EXPERIMENTAL BIOLOGY

Cytological Characteristics of Red Bone Marrow of BALB/c Mice after Implantation of Human Fetal Tissues

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Changes in erythroid and myeloid hemopoietic stems, T and B lymphocyte counts, and cell mitotic activity were observed in red bone marrow of BALB/c mice injected with human fetal tissues (placental extract and fetal liver suspension). The changes depended on the time postimplantation and, to a lesser extent, on the type of fetal tissue.

Key Words: *human fetal tissues; myelogram; mice*

Red bone marrow (RBM) is the central organ of hemopoiesis and immune system [1-4,6]. Blockade of RBM causes hypoplasia or aplasia of the erythroid, myeloid, lymphoid, monocytic, and megakaryocytic hemopoietic stems, which often leads to the development of states incompatible with life [3,4,6]. The purpose of this study was immunomorphological characterization of mouse RBM after injection of human fetal tissues (HFT).

MATERIALS AND METHODS

Placental extract and suspension of human fetal liver were prepared at Institute of Biological Medicine by an original method. Experiments were carried out on 27 female BALB/c mice aged 8 months; 12 mice received a single intramuscular injection of placental extract and 12 of fetal liver suspension into the thigh. The dose of HFT 20 times surpassed the therapeutic dose for humans. Controls (n=3) were injected with medium 199. On days 7, 14, 42, and 64, 3 mice from each experimental series were decapitated. RBM smears from the femorum were dried on air, fixed in absolute methanol, and stained according to Romanowsky—Giemsa. Cell types were identified using common criteria [1]. T lymphocytes (θ^+-cells) were detected in the cytotoxic test [12] with anti-θ-antiserum prepared by previously described method [11], B lymphocytes (Ig^+-cells) were detected by direct antiglobulin rosette formation test [5,7].

Results were processed using Student's t test. The animals were sacrificed, material fixed, and all cytological and immunological studies were carried out after 15.00, because circadian rhythms of hormonal and immune systems in humans and mice are opposite [10].

RESULTS

Implantation of placental extract to mice significantly increased the number of karyocytes per femur starting from day 14 postinjection. Injection of fetal liver suspension caused no such effect. At late stages of the experiment (days 42 and 64) the count of B lymphocytes decreased after implantation of both HFT preparations (7.0±0.6% in experiment *vs.* 15.7±1.2% in control, $p<0.01$). This decrease may be due to their increased migration to the periphery, *e. g.*, into the spleen, where the content of B lymphocytes notably increased, but their mitotic activity remained unchanged. The number of T cells in RBM reached the peak on day 64 after implantation of both HFT preparations (34.0±5.6% in experiment *vs.* 17.3±1.8% in control,

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TABLE 1. RBM Myelogram (%) of BALB/c Mice Injected with Human Fetal Liver Suspension ($M\pm m$, n=3)

