System of Hemopoiesis and Morphogenesis

B. G. Yushkov, V. A. Chereshnev, N. V. Tyumentseva, O. S. Artashyan, and A. I. Kuz'min

Translated from *Kletochnye Tekhnologii v Biologii i Meditsine,* Vol. 1, No. 4, pp. 226-229, November, 2005
Original article submitted March 1, 2005

Method of transplantation of hemopoietic cells is proposed for acceleration of recovery of damaged tissue structures. The morphogenetic effect of transplantation depended on the state of damaged tissue and was determined by not only hemopoietic stem cells, but also lymphocytes, macrophages, and mast cells.

Key Words: *hemopoiesis; morphogenetic function; autoprostheses*

Bone marrow-derived cells can participate in the regeneration of various cell types, including cardiomyocytes, hepatocytes, neurons, neuronal cerebral cells, endotheliocytes, and osteoblasts. However, it is not clear whether each of these cell lines originates from its true bone marrow progenitor or the bone marrow possesses a population of polypotent cells, which develops during embryogenesis as a kind of biological reserve for postnatal compensation and recovery of damaged tissues [3]. Apart from hemopoietic cells, the bone marrow contains stromal and vascular cells, adipocytes, osteoblasts, osteoclasts, and mastocytes. Functional state of these cells can significantly affect the morphogenetic function of the bone marrow.

Our aim was to study morphogenetic function of hemopoietic cells during the development of microvessels and autoprostheses of large vessels.

MATERIALS AND METHODS

The experiments were carried out on random-bred albino rats weighing 200-250 g. In group 1 rats, 10^{-7} myelokaryocytes were injected into the left hindleg on day 21 after ligation of the femoral artery; physiological saline was injected into the control right hindleg. Polyvinyl chloride (PVC) or Millipore tubes 2 mm in diameter and 20 mm in length (Millipore cellulose filter, NA 0.45 μ) were implanted under the skin on the back in group 2 rats as a matrix for the formation of connective tissue prosthesis. After removal of the matrixes, the prostheses were examined by macro- and microscopy. Histological sections (3-5 μ) were stained with hematoxylin and eosin (and with picrofuchsin to examine the collagen fibers) and used for morphological and morphometrical characterization of prostheses.

For modulation of functional and metabolic activity of macrophages, Tamerit was injected intramuscularly (0.4 mg), and carrageenan was administered intraperitoneally (1.5 mg); for lymphocyte stimulation, dalargin was injected intramuscularly (0.01 mg) for 4 weeks.

RESULTS

On day 28 after injection of the bone marrow cell suspension, individual endotheliocytes and newly-formed vessels were revealed in the ischemic zone. Myelokaryocytes accelerated vessel formation in site of their injection (Fig. 1, Table 1).

Accumulation of endotheliocytes and formation of capillaries in legs with ligated artery can result from either differentiation of hemopoietic stem cells into endothelial cells [6], or existence of common progenitor of endotheliocytes and hemopoietic cells [5], or the presence of endotheliocyte

Institute of Immunology and Physiology, Ural Division of Russian Academy of Sciences; A. M. Gorky Ural State University; Ekaterinburg

