Effect of Macrophage Migration Inhibition Factor on the Content of Stromal Precursor Cells in Mouse Bone Marrow and Efficiency of Bone Marrow Precursor Cell Cloning *In Vitro*

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The content of stromal precursor cells in the bone marrow of mice decreased 2-5.7 times 24 h after injection of macrophage migration inhibition factor in doses of 0.1-50 ng/kg, this reduction depending on the dose of inhibition factor. The content of precursor cells in the bone marrow of mice increased 2-fold 24 h after injection of *S. typhimurium* bacterial mass. One day after injection of *S. typhimurium* bacterial mass, the count of precursor cells in mouse spleen was 7-fold higher than 24 h after injection of macrophage migration inhibition factor. The efficiency of cloning of mouse bone marrow stromal precursor cells *in vitro* was suppressed 1.7-2.8 times in the presence of macrophage migration inhibition factor in doses of 0.1 to 50 ng/ml culture medium. The effect of cloning inhibition was preserved, if macrophage migration inhibition factor was added to the culture medium after 2 days of bone marrow cell culturing. In general, macrophage migration inhibition factor inhibits stromal precursor cells *in vivo* and *in vitro*. The data also indicate that macrophage migration inhibition factor is not responsible for rapid and sharp increase in the count of stromal precursor cells after immunization of animals.

Key Words: *bone marrow stromal cells; immune response; macrophage migration inhibition factor*

The immune system reacts to signals by rapid shifts in the composition and volume of various cell populations. These shifts also involve the population of stromal precursor cells (CFU-F) in the hemopoietic and lymphoid organs. The count of CFU-F increased 8-fold in the guinea pig lymph nodes 1-7 days after injection of diphtheritic antitoxin and ovalbumin, this increase being more pronounced during repeated immune response [7]. Injection of *S. typhimurium* antigens causes a significant (up to 10-fold) increase in the count of CFU-F in mouse spleen [1]. Repeated immunization of mice with type 5 group A Streptococcus antigens leads to a 2-4-fold increase in the count of CFU-F in the bone marrow of mice [2]. Addition of allogenic splenic cells and *in vitro* mitogenic stimulation of mouse splenic (but not bone marrow) cell suspensions decreases CFU-F cloning efficiency (CFE-F) in cultures [1]. A close relationship between the mechanisms of natural and adoptive immunity during realization of the immunological defense became obvious now. One of the key and earliest mediators of na-

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tural immunity is macrophage migration inhibition factor (MIF) produced within the first hours after contact of the organism with foreign agents. Since the shifts in CFU-F count in immune reactions are fully realized within the first 24 h, it is interesting to clear out, whether MIF is responsible for this effect. The bone marrow CFU-F population contains osteogenic precursor cells. Retransplantation of bone marrow fragments and cultured bone marrow CFU-F descendants (stromal fibroblasts after passages in culture) into the body leads to the development of a bone marrow organ populated with hemopoietic cells [4,5]. Absorption of old and formation of new bone tissue is a dynamic process continuing throughout the entire lifespan in normal and damaged bone tissue. This process is regulated by several cytokines, growth factors, and hormones; MIF can also be involved in bone tissue metabolism. An important role of metalloproteinase in bone matrix degradation associated with bone tissue restructuring is known. It was found that MIF elevated the content of MMP-13 (collagenase 3) mRNA in rat osteoblasts [10]. High level of MIF mRNA expression in MC3T3-E1 mouse osteoblasts was noted [11].

It was shown that MIF activated macrophages and stimulated the secretion of proinflammatory cytokines (TNF-α and IL-1) essential for bone metabolism [9]. It was reported that TNF-α and IL-1 not only absorbed the available bone tissue, but also inhibited the formation of new bone tissue [12]. It was also reported that at least some of these cytokines modulated the growth and proliferation of bone marrow stromal precursor cells and their cultured descendants (fibroblasts from strains cultured during several passages) *in vitro*. For example, it was shown that IL-1 suppressed and IL-3 and TNF-α had biphasic effects on the stromal cell growth *in vitro*. Low doses of TNF-α slightly (by 50%) stimulated the growth of CFU-F and their cultural descendants, while higher doses of IL-3 and TNF-α virtually completely suppressed the growth of stromal cells in cultures [3]. These data suggest that bone marrow CFU-F can be the targets for MIF.

Here we evaluated changes in the count of CFU-F in the bone marrow of mice after injections of MIF in various doses, changes in CFU-F content in the bone marrow of mice after injection of the antigen (*S. typhimurium* bacterial mass), compared changes in CFU-F count in the spleen of mice after injections of MIF and *S. typhimurium* bacterial mass, and evaluated changes in CFE-F in mouse bone marrow cultures in the presence of various MIF doses in culture medium.

MATERIALS AND METHODS

Experiments were carried out on CBA mice (2-3 months) and guinea pigs (4-5 months) from Kryukovo Breeding Center. *S. typhimurium* bacterial mass (500 μg in 0.5 saline) and MIF (0.1-50 ng/g in 0.2 ml saline) were injected intraperitoneally 1 day before bone marrow and splenic cell explantation into cultures. Ten minutes or 2 days after cell explantation into cultures, MIF in doses of 0.5-50 ng/ml culture medium was added into some of mouse bone marrow cultures. Native human MIF was derived from human bone marrow as described previously [6].

