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Reprint

Impact of bone marrow allotransplantation on functional state of mast cells in lymph nodes

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Abstract: Objective: to analyze the functional state of mast cells (MC) after bone marrow allotransplantation at the early stages of the experiment.
Materials and Methods. The experiments were carried out on 40 outbred male mice, which were divided into 3 groups: Group 1 with intact animals (n=10); Group 2 with control animals injected with 0.85% sodium chloride solution (n=10); Group 3 with experimental animals (n=30) injected into their tail vein with a cell suspension from another mouse under ether anesthesia. The state of mast cells was evaluated by the method of staining with polychrome toluidine blue sensu P.G. Unna.
Results. Forty minutes after the introduction of foreign bone marrow in the medulla, metachromatic MC were found near the vessels, and their proportion (60%) prevailed over orthochromatic MC (40%). MC were most often located in groups, especially near the vessels, in contact with each other. The morphometric parameters of MC were changing (diameter, 8.00-17.00 μm 6.00-12.00 μm vs in intact MC). Two hours after the experiment, in accordance with the degree of heparin sulfation, metachromatic mast cells were predominantly detected (92%), and extensively degranulated MC predominated (61%), $p<0.05$. The size of MC was changing (diameter: 3.00-9.00 μm; distance between cells: 29.00-53.00 μm). One-way analysis of variance established that there was a significant effect of the experiment duration on the change in the proportion of MC with metachromatic staining.
Conclusion. An introduction of bone marrow transplant significantly changes the functional profile of mast cells.

Keywords: bone marrow transplantation, lymph nodes, mast cells.

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Introduction

The lymphatic system is an integral part of the vascular system of the human body [1-4]. Lymph nodes constitute major structural components of the lymphatic system. They are an important link in lymph circulation pathways and are peripheral organs of the hematopoietic and immune systems. The study of the lymphatic system organs at the cellular level, under the effect of various factors, makes it possible to identify the degree of their morphological changes in response to a specific impact [1-4].

MC, located in septa and near vessels, are among the structures actively involved in the processes of immunity and hematopoiesis; therefore, their study has attracted close attention of researchers in recent years [5-7]. For over a hundred years of MC research, the collected data clarified their main functions: MC are involved in the formation of intercellular substance by synthesizing sulfated glycosaminoglycans; also, they synthesize, deposit, and release biogenic amines by exocytosis, which affects the microenvironment, thereby regulating local homeostasis [3]. The functional state of MC depends on the presence of biologically active substances secreted by them. There are preformed MC mediators, which include histamine, serotonin, glycosaminoglycans (heparin and chondroitin sulfates), as well as enzymes (tryptase, chymase, etc.) [1-4]. We know that substances from MC are released by diffusion or degranulation. There are several types of MC degranulation. Piecemeal degranulation with selective release of mediators from MC granules is necessary for regulating physiological processes aimed at maintaining homeostasis, whereas in anaphylactic degranulation occurring via exocytosis, the granules merge with each other and with cell membrane, which leads to an accelerated release of mediators. This type of degranulation occurs in response to any changes that take place in the body [2-5]. Hence, first of all, it is necessary to investigate the state of MC in lymph nodes in terms of experimental environmental impact on the body. Using polychrome toluidine blue staining sensu the method of P.G. Unna, we were evaluating the state of MC under the conditions of a foreign antigen introduction.

Objective: to analyze the functional state of MC after bone marrow allotransplantation at the early stages of the experiment.

Materials and Methods

The experiments were carried out on 40 outbred male rats, which were divided into 3 groups: Group 1 with intact animals (n=10); Group 2 with control animals injected with 0.85% sodium chloride solution (n=10); Group 3 with experimental animals (n=30) injected into their tail vein with a cell suspension from another mouse under ether anesthesia. We used the following method: 1 mL of bone marrow was removed from the epiphysis of the femur in a rat, mixed with 2 mL of 0.85% sodium chloride solution. Then, 1 mL of the resulting suspension was injected into the tail vein of another rat. Under ether anesthesia, the cervical lymph nodes were removed after 40 min, 2 h and 4 h from the start of the experiment. For histological examination, the lymph nodes were fixed in 10% neutral formalin. After histological examination, the material was embedded in paraffin, and serial sections 5 μm thick were prepared on the Accu-Cut SRM 200 rotary microtome, and then stained with toluidine blue sensu P.G. Unna method [3]. Their state was evaluated by the degree of MC staining: α-orthochromatic color is typical for non-sulfated (immature) heparin; inky blue color is indicative of β-metachromatic heparin; purple color implies γ-metachromatic (mature) heparin. The morphometric parameters of MC were studied (diameter, μm; distance between MC, μm). An idea of the quantitative MC distribution is given by the method of counting them in five fields of view of a microscope with magnifications of lens of ×40, and of eyepiece of ×10. Histological preparations were studied using the Leica DM4000B microscope with a Leica DFC 425 color camera and the Leica Application Suite 3.8.0 licensed program.