0007-4888/05/1405-0627

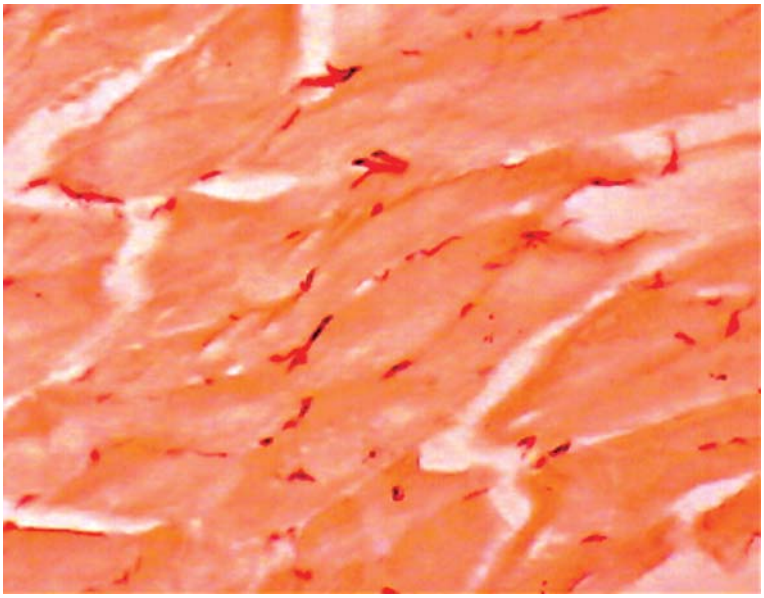

Fig. 1. Muscle of rat shin 28 days after injection of myelokaryocytes. Staining of endotheliocytes for alkaline phosphatase, ×496.

precursor cells in the cell suspension in addition to hemopoietic stem cells [4].

The morphogenetic function of hemopoietic cells is clearly demonstrated during the formation of vascular autoprostheses. The formation of these prostheses begins from infiltration of the adjacent tissues with neutrophils, lymphocytes, monocyte-macrophages, and fibroblasts (reaction typical of foreign body encapsulation). During the second week after marrow injection, the implant was covered with a connective tissue capsule, which could be easily detached from the matrix with preserved tube structure. This tube can be used as a vascular autoprosthesis.

The wall of the connective tissue tube thinned gradually and attained a minimum to postinjection week 6. At the early terms (week 1), fibroblasts prevailed in the prosthesis (Table 2). They were located predominantly near the inner and outer surfaces of the tube in sites of active formation of the connective tissue, while fibrocytes occupied the central region. Initially, the prosthesis wall consisted of loose connective tissue, where most fiber-like structures had no definite orientation. Infiltration of the wall with segmented neutrophils and lymphocytes indicated acute inflammation (Table 2).

TABLE 1. Development of Capillaries in Ischemic Muscle Fragment after Injection of Bone Marrow Cells (10^7 per 0.1 mm^2)

Parameter	Without bone marrow cells (control)	Injection of bone marrow cells
Individual endotheliocytes	4.02±0.60	4.22±0.40
Formed vessels	2.76±0.40	6.02±0.70*
Total	6.78±0.80	10.24±0.80*

Note. *p<0.05 compared to the control.

The prostheses obtained at the later terms were characterized by more ordered morphofunctional structure. At this period, their wall consisted of fibrous connective tissue. The collagen fibers of different maturity were predominantly oriented along the autoprosthesis. When the fibroblast is encompassed by self-synthesized intercellular substance, it loses its ability to divide and turns into fibrocytes. Therefore, the number of fibroblasts in the prosthesis gradually decreases, while that of fibrocytes increases (Table 2). In addition, the intensity of inflammatory reaction also gradually decreases.

Among cells regulating the development and secretory activity of fibroblasts, the major role is played by macrophages, which suggests a certain influence of their functional state on the formation of autoprostheses. Indeed, stimulation of macrophages with Tamerit inhibited inflammation and decreased the total number of cells of the fibroblast lineage at the expense of fibroblasts and with increasing the percentage of functionally active fibrocytes (Table 3).

Four weeks after implantation of PVC tube, the prosthesis wall consisted of fibrous connective tissue, and was thinner than that in control rats not treated with the drug; the collagen fibers of various degree of maturity were oriented longitudinally.

Blockade or inhibition of macrophages with carrageenan decelerated the development of the connective tissue tube: on week 4 it was 3-fold thicker than in rats not treated with the drug. More-

TABLE 2. Development of Connective Tissue Autoprostheses after Subcutaneous Implantation of Millipore Matrix (*M*±*m*)

Parameter	Time after implantation of Millipore tube, weeks					
	1	2	3	4	5	6
Wall thickness, mm	0.63±0.05	0.37±0.03*	0.28±0.02*	0.33±0.02*	0.22±0.02*	0.13±0.01*
Number of cells	91.03±3.90	78.66±2.71*	96.98±3.20	78.22±3.90*	100.42±3.60	65.11±2.10*
Fibroblasts+fibrocytes	58.62±8.50	61.04±3.10	75.06±6.10	66.72±3.97	81.3±6.9	57.2±2.8
Fibroblasts	41.24±6.97	28.08±3.70	34.23±4.30	31.6±4.2	23.7±2.0	18.9±1.9*
Fibrocytes	17.39±5.60	32.96±6.20	40.83±7.60*	35.12±7.39	57.6±8.7*	38.3±4.0*
Lymphocytes	5.64±1.30	3.62±0.70	3.88±0.96	2.58±0.76*	3.2±1.4	4.7±2.0
Segmented neutrophils	26.03±8.70	11.01±2.82	16.10±5.71	8.29±3.70	12.9±5.7	3.0±1.2
Macrophages	0.55±0.30	2.99±0.7*	1.84±0.95	0.63±0.50	3.0±1.3*	0.2±0.2
Plasma cells	0.18±0.10		0.10±0.09			

