Experimental Evaluation of Rat Cord Blood as a Source of Stem Cells Stimulating Regeneration of the Bone Tissue

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We studied subpopulation structure of rat cord blood and its effects on regeneration of the bone tissue. In cord blood leukocytes, cell fractions with phenotypes $CD45^+/CD90^-$, $CD45^-/CD90^+$, $CD34^+/CD45^+$ were determined. The cells adhered to plastic during culturing and had a fibroblast-like morphology. Transplantation of rat cord blood cells into modeled femoral bone defects stimulated regeneration of the bone tissue and led to recovery of its anatomical integrity, which was confirmed by X-ray examination and histological analysis. No complete recovery of the bone structure was observed in controls (without cell implantation).

Key Words: *stem cells of the blood; bone regeneration*

Numerous studies showed that cord blood contains hemopoietic, mesenchymal, and unrestricted somatic stem cells (USSCs) capable of differentiating into the bone tissue during culturing in special media, which makes this material a promising means for stimulation of regeneration [1-6,10]. At the same time, the effect of these cells on damaged bone tissue is poorly studied.

Here we studied subpopulation structure of rat umbilical cord blood and its effects on regeneration of the bone tissue.

MATERIALS AND METHODS

Experiments were carried out on mature Wistar rats weighing 250-400 g. In pregnant females (gestation days 21-24), the blood from the umbilical cord was taken with a syringe washed with 6% EDTA.

Immunophenotyping (FACS Calibur) was performed using FITC- and propidium iodide-labeled monoclonal antibodies to rat CD34, CD45, CD90 antigens (PE; Catlag, Invitrogen). Mouse IgG_1 FITC and IgG_{2a} PE were used as negative isotypic control. The data were processed using Cell Qest software.

Washed umbilical cord blood cells were seeded in sterile plastic Costar flasks at a seeding density of 1.5 mln cells per 1 ml. The nutrient medium contained 90% DMEM, 10% bovine serum (Biolot) and 40 mg/ml gentamicin. The cells were cultured in a CO_2 incubator (Jouan; 5% CO_2) at 37°C (days 16). Nonadherent cells were removed 1 day after seeding and a new portion of the nutrient medium was added; the medium was then replaced every 3 days. The cultures were analyzed under an inverted microscope equipped with the appropriate software (Leica).

For transplantation into the bone defect, a suspension of nucleated cells was isolated from umbilical cord blood cells by sedimentation in 33% polyglucin (1:2 ratio). In experimental animals (n=32), a bone defect (5 mm length and 3 mm width) was made, the bone marrow channel was opened, and the bone marrow was removed. The bone defect was filled with 0.2 ml cell suspension ($3.6\text{-}4.2\times10^5$ cells), the defect was covered with a collagen film. On days 5 and 10 after surgery, 0.1 ml cell suspension was injected into the defect area. In control animals (n=16), the defect was sealed with autoblood clot. X-ray examination and histomorphological analysis of the operated extremities were performed on days 15, 30, 60, and 120.

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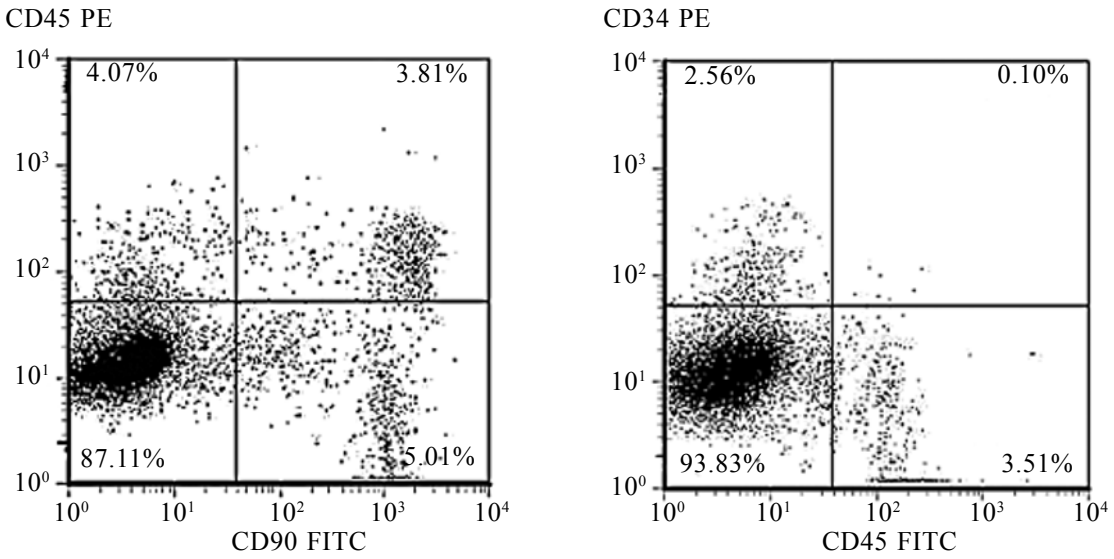

