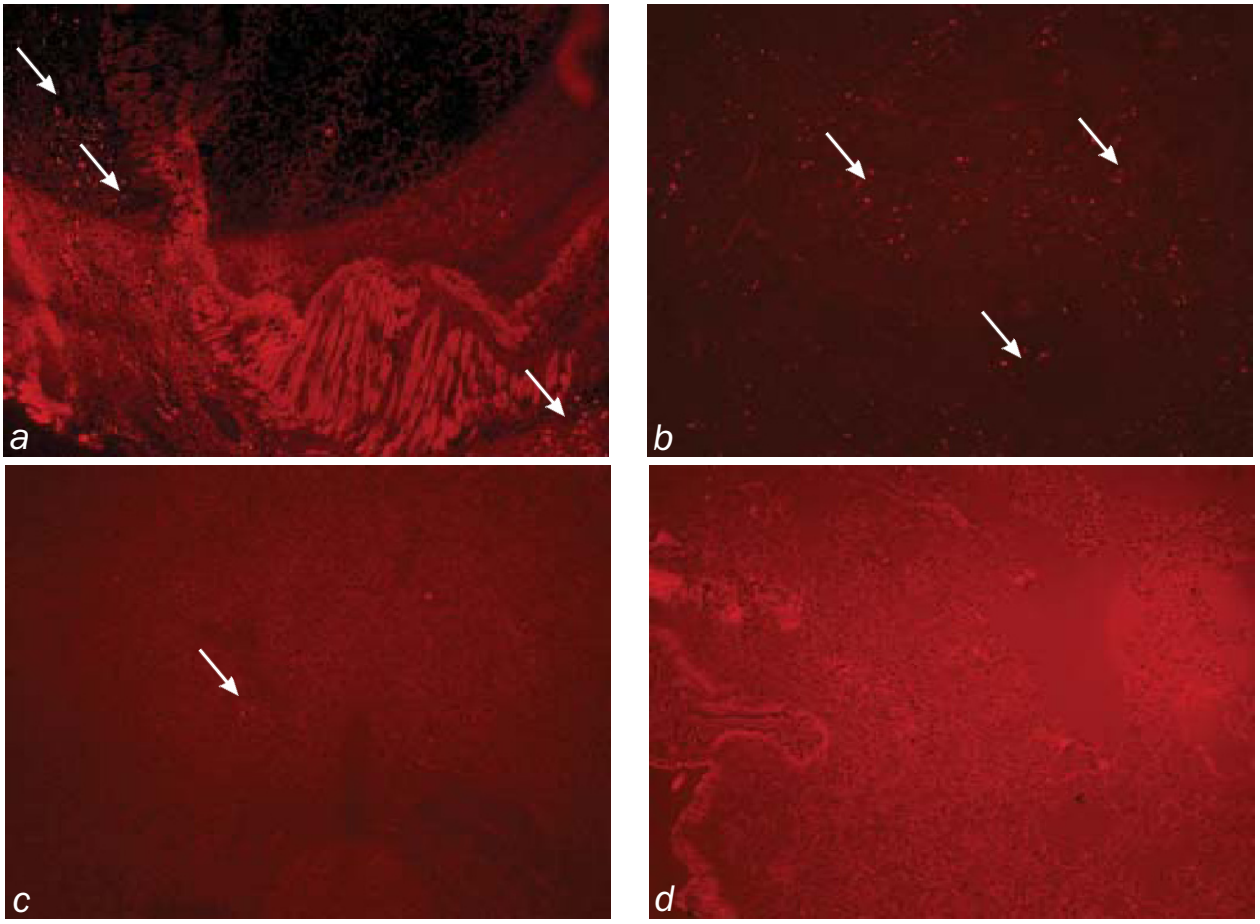

Fig. 4. Mononuclears vitally labeled with PKH26 four weeks after transplantation (×100). *a*) myocardium; *b*) spleen; *c*) liver; *d*) lungs. Arrows: labeled cells.