Cell types	Control	Time postinjection, days			
		7	14	42	64
Nucleated erythrocytes	13.3±1.5	25.7±1.8**	25.7±2.2***	9.7±0.3	20.7±1.6***
erythroblasts	0.7±0.7	2.7±0.9	1.7±1.2	0	1.3±0.9
pronormoblasts	0	3.7±1.9	0	0	0
basophilic normoblasts	0	0	2.3±1.5	2.3±0.3**	3.7±0.9***
polychromatophilic normoblasts	0	7.7±1.5**	6.3±2.0***	2.3±0.3**	3.3±0.7**
oxyphilic normoblasts	12.7±1.5	11.7±2.7	15.3±4.8	5.0±0.2***	12.3±3.8
Neutrophils	74.0±1.0	62.0±2.3**	67.0±1.2**	80.3±1.5**	67.2±1.0**
myeloblasts	1.3±0.3	3.7±0.7***	4.0±2.0	0.7±0.7	1.3±0.3
promyelocytes	1.0±0.6	4.0±2.0	2.7±0.9	3.3±0.3***	4.0±0.6***
myelocytes	14.7±0.9	9.3±2.3	4.7±0.3**	9.3±0.9***	7.0±2.0***
metamyelocytes	15.0±1.7	12.3±3.1	8.7±1.2**	13.7±2.8	8.3±0.9***
immature	19.3±0.9	20.3±1.8	23.7±1.8	28.3±2.7	22.7±1.8
mature	22.7±1.8	17.7±5.4	21.3±3.3	25.0±1.5	26.7±3.3
Neutrophils/erythrocytes	5.6	2.4	2.6	8.2	3.0
Monocytes/macrophages	2.3±0.3	3.7±0.3***	3.0±0.6	1.7±1.7	2.0±0.6
Lymphocytes	6.7±2.0	4.7±0.9	4.3±0.3	9.7±4.1	4.3±0.3
Osteoblasts	0.3±0.3	0	0.3±0.3	0.3±0.3	0.7±0.7
Osteoclasts	2.7±0.3	2.0±0.6	2.7±0.7	1.7±0.3	3.0±0.6
Megakaryocytes	0.3±0.3	0	0	0	0
Mitotic index	0.15	0.4	N. d.	0.13	0.12

Note. Here and in Table 2: *p<0.001, **p<0.01, ***p<0.05 *vs.* the control in table 1. N. d.: not determined

p<0.05). These changes can qualitatively modify hemopoiesis, because T lymphocytes regulate proliferation and differentiation of early hemopoietic precursors [6,9].

A sharp increase in the erythroid stem, involving virtually all nucleated erythrocyte forms and occurring soon after implantation of HTF, deserves special attention (Tables 1 and 2). This phenomenon can be due to stimulation of mononuclear phagocyte system (MPS) which manifests, among other things, by a drastic increase in the count of Kupffer' cells intensively producing erythropoietin and some colony-stimulating factors (granulocytic, granulocytic-macrophagal). These substances activate the proliferation of the respective hemopoietic stems [8]. This fact is confirmed by an increase in the percentage of young cell forms of the myeloid hemopoietic stem at certain stages of experiment (Tables 1,2). Prostaglandins (PG) according to published data also stimulate colony formation by RBM hemopoietic stem cells [8]. On the other hand, the increase in the red hemopoietic stem can be attributed to increased count of T lymphocytes in the RBM especially at the late stages of the experiment. We earlier demonstrated that T lymphocytes promote activation of hemopoietic erythroid stem by stimulating the growth of early colony-forming units (CFU) in mouse bone marrow (CFU-s, BFU-e, CFU-e) [3,6,8].

The number of myeloblasts increased until day 14 postimplantation, followed by an increase in the percentage of promyelocytes (Tables 1, 2). The number of myelocytes decreased virtually at all stages of the experiment, while the content of immature and mature neutrophils remained unchanged. This kinetics of hemopoietic myeloid stem led to a decrease in neutrophil count in RBM, but this had no effect on relative and absolute count of mature neutrophils in the peripheral blood, which did not differ from the control throughout the experiment. The increase in the number of early myeloid precursors may be due to enhanced production of colony-stimulating factors by activated MPS, while decreased number of myelocytes can be caused granulocytic chalone, which prolongs G1 phase of the cell cycle and delays the entry and passage of early myeloid precursors (myeloblasts, promyelocytes) through the S phase; this however does not completely block proliferation [6]. In addition, activated macrophages produce some granulocyte inhibitors, including prostaglandin E, a potent inhibitor of granulocyte colony formation from human and animal blood CFU [6,8]. In our experiments the level of

TABLE 2. RBM Myelogram (%) of BALB/c Mice Injected with Human Placenta Suspension ($M\pm m$, n=3)