Fig. 1. Dot-plot cytograms: subpopulation structure of rat umbilical cord blood.

The data were processed statistically using Statistica 6.0 software.

RESULTS

The umbilical cord blood contained 18.4±1.6×10^9 leukocytes, 60.3±3.3% lymphocytes, 20.3±2.3% monocytes, and 19.6±0.4% granulocytes. Isolation of the fraction of nucleated cells by sedimentation in polyglucin yielded 92% viable cells, which was confirmed by supravital trypan blue staining.

Immunophenotyping of umbilical cord blood cells revealed the following cell populations: 3.05±2.18%

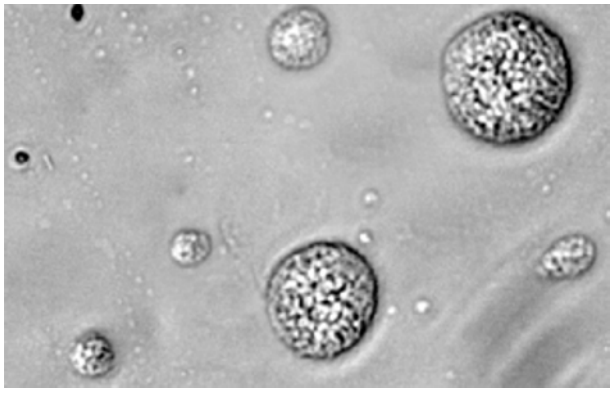

Fig. 2. Colonies of stem cells on day 15 of culturing: phase-contrast microscopy (×100).