Bone marrow cell suspensions from the femoral bones of mice and guinea pigs were prepared with a syringe as described previously [8]. Bone marrow ($1\text{-}5\times10^6$) or splenic cells ($5\text{-}10\times10^6$) were explanted in 25-cm^2 flasks in 5 ml α-MEM with 20% FCS (Paneco) and antibiotics (penicillin and streptomycin, 100 g/ml each). Bone marrow cells (10^7) from guinea pigs irradiated in a dose of 60 Gy (^{60}CO, 10 Gy/min) were added to some cultures as a feeder. The cultures were incubated for 10-12 days in a CO_2 incubator at 37°C, fixed with ethanol, stained with azur and eosin, and the colonies containing at least 50 fibroblasts were counted. CFE-F (number of colonies formed by 10^5 explanted cells) was estimated.

RESULTS

The morphology of mouse bone marrow cell cultures did not differ from that described previously

TABLE 1. Counts of CFU-F in the Bone Marrow of Mice Injected with MIF

MIF dose, ng/g	CFE-F per 10^{-5} explanted cells	Count of nucleated cells, $\times10^6$	Count of CFU-F per organ
—	7.5±1.5	12.4±2.1	934±197
0.1	3.5±0.3	13.5±1.1	480±34
5	2.7±0.2	13.4±1.3	362±20
50	0.9±0.1	17.5±1.1	164±11

Note. "—": not injected.

TABLE 2. Counts of CFU-F in the Bone Marrow and Spleen of Mice Injected with *S. typhimurium* Bacterial Mass and MIF

Organ, treatment		Dose per gram body weight	CFE-F, per 10^{-5} explanted cells	Count of nucleated cells, $\times 10^6$	Count of CFU-F per organ
Bone marrow	intact	500 μg	2.4±0.5	17.0±1.1	415±95
	MIF	10 ng	2.4±0.5	15.5±1.3	240±60
	S. typhimurium	500 μg	1.6±0.4	17.2±1.8	785±185
Spleen	MIF	10 ng	0.05±0.01	282±21	280±54
	S. typhimurium	500 μg	0.40±0.05	260±22	1040±163

[8]. Discrete colonies of fibroblasts, 0.2-0.5 cm in diameter, were seen in the cultures on days 10-14; the colonies contained an appreciable admixture of macrophages. The counts of nucleated cells in mouse bone marrow and spleen virtually did not change after injection of *S. typhimurium* bacterial mass or MIF (Tables 1, 2). The counts of stromal precursor cells in the bone marrow decreased 2-5.7 times in a dose-dependent manner 1 day after MIF injection (0.1-50 ng/kg). The count of CFU-F in mouse bone marrow increased 2-fold in comparison with intact animals 1 day after injection of *S. typhimurium* bacterial mass (Table 2). These data suggest that MIF modifies the count of bone marrow CFU-F. However, taking the inhibitory character of this influence, the increase of CFU-F count after immunization can hardly be attributed to the effect of MIF. This conclusion is confirmed by the fact that CFU-F count in the spleen after immunization of mice with *S. typhimurium* bacterial mass was 7-fold higher than after injection of MIF (Table 2). It seems that some other immunity factors are responsible for the increase in CFU-F count after immunization.

The efficiency of mouse bone marrow CFU-F cloning *in vitro* is inhibited 1.7-2.8 times in the presence of MIF in doses of 0.1-50 ng/ml culture medium (Table 3). These results are in line with the data demonstrating the effects of MIF, bacterial v-antigen, and type 1 collagen (the major extracellular matrix protein) on CFE-F in rat bone marrow cell culture. All the studied proinflammatory protein factors exhibited similar effects on CFU-F growth in rat bone marrow culture, suppressing it to a different degree: MIF and v-antigen showed slight inhibition (18 and 11%, respectively), while the effect of collagen was stronger (30-62%) [6]. The effect of MIF on bone marrow stromal fibroblasts multiplication in cultures after several passages depended on its concentration: in a concentration of 10 ng/ml MIF stimulated cell proliferation by 30-51%, while in a concentration of 50 ng/ml it inhibited cell proliferation by 16-30% [6].

TABLE 3. Stromal Precursor Cell CFE-F in Mouse Bone Marrow Cultures in the Presence of MIF in Culture Medium

Time of MIF addition after the start of culturing of bone marrow cells	MIF dose, ng/ml culture medium	CFE-F, per 10^{-5} explanted cells
10 min	—	10.2±1.1
	0.5	6.0±1.4
	5	5.6±1.0
	25	3.6±0.8
	50	4.7±1.1
2 days	—	11.4±1.1
	0.5	8.0±0.2

Note. "—": nothing added.

Hence, the degree of inhibition of CFU-F growth in primary cultures of mouse bone marrow (Table 3) was higher in comparison with suppression of the growth of these cells in primary cultures of rat bone marrow cells. Suppression of CFE-F was similar after addition of MIF during cell explantation in cultures and 2 days after explantation (Table 3). The latter fact suggests that MIF suppressed the growth of not only CFU-F, but also their cultured descendants, because it is known that after 2 days of culturing CFU-F pass through at least one mitosis. MIF in a concentration of 50 ng/ml inhibits the growth of rat bone marrow stromal fibroblasts from strains cultured during several passages and free from admixtures of other cell categories, but the degree of this inhibition does not exceed 30% [6]. Moreover, MIF in a concentration of 10 ng/ml stimulated (by 30-50%) prliferation of these cells [6]. However, it remains unclear whether MIF directly modified stromal cells *in vitro* or its effect was to a certain degree mediated by other cells (for example, macrophages) present in cultures or in the body. The latter hypothesis is confirmed by the fact that the maximum suppression (5.7 times) of CFE-F after MIF injection significantly surpassed *in vitro* effect of MIF (by 2.7 times).

In general, MIF regulates bone marrow stromal precursor cells *in vivo* and *in vitro*, its effect being inhibitory. It seems that MIF is not responsible for rapid and drastic increase in the count of CFU-F after immunization of animals.

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