Cell types	Time postinjection, days			
	7	14	42	64
Nucleated erythrocytes	23.3±1.9***	23.3±1.1**	14.7±0.8	20.3±1.1***
erythroblasts	0	1.3±0.7	1.0±0.15	0.3±0.3
pronormoblasts	2.7±0.3*	1.3±1.3	0	1.0±1.0
basophilic normoblasts	0	2.00±0.01*	4.0±1.2***	4.3±1.2***
polychromatophilic normoblasts	8.0±2.6	6.3±1.2	3.7±0.7	3.7±0.9
oxyphilic normoblasts	10.0±2.9	12.3±2.3	6.0±1.0***	11.0±2.0
Neutrophils	70.0±2.7	65.7±1.8**	76.3±1.6	71.0±1.2
myeloblasts	6.7±1.5***	4.7±0.9	2.7±0.9	2.0±0.6
promyelocytes	4.0±1.2	1.00±0.01	3.3±1.2	4.0±0.6***
myelocytes	10.3±2.4	6.7±2.3***	7.7±1.7***	8.0±1.5***
metamyelocytes	12.3±2.2	9.3±2.7	15.7±2.3	9.0±2.5***
immature	19.3±4.9	21.0±1.0	21.0±0.6	22.7±1.8
mature	17.0±3.8	23.0±2.6	26.0±2.9	25.3±0.3
Neutrophils/erythrocytes	3.0	2.8	5.2	3.5
Monocytes/macrophages	4.3±1.2	5.7±0.3**	2.00±0.01	2.3±0.9
Lymphocytes	3.0±0.6	9.3±2.7	3.7±0.9	4.7±0.3
Osteoblasts	0.3±0.3	0	2.3±0.9	0.7±0.3
Osteoclasts	2.00±0.01	0	2.3±0.3	2.7±0.6
Megakaryocytes	0	0	0	0
Mitotic index	0.35	N. d.	0.1	0.1

mature neutrophils in RBM remained normal, which can be explained by their slow release (and probably maturation) from RBM into peripheral blood, where these karyocytes are present in abundance as parietal neutrophil pool. This pool is usually not included in active circulation, but under certain circumstances can compensate for cell deficiency in circulating blood [6].

Mitotic activity of mouse RBM cells after implantation of HFT increased almost 3-fold in comparison with the control at early stages of the experiment (day 7) and later virtually did not differ from the control (Tables 1, 2). The increase in mitotic activity coincided with the increase in the total number of erythrokaryocytes and, to a certain extent, the percentage of young myeloid cells. Presumably such time course of cell mitotic activity is physiologically justified: after the increase in the total count of young karyocytes characterized by relatively high proliferative capacity further increase in their absolute count is limited via the feedback regulation [3,6].

REFERENCES

1. M. G. Abramov, *Hematological Atlas* [in Russian], Moscow (1985).
2. V. G. Galaktionov, *Immunology* [in Russian], Moscow (1998).
3. P. D. Gorizontov, O. I. Belousova, and M. I. Fedotova, *Stress and Blood System* [in Russian], Moscow (1983).
4. I. A. Kassirskii and G. A. Alekseev, *Clinical Hematology* [in Russian], Moscow (1970).
5. D. Klaus, *Lymphocytes. Methods* [in Russian], Moscow (1990).
6. G. I. Kozinets and E. D. Gol'dberg, *Kinetic Methods of Hemopoiesis* [in Russian], Tomsk (1982).
7. E. R. Kudryavtseva, *Immunologiya,* No. 6, 71-73 (1983).
8. A. N. Mayanskii and D. N. Mayanskii, *Essays on the Neutrophil and Macrophage* [in Russian], Novosibirsk (1989).
9. O. O. Romashko, *Uspekhi Sovrem. Biol.,* No. 2 (5), 273-286 (1985).
10. A. V. Shurlygina, *Vestn. Akad. Med. Nauk SSSR,* No. 12, 45-50 (1991).
11. E. S. Golub, *Cell. Immunol.,* **2**, 353-361 (1971).
12. K. Komuro and J. A. Boyse, *J. Exp. Med.,* **138,** No. 2, 479-482 (1973).