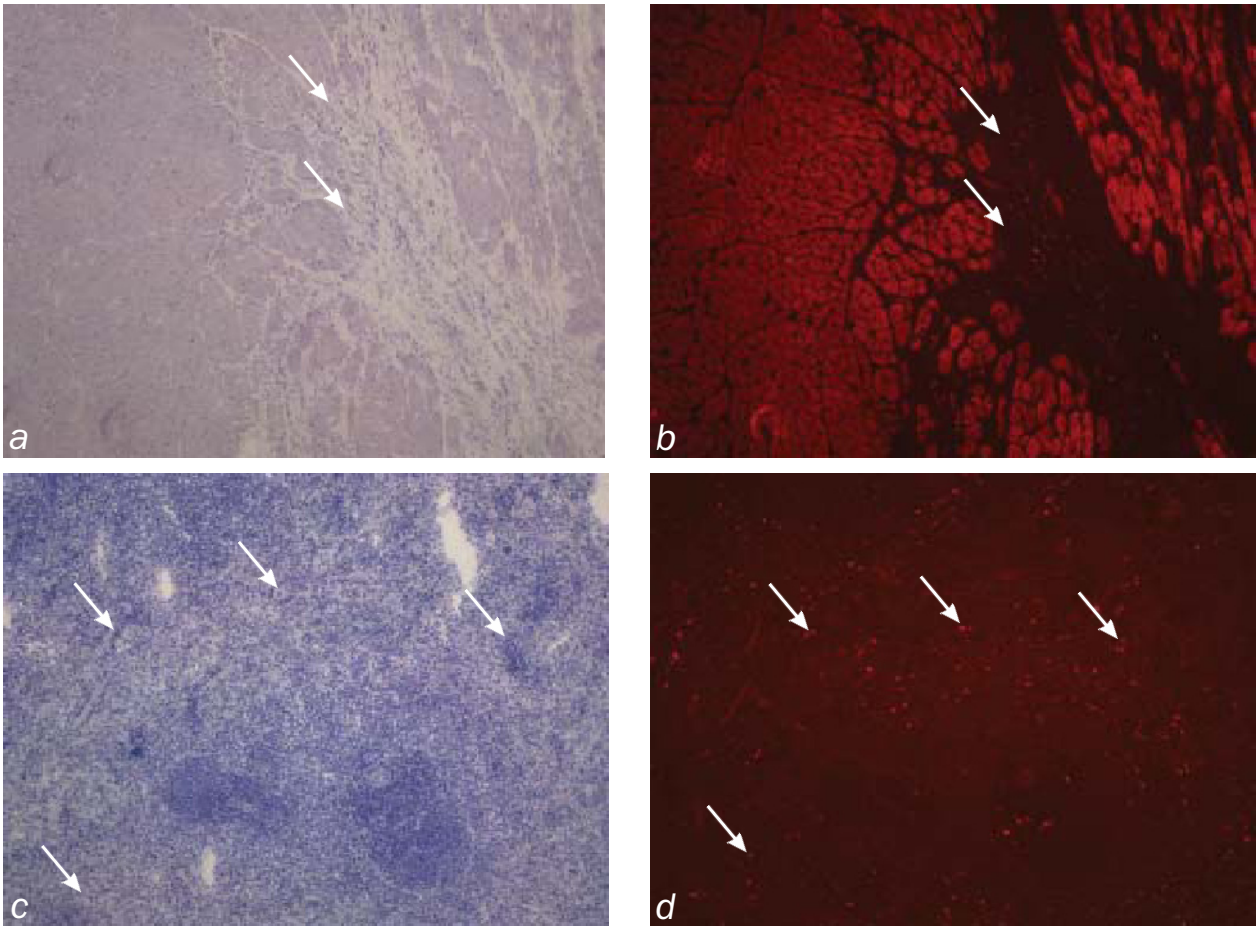

Fig. 5. Localization of labeled mononuclears in the heart (*a*, *b*) and spleen (*c*, *d*) evaluated by light microscopy (*a*, *c*) and fluorescent microscopy (*b*, *d*) (×100). Arrows show labeled mononuclears located in the scar tissue (*a*, *b*) or in the red pulp (*c*, *d*).

field of view, respectively (Fig. 4). In the myocardium and spleen, the number of labeled cells per field of view considerably surpassed the number of labeled mononuclears in the liver and lungs.

