Mechanism of Macrophage-Mediated Regulation of Osteogenesis

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Phase changes of the functional activity of cells of the mononuclear phagocyte system are shown on a model of closed fracture of the femur in laboratory rodents. Analogous changes included the dynamics of both number and activity of the bone marrow hemopoietic islets. The parameters studied fall in the early post-traumatic stage and rise during the active phase of osteogenesis. It is concluded that there is an interrelationship between bone tissue and the hemopoietic and macrophage systems during osteogenesis, which is, in particular, realized via changes in hemopoietic islet formation.

Key Words: *macrophages; hemopoietic islets; osteogenesis*

Data indicating that the mononuclear phagocyte system (MPS) participates in the regulation of repair processes, including repair of osseous tissue, are convincing [1,7]. An important role of intercellular cooperation between macrophages, osteogenic cells, and hemopoietic cells in the mechanisms of osteogenesis has been shown [5]. However, little attention has been paid to the systemic reaction of mononuclear phagocytes, including the reaction of resident stromal macrophages present in special structural-functional associations in the bone marrow, i.e., hemopoietic islets (HI), in reparative osteogenesis. This was the topic of the present investigation.

MATERIALS AND METHODS

The work was carried out on mice of the CBA strain (Animal Breeding Center, Institute of Plant and Animal Ecology, Russian Academy of Sciences) maintained under the usual spring-summer vivarium conditions. A closed fracture of the right femoral diaphysis was inflicted under short-term ether anesthesia using surgical forceps. The studies were made before the trauma (control) and 3 hours and 2, 4, and 8 weeks after it.

The first series of experiments was carried out on 30 mice. Bone marrow was taken from the left femur and treated with collagenase, and the number of HI was counted [6]. The count of macrophage-type islets was performed in vital preparations stained with 0.1% neutral red in a Goryaev chamber.

The second series of experiments was conducted on 30 male outbred Wistar rats (Kryukovo Animal Breeding Center). The aim of the experiments was to evaluate the absorbing activity of the MPS using the india ink clearance test [4].

The course of osseous regeneration was monitored by roentgenography. Animals were also checked for conventional hematological parameters including the differential leukocyte count.

The reliability of results was assessed using the methods of variational statistics.

RESULTS

Many regulatory reactions of hemopoietic tissue are realized on the level of HI [2,3]. However, the state of the islets during reparative osteogenesis has not

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Table 1. Clearing Activity of Macrophages and Content of Bone Marrow HI during Reparative Osteogenesis ($M \pm m$)

Parameter	Time of testing				
	control	3 hours	2 weeks	4 weeks	8 weeks
India ink clearance	0.060±0.010	0.031±0.008*	0.084±0.011*	0.040±0.015	0.046±0.010
Peripheral blood monocyte count, 10/liter	0.22±0.07	0.26±0.10	0.49±0.07*	0.30±0.05	0.32±0.04
HI content in femur, 10/liter	11.4±0.8	6.5±1.2*	19.0±1.0*	10.3±1.2	12.7±0.7

Note. * Results reliably differ from the control ($p<0.05$).

been adequately investigated. The experiments presented show that the number of macrophage-type HI drastically falls during the first post-trauma hours (Table 1). Two weeks after trauma, when roentgenograms reveal active osteogenesis, the number of these HI considerably increases. Later this index reaches the normal level, this coinciding with the completion of the osseous-cartilage callus formation.

Similar results were obtained in the study of MPS absorbing capacity. This was strongly inhibited in the early post-traumatic period, apparently being one of the causes of the development of post-fracture immunodeficiency. Activation of osteogenesis was accompanied by an increase of india ink clearance followed by the return of the latter to the normal level after the completion of reparative osteogenesis.

No significant alterations in the quantity of peripheral blood monocytes was found during the period of observation, although a moderate monocytosis occurred two weeks after fracture.

Thus, the activation of osteogenesis is attended by a systemic MPS reaction, which is manifested in a change of the state of the hemopoiesis-stimulating microenvironment and of the quantitative and functional parameters of mature macrophages. The lack of marked shifts in the peripheral blood monocyte count does not exclude the possible participation of the latter in osteogenesis, since these cells are capable of migrating and accumulating in the regenerating organ [1].

It is known that monocytes-macrophages participate in osteogenesis via their capacity for osteoclastic resorption, intercellular cooperation with osteogenic cells, and the production of interleukins promoting osseous regeneration [1,5,7]. Moreover, an important role in the regulation of osteogenesis is played by the hemopoietic system, including both precursors and mature cells [5]. In the present work we have shown for the first time the reparative osteogenesis-related changes of the HI level, indicating the recruitment of the hemopoiesis-regulating macrophages residing in the bone marrow in the control of osteogenesis.

The results demonstrate that there are common regulatory mechanisms of hemopoiesis and osteogenesis realized by macrophages. Apparently the osseous, hemopoietic, and monocyte-macrophage systems work in concert to regulate regeneration [1,5] via functional changes of the bone marrow hemopoietic islets.

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