Functional Activity of Blood Mononuclear Cells during Regeneration of Bone Tissue

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It is established that, during elongation of a segment of the extremity, normal regeneration of the bone tissue is attended by a slight decrease in monocyte adherence during the postoperative period and by an increase of the functional activity of lymphocytes during subsequent activation of osteogenesis. Suppression of the lymphoid apparatus is observed in the case of disturbed osteogenesis.

Key Words: *monocytes; lymphocytes; distraction osteogenesis*

Persuasive evidence of the involvement of immunocompetent cells in the regulation of reparative processes, including those in the bone tissue, has now been gathered. For instance, during repair of the tubular bones, morphofunctional characteristics of lymphocytes and monocytes alter, and immunomodulators modify the rate of formation of the osseous callus [1]. The question as to the status of cell immunity during elongation of a segment of the extremity by the method of distraction osteosynthesis in Ilizarov apparatus has been less studied. A specificity of this type of osteogenesis is a definite order of stages of regenerate formation with the development of an active zone of growth, which comprises a great number of fibroblasts, and with prolonged and synchronized collageno- and osteogenesis. Phasic alterations of the cell composition in the bone marrow and peripheral blood, as well as the population and enzyme status of lymphocytes have been established to correlate with clinico-functional stages of bone tissue regeneration during transosseous osteosynthesis (postoperation and during distraction and fixation), on which some methods of assessing osteogenesis have been based [3,6]. Further investigation of the mechanisms of the lymphocyte/macrophage regulation of osteogenesis required more in-depth research into the functional activity of blood mononuclears for the normal and disturbed course of distraction osteogenesis, and this prompted the present.

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MATERIALS AND METHODS

The experiments were carried out on 18 mongrel dogs of both sexes weighing 8-12 kg and aged 1-2 years. Tibial osteostomy was performed under thiopental anesthesia and osteosynthesis was carried out with Ilizarov's device by routine methods. Distraction was started 10 days postoperative, at a rate of 1 mm a day. The shin was stretched 25% vis-a-vis its initial size for 40 days. Ilizarov's device was then stabilized, and the fixation period lasted 270 days. Repair of the bone tissue was monitored by X-ray examination. A retrospective analysis of the roentgenograms allowed for dividing the animals into two groups: with a normal (group 1) and with a disturbed (group 2) course of osteogenesis.

Blood sampling was performed from the subcutaneous vein in the shin before surgery, every 10

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days before the onset of the stabilization period, and on fixation days 30, 180, and 270. Monocytes were isolated by centrifugation in a Ficoll-verographin gradient. The functional state of the cells was assessed according to the adherence [8] in loading tests of rosette formation [4] with theophylline and tactivine.

The results were statistically processed by the methods of variational statistics.

RESULTS

The adherence of mononuclears is one of the integral parameters of their functional activity [7]. A study of this parameter showed that before surgery the state of monocytes in group 1 differed from that in group 2 (Table 1). This corroborated our assumption that the course of distraction osteogenesis is determined not only by the degree of damage to the osteogenic tissues and by preservation of the circulation, as was reported earlier [2], but also by some endogenous factors, specifically immunological factors. We noted that during the postoperative period the ability of monocytes to adhere to the glass slightly dropped in the animals with the normal course of bone tissue regeneration, which evidently reflected the reaction to operation-induced trauma. During the periods of distraction and fixation, pronounced changes of this parameter were absent; however, this did not rule out the involvement of monocytes in osteogenesis, since it was discovered previously that their production in the bone marrow is enhanced, and antimacrophage serum markedly affects the formation of distraction regenerate [5,6]. The initially reduced adherence of monocytes was restored during active osteogenesis. This probably resulted from adaptive shifts in the monocyte/macrophage system directed toward optimization of cell homeostasis (notably, when it is disturbed) and is indicative of the important role played by the adherence molecules in achieving interaction between the immunocompetent cells and the regenerating tissue.

TABLE 1. Adherence (%) of Monocytes from Dog Blood for Distraction Osteosynthesiss ($M \pm m$)

Time of examination	Group 1	Group 2
Preoperation	89.1±2.3	70.6±6.4*
Onset of distraction	80.5±3.1	68.8±4.3
Distraction day 10	81.4±1.1	62.4±7.3*
Distraction day 40	78.1±5.4	78.5±4.1
Fixation day 30	81.7±3.2	81.5±6.0
Fixation day 180	77.2±10.6	70.5±8.5
Fixation day 270	77.2±5.3	71.5±8.8

Note. Differences between groups for $p<0.05$ marked by an asterisk.