Hence, transplanted mononuclears migrated primarily into the myocardium and spleen: 38.7% cells were detected in the scar, 49.2% cells were located in the spleen, 8.6% and 3.5% cells were detected in the liver and lungs, respectively. The cells still migrated to the scar, survived, and differentiated into fibroblasts. No data on possible differentiation of the transplanted cells into vascular cells or CMC were obtained (Fig. 5, *a*, *b*) In the spleen, transplanted cells were primarily located in the red pulp, only solitary cells were detected in the white pulp (Fig. 5, *c, d*).

Comparison of the parameters obtained 2 and 4 weeks after transplantation showed that the number of cells in the heart and other organs decreased (Fig. 6). After 4 weeks, the number of cells detected in all organs significantly decreased by 28% compared to that 2 weeks after transplantation (p=0.02). This is most likely due to label discoloration, because the number of labeled cells evenly decreased in all organs.

The method of transventricular intracoronary transplantation of cells without X-ray control of cathe-

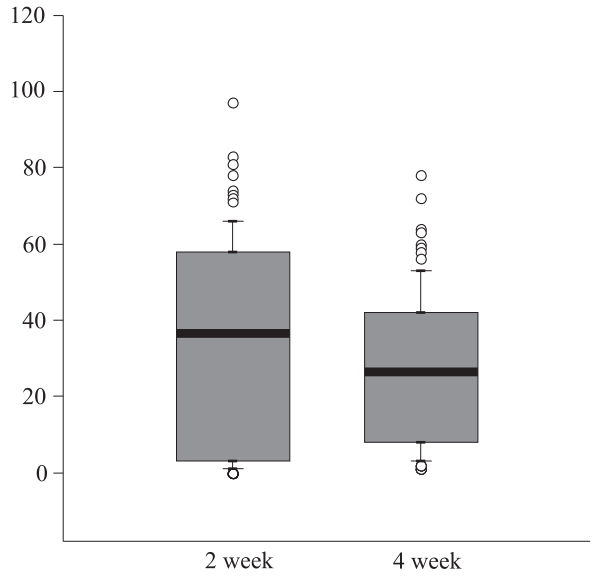

Fig. 6. Dynamics of the total number of transplanted cells 2 and 4 weeks after injection.

ter localization is effective and ensures cell delivery to heart tissues in small laboratory animals. Intracoronary injection is now considered to be the optimal route of cell transplantation, because it corresponds by intramyocardial transplantation by its efficiency, but is less invasive [6]. Moreover, we showed that the cells were located only in the damaged area, but were not equally distributed in all heart tissues even after nonselective administration into the left and right coronary arteries [13]. Localization of transplanted mononuclears in the scar attests to their homing into the damaged zone. Previous studies showed that precursor cells migrate to the zones with high concentration of chemoattractants, *e.g.* sdf-1 (stromal-derived factor), stimulating their migration into the damaged area [2].

The transplanted cells enter the systemic circulation, but predominantly populate hemopoietic organs, in particular, the spleen, but not the liver and lungs as after intravenous injection [8,9]. In the spleen, the cells were predominantly located in the red pulp and massive homing of cells into the spleen was determined by the same mechanisms, because the spleen is the hemopoietic organ and a natural niche of transplanted cells.

The transplanted autogenic mononuclears were viable throughout the observation period and were not eliminated by the immune system. First, viability of cells was confirmed by the fact of their homing, second, the cells carrying the fluorescent label looked undamaged on sections, and third, the transplanted cells were not surrounded by lymphocytic and macrophage infiltration.

In the myocardium, the cells were located only in the scar tissue, had spindle shape, and were located between collagen fibers. Labeled cells were not located in the tunica media or adventitia of blood vessels. There were no labeled cells near CMC. The distribution of transplanted cells in cardiac tissues demonstrates that they did not fuse and did not differentiate into CMC, endotheliocytes, and smooth muscle cells. We can hypothesize that small fraction of stromal mononuclears migrated into damaged area of the myocardium, while hemopoietic cells ($CD34^+$) migrated with blood flow into the spleen and other hemopoietic organs; this explains localization of labeled cells in the scar and their fibroblast-like shape (cells in the spleen had round shape).

Our findings disagree with some reports that mononuclears containing fractions of hemopoietic stem cells and multipotent stromal cells can differentiate into practically all cardiac tissues [10,12]. However, impossibility of replacement of damaged CMC with transplanted mononuclears does not rule out their therapeutic effect consisting in stimulation of myocardial reparation due to production of paracrine factors by transplanted cells.

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