Note. *p<0.05 compared to postsurgery week 1.

TABLE 3. Effect of Macrophage Functional Activity on Parameters of Autoprostheses on Week 4 after Subcutaneous Implantation of Polyvinyl Chloride Matrix (*M*±*m*)

Parameter	Control	Tamerit, 2 mg/kg	Carrageenan, 20 mg/kg
Wall thickness	0.10±0.01	0.020±0.001*	0.30±0.02*
Number of cells	57.46±1.60	48.93±1.68*	65.31±1.60*
Fibroblasts+fibrocytes	53.2±1.6	48.93±0.10	62.24±0.90*
Fibroblasts	21.9±4.5	7.3±1.5*	44.15±5.10*
Fibrocytes	31.3±3.8	41.6±1.5	18.09±5.00
Lymphocytes	2.6±1.1		1.04±0.51
Segmented neutrophils	0.9±0.6		2.02±0.83
Macrophages	0.7±0.5		

Note. *p<0.05 compared to the control.

over, instead of fibrous tissue, the internal surface of the autoprosthesis was composed of loose connective tissue, which is a typical feature at early terms of prosthesis development (Table 3).

Mast cells also modulate functional activity of fibroblasts. These cells activate skin [1] and pulmonary [2] fibroblasts. During the development of autoprosthesis, the number of mast cell varied insignificantly from 4.37±0.33 to 3.94±0.25 per 0.01 mm^2 unit area. However, while the acute phase of inflammation faded, the percentage of degranulated cells increased and degranulation coefficient increased from 0.18±0.02 to 0.33 to 0.04 (p<0.05).

When lymphocytes were stimulated with dalargin, the prosthetic wall on Millipore matrix consisted of loose connective tissue characteristic of the early terms of prosthesis development. The prosthesis wall was markedly thicker than in control rats (0.44±0.02 mm, p<0.001). The number of fibroblast cells was virtually the same as in the control, but maturation of fibroblasts into fibrocytes was delayed. The number of lymphocytes did not differ from the control level.

The 5-week connective tissue prostheses were used for angioplasty of the carotid arteries in those rats, where they were grown. The prostheses were stitched end-to-end into the cut arteries. According to ultrasonic scanning and angiography data, the lumen of the prostheses remained open and worked normally over 3 and 6 months after surgery. There were no signs of thrombosis or occlusion, which was corroborated by histological examination. During this period, the prosthetic wall was formed by fibrous connective tissue. Endotheliocyte-like cells were formed on the inner side of the prosthetic wall.

Thus, the morphogenetic effect of bone marrow transplantation depends on the state of the damaged tissue and is determined not only by hemopoietic stem cells, but also by lymphocytes, macrophages, and mast cells.

REFERENCES

1. S. Chujo, F. Shrasaki, S. Kawara, *et al*., *J. Cell. Physiol*., **203**, No. 2, 447-456 (2005).
2. E. Garbuzenko, I. Puxeddu, F. Levi-Schaffer, *et al*., *Exp. Lung Res*. **30**, No. 8, 705-721 (2004).
3. K. K. Hirschi and M. A. Goodell, *Gene Ther*., **9**, No. 10, 648-652 (2002).
4. M. A. Moore, K. Hattori, B. Heissig, *et al*., *Ann. New York Acad. Sci*., **938**, 36-45 (2001).
5. S. I. Nishikawa, *Curr. Opin. Cell. Biol*., **13**, No. 6, 673-678 (2001).
6. D. Orlic, J. Kajsttura, S. Chimenti, *Proc. Natl. Acad. Sci. USA*, **98**, No. 18, 10,344-10,349 (2001).