Morphofunctional Characteristics of Blood Mononuclear Cells during *in Vitro* Culturing under Dynamic Conditions

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The primary culture of human blood mononuclear cells was incubated on fibrous membranes under a constant directional flow of the nutrient medium modulating intraocular fluid flow. Incubation was performed for 24, 48, and 72 h. Intracellular enzyme activity of mononuclear cells increased under dynamic conditions. Differentiation of blood monocytes and circulating fibroblast precursors into macrophageal cells and mature cells, respectively, was accelerated.

Key Words: *directional flow of nutrient medium; mononuclear cells; fibroblasts*

Morphofunctional activity of cells mediating initiation, progression, and outcome of any pathological process strongly depends on the modulating effect of microenvironmental factors [7,8]. Microenvironmental conditions in the eyeball are determined by its anatomical and physiological characteristics and influence of extrastromal regulatory elements [6,10]. Anatomical-and-physiological characteristics depend on a directional flow of the intraocular fluid and fibrillar structure of the vitreous body [2,5,12]. Extrastromal elements are presented by cells migrating into the vitreous cavity (retinal pigment epithelial cells, monocytes/macrophages, lymphocytes, *etc*.) and humoral factors (*e.g.*, cytokines and growth factors) [4,9,11]. A directional fluid flow in the eyeball provided by the pressure-gradient force is of particular interest in this respect [2]. Here we studied the effect of a directional fluid flow on morphofunctional activity of human peripheral blood mononuclear cells.

MATERIALS AND METHODS

In vitro modeling of the nutrient medium flow similar to fluid flow in the eyeball was performed using a special device [5]. This device (Fig. 1) was constructed as a closed-loop system with a chamber (*2*) that had a semipermeable filter (*3*). The system was prefilled with the nutrient medium delivered from a special reservoir (*1*). It contained 80% McCoy 5A medium, 20% fetal bovine serum, and gentamicin (0.02 ml per 10 ml medium). The blood was taken from the cubital vein of healthy volunteers (4-5 ml) and placed in a sterile tube containing 1 ml heparin. Mononuclear cells were isolated by fractionation in a Ficoll-Verografin density gradient (1.067-1.077 g/ml) [1]. The final concentration of cells was brought to 3×10^6 nuclear cells/ml using the nutrient medium. Cell viability in the trypan blue test was 95-97%. These cells were introduced to the chamber (*2*) via a valve (*4*). The chamber (*2*) was communicated with a nutrient medium-containing reservoir (*1*) via a roller pump (*6*) with a support valve (*7*; *e.g.*, Aspirator-01 device, *5*). The roller pump provided a continuous directional flow of the nutrient medium (flow rate 2.1-2.4 mm^3/min). The primary culture was incubated at a constant flow of the nutrient medium for 24, 48, and 72 h under specific conditions. Control mononuclear cells were cultured on a semipermeable filter placed in a petri dish with the nutrient medium at 37°C, 5-7% CO_2, and 100% humidity.

The cells were studied by histological and cytochemical methods. Examination and photographing were performed using IBM PC Pentium, EPSON digital camera, and Karl Zeiss-Yena microscope. Optical density of mononuclear cells adhering to a filter was

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estimated by computed morphometry of digital images with PhotoShop 6.0 software [3,13]. This characteristic was expressed in arbitrary units. The results were analyzed by means of nonparametric Mann--Whitney U test (p_U, Statistica software). The type of data distribution was predetermined.

RESULTS

After 24 h the culture of mononuclear cells on a filter was presented by adherent round cells with a basophilic cytoplasm and large bean-shaped or round nucleus that sometimes had dentate invagination. The nucleocytoplasmic ratio approached 1. A cytochemical (Table 1) study revealed high activity of intracellular α-naphthyl acetate esterase, which was inhibited by sodium fluoride. Alkaline phosphatase was undetected in cultured cells.

Twenty-four hours after the start of study intracellular α-naphthyl acetate esterase activity increased compared to the control and culturing under standard conditions (p_U<0.01). The reaction with sodium fluoride allowed us to visualize individual adherent cells with resistant nonspecific esterase and moderate activity of alkaline phosphatase. Most morphological characteristics (cell shape, shape and volume of the nucleus, nucleocytoplasmic ratio ≈1) indicated that these cells belong to young fibroblasts.

Seventy-two hours after the start of study the culture of cells on a filter mainly included lymphocytes and macrophages with a foam vacuole-containing cytoplasm. The bean-shaped or round nucleus had dentate invagination. The nucleocytoplasmic ratio was below 1. We revealed not only lymphocytes and macrophages, but also individual large cells of irregular shape (mainly spindle cells). Cytochemical analysis revealed an increase in α-naphthyl acetate esterase activity, which surpassed enzyme activity in cells cultured for 48 h or under standard stationary conditions (Tables 1 and 2, p_U<0.01). Nonspecific esterase was resistant to sodium fluoride. Intracellular alkaline phosphatase activity was higher than 48 h after the start of the study (Table 1, Fig. 2, p_U<0.01). These cells were morphologically and cytochemically similar to actively synthesizing fibroblasts. Functional activity of cells was confirmed by the presence of thin connective tissue fibers on a filter (Fig. 3).

During culturing of peripheral blood mononuclear cells under standard stationary conditions the culture of cells on a filter was presented by round cells with a bean-shaped or round nucleus that had dentate invagination. The nucleocytoplasmic ratio was equal to or below 1. Cytochemical analysis revealed moderate activity of α-naphthyl acetate esterase, which progressively increased during culturing (Table 2, p_U<0.05).