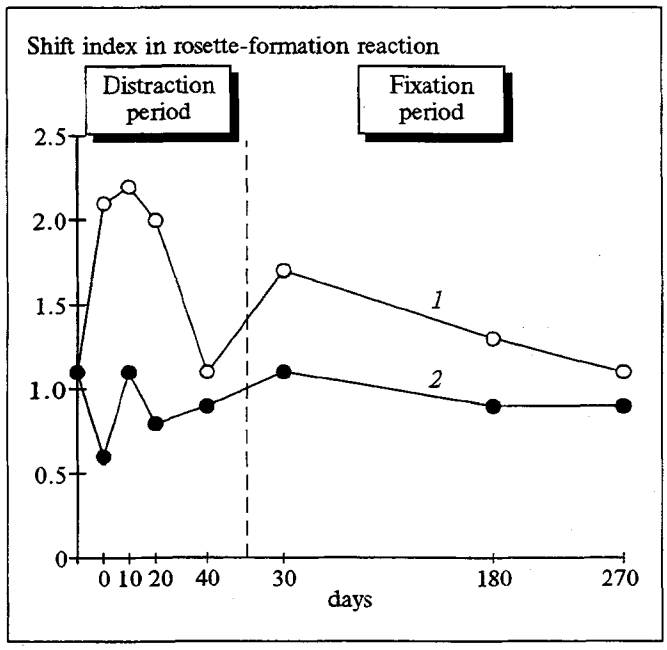

Fig. 1. Functional state of blood lymphocytes in loading rosette test with Tactivine for distraction osteosynthesis. *1*) group 1; *2*) group 2.

The loading rosette tests demonstrated that the functional activity of lymphocytes was increased during the periods corresponding to the maximum activity of osteogenesis (distraction days 10-40) and to the rearrangement of the distraction regenerate (fixation day 30). This manifested itself as an increase in the index of the shift of rosette formation during incubation of lymphocytes with tactivine (Fig. 1). In the case of a disturbed course of bone tissue regeneration a slight reduction of this parameter at the onset of distraction was followed by an increase to the normal level. The shift index in the test with theophylline changedo to a lesser degree in the animals of both groups. The morphogenetic function of lymphocytes is evidently more sensitive to thymic hormones. The responsiveness of the receptor apparatus of lymphocytes in the loading rosette tests was reduced in the animals with the disturbed course of bone tissue regeneration.

Thus, normal regeneration of osseous tissue during elongation of a segment of the extremity goes along with a slight decrease of monocyte adherence during the postoperative period and an increase of lymphocyte functional activity during the period of activated osteogenesis. The lymphoid apparatus is suppressed for a disturbed course of osteogenesis.

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Effect of a Static Magnetic Field on the Growth Rate and *in Vitro* Angiogenesis of Endothelial Cells

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It is shown that a static magnetic field accelerates the growth rate of endothelial cells from the bovine pulmonary artery, but has no effect on the attachment and growth of cells from the human umbilical vein. A static magnetic field markedly stimulates the differentiation of endotheliocytes from the human umbilical vein to capillary-like structures.

Key Words: *static magnetic field; endothelial cells; angiogenesis*

There are some data on the effect of a strong magnetic field on the function and morphology of various cells. Exposure to a static magnetic field (SMF) has been shown to inhibit the growth and to increase the number of chromosomal aberrations in human lymphocytes [4]. Under similar conditions no changes were observed in the growth rate and morphology of WI-38 cells and human skin fibroblasts [7]. However, SMF was reported to accelerate the growth of mouse pulmonary fibroblasts [12]. Pulsed electromagnetic fields are able to stimulate osteogenesis [5], DNA synthesis [9], and cell proliferation [13], and to affect the production of extracellular matrix components in various culture systems [11].

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Endothelial cells (EC) are at present among the best studied cultured cells. These cells are responsible for athrombogenicity of the vascular bed, and are metabolically active [8]; moreover, they possess the capacity for differentiation in culture (*in vitro* angiogenesis) [10]. Endothelium is known to play an active and important role in the interaction with blood leukocytes, as well as in acute and chronic inflammation [8]. Numerous functions of EC allow for using these cells as a convenient model for studying the effects of various agents. However, little is known about the effect of SMF on EC proliferation.

The purpose of the present study was to investigate the effect of SMF on the growth and differentiation of cultured EC from human umbilical vein (HUV) and bovine pulmonary artery.

MATERIALS AND METHODS

EC isolated from HUV by dispase digestion [1] were cultured at 37°C in 5% a CO_2 atmosphere

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