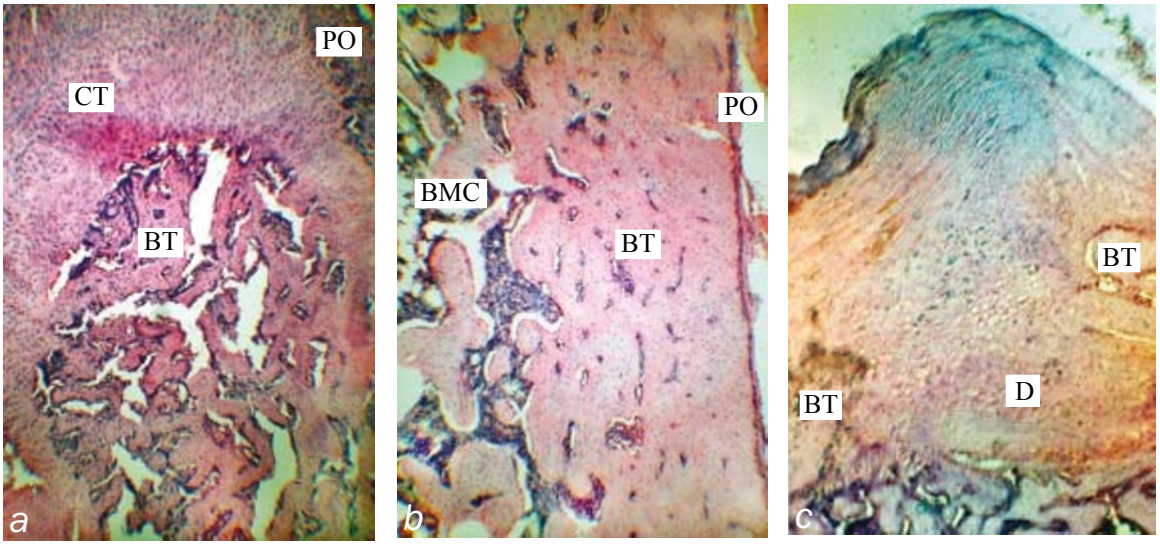

Fig. 3. Micropreparations of rat femoral bones. Hematoxylin and eosin staining, ×100. *a*) formation of the cartilaginous tissue (CT) and primitive osteal plates (OS) at the edges of the defect, formation of the periosteum (PO) on day 15 after transplantation of the umbilical cord blood cells; *b*) completely formed bone tissue (OS), PO, and bone marrow channel (BMC) on day 90 after transplantation of umbilical cord blood cells; *c*) dense connective tissue scar (D) between the edges of the cortical plate (OS) on day 90 after surgery (controls).

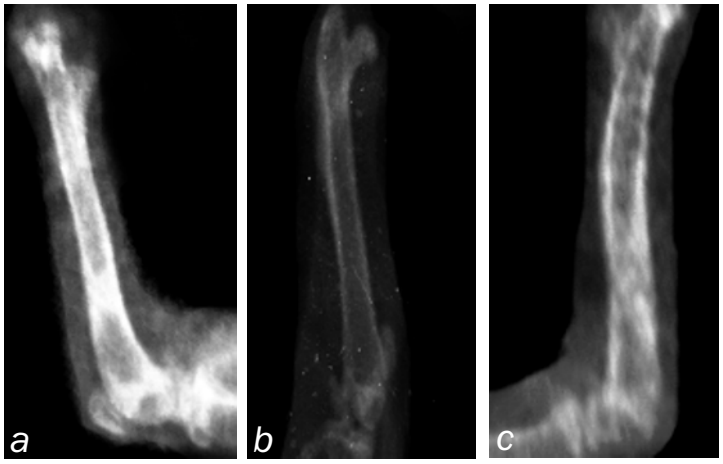

Fig. 4. X-ray films of rat femoral bones. *a*) shadow at the site of bone defect on day 30 after transplantation of the umbilical cord blood cells; *b*) recovery of the bone structure on day 60 after transplantation of the umbilical cord blood cells; *c*) inhomogeneous structure of the bone regenerate on day 60 after surgery (controls).

$CD45^+/CD90^-$ cells (committed leukocyte precursors [1]), 3.78±2.15% $CD45^-/CD90^+$ [1,2,4,5,8]), and 0.09±0.11% $CD34^+/CD45^+$ cells (linear non-committed hemopoietic stem cells [1,2]; Fig. 1).

Morphological analysis of umbilical cord blood cells at early stages of culturing revealed numerous colonies of small round cells and solitary large multinuclear cells. By day15, sparse colonies of fibroblast-like spindle-shaped cells appeared (Fig. 2).

X-ray examination and histological analysis of femoral bone regenerates demonstrated early formation of organ-specific regenerate after transplantation of umbilical cord blood cells into experimental bone tissue defect. On day 30 after surgery, the defect in experimental animals was filled with cartilaginous and coarse-fibered bone tissue (Fig. 3, *a*; Fig. 4, *a*), while by days 60-90 after surgery, the reparative processes eventuated in complete recovery of the anatomical and functional integrity of the bone (Fig. 3, *b*; Fig. 4, *b*).

In the control group (without transplantation of umbilical cord blood cells), the bone structure was not restored throughout the observation period (4 months), which was confirmed by the results of X-ray and histological examination of the operated extremities (Fig. 3, *c*; Fig. 4, *c*).

Acceleration of reparative processes in the bone tissue after transplantation of umbilical cord blood cells can be related to some factors. First, osteogenic potential is intrinsic of umbilical cord blood cells due to the presence of mesenchymal stem cells, which was confirmed in experimental studies demonstrating differentiation of human and animal umbilical cord blood cells into bone tissue cells during culturing in special media [2-6,8,10]. Hemopoietic stem cells also play a role in reparative osteogenesis. There are published data that $CD34^+$ cells in the peripheral blood accelerate healing of bone fractures via stimulation of osteogenesis [7,9].

Thus, we found that umbilical cord blood cells containing subpopulations of hemopoietic and mesenchymal stem cells produce a pronounced stimulating effect on reparative regeneration of the bone tissue.

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