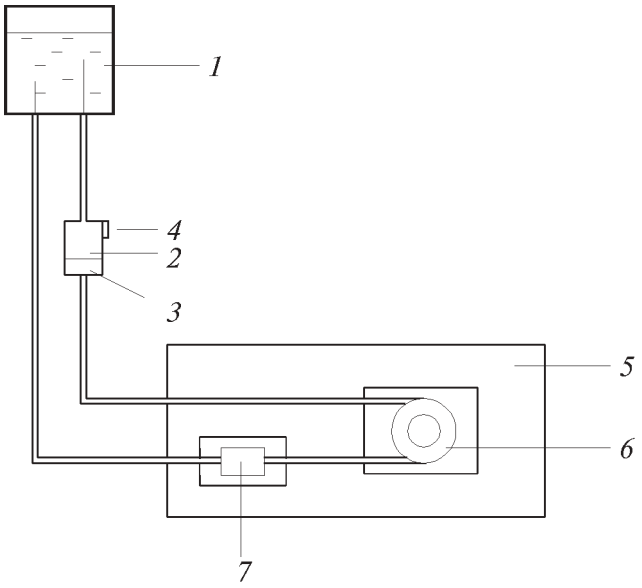

Fig. 1. Device for *in vitro* culturing of blood mononuclear cells: reservoir with nutrient medium (*1*), chamber (*2*), semipermeable filter (*3*), valve (*4*), Aspirator-01 device (*5*), roller pump (*6*), and support valve (*7*).

It was associated with differentiation of monocytes into macrophages. Nonspecific esterase was inhibited by sodium fluoride. Alkaline phosphatase was undetected.

Our results show that *in vitro* culturing of peripheral blood mononuclear cells under a directional

TABLE 1. Cytochemical Activity of Blood Mononuclear Cells during *in Vitro* Culturing under Conditions of Directional Fluid Flow (optical density units)

Cytochemical reaction	Time of culturing, h		
	24	48	72
Nonspecific esterase	63.81	69.17*	73.39°
Fluoride-resistant nonspecific esterase	0	64.93	71.45+
Alkaline phosphatase	0	40.17	69.07+

Note. p<0.01: *compared to 24 h; +compared to 48 h; °compared to 72 h.

TABLE 2. α-Naphthyl Acetate Esterase Activity in Blood Mononuclear Cells during *in Vitro* Culturing under Various Conditions (optical density units; n=25)

Culturing conditions	Time of culturing, h		
	24	48	72
Directional fluid flow	63.81	69.17*	73.39*
Stationary culture	62.38	63.95	64.08

Note. *p<0.01 compared to stationary culture; n, counted cells.

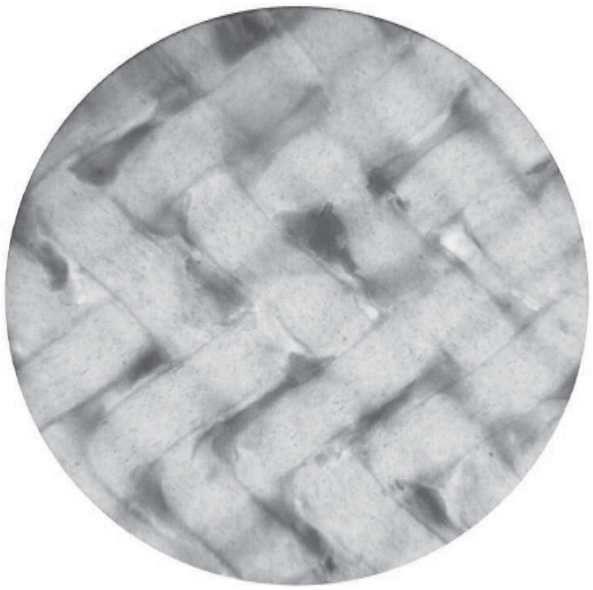

Fig. 2. High activity of alkaline phosphatase in individual cells of irregular shape after *in vitro* culturing of blood mononuclear cells under directional flow of the nutrient medium for 72 h (×800).

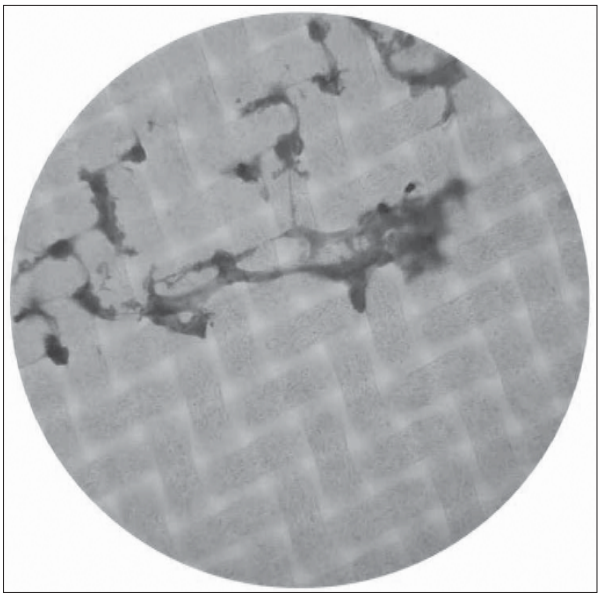

Fig. 3. Thin connective tissue fibers on a filter after *in vitro* culturing of blood mononuclear cells under directional flow of the nutrient medium for 72 h (Van-Gieson staining, ×600).

flow of the nutrient medium was accompanied by the increase in intracellular enzyme activity. Modulating influences of microenvironmental factors (directional fluid flow, extracellular matrix) accelerated differentiation of monocytes into macrophages. Besides this, young mesenchymal cells revealed among mononuclear cells underwent rapid differentiation into mature collagen-synthesizing cells. These data extend our knowledge the effect of microenvironmental factors on morphofunctional characteristics and fibrogenic activity of blood mononuclear cells.

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