Statistical data processing was carried out using the Statistica 6.0 software (StatSoft, USA). The research materials were subjected to statistical analyses using standard methods of parametric and nonparametric statistics. The nature of the distribution was established via the Kolmogorov-Smirnov test with the Lilliefors correction. To describe the data, such standard statistical parameters as the mean and standard deviation (M±σ) were used. Since the distribution in some of the studied samples was non-normal, the criteria of nonparametric statistics were used. To compare the mean values of two independent samples, the nonparametric Mann-Whitney U test was employed. Differences were considered significant at $p<0.05$. Whenever there was a significant difference between the time intervals as a whole, pairwise comparisons of the average values at available time intervals (40 min, 2 h and 4 h) were carried out via Tukey's test. Relationships between time interval and MC parameters were assessed using Spearman's nonparametric rank correlation analysis. The closer the absolute value of the correlation coefficient was to 1, the stronger was the relationship between the measured values. A one-way analysis of variance was carried out whenever the duration of the experiment was used as a factor.

Our studies complied with the provisions of the European Convention for the Protection of Vertebrate Animals, the requirements of the national guidelines for the maintenance and care of laboratory animals, and were conducted with the approval of the Ethics Committee of the School of Medicine at I.N. Ulyanov Chuvash State University named (Protocol No. 5/8 of June 18, 2015).

Results

In control animals (Group 2), injected with 0.85% sodium chloride solution (n=10), the changes in the MC morphometric parameters up to 30 minutes after the experiment were revealed. Further on, the parameter values were identical to those of intact rat pups. Consequently, the duration of the experiment in the Group 3 was extended beyond 40 minutes.

When studying the lymph nodes of intact animals using the Unna method, we discovered that the crown of lymph nodules (follicles) and diffusely located internodular lymphocytes had an orthochromatic color. Two types of lymph nodules were identified: superficial, in which their reactive centers were stained; and deeper-lying nodules, in which their central part was not stained. In the reactive center, we did not detect cells positive for heparin. Glycosaminoglycan-positive lymphocytes were found in the paracortex and pulpy cords.

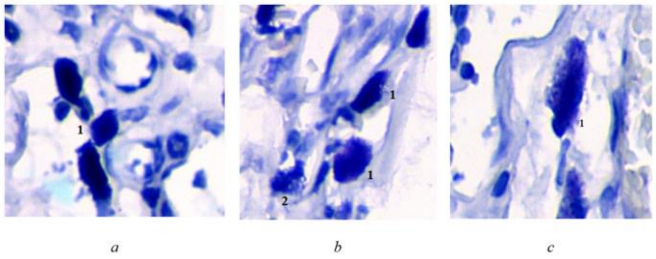
In the lymph node, MC were detected with varying degrees of metachromasia: orthochromatic (64±1.4%), β-metachromatic (20±1.8%), and γ-metachromatic (16±0.9%). The predominant cell type was non-degranulated and little degranulated (*Figure 1*).

When examining lymph nodes 40 minutes after bone marrow allotransplantation, their orthochromatic color was observed. The number of macrophages increased sharply. Orthochromatic and metachromatic MC were found in the capsule, along the septa, and in the medulla near the vessels. MC were most often located in groups, and in some places (e.g., near the vessels) they were in contact with each other. The morphometric parameters of MC were changing (*Figure 2*). According to the degree of metachromasia, MC with highly sulfated heparin predominated (*Figure 3*).

Forty minutes after bone marrow allotransplantation, we observed MC with different degrees of degranulation (*Figure 4*), and the proportion of moderately and extensively degranulated MC prevailed over non-degranulated MC (*Figure 5; Table*). Significant differences in cell sizes were detected in MC with orthochromatic coloration (by 1.28 times, $p<0.001$), and in MC without nuclei (by 1.19 times, $p<0.001$).

Two hours after bone marrow allotransplantation, resident macrophages appeared in the paracortical zone of the lymph node. In terms of the degree of heparin sulfation, predominantly metachromatic MC were detected (Figure 4). MC with varying degrees of degranulation were occurring, and the proportion of moderately and extensively degranulated MC prevailed over non-degranulated MC (Figure 5; Table).

A one-way analysis of variance revealed a significant effect of the time interval after the bone marrow allotransplantation on the change in the proportion of MC with metachromatic coloration. Spearman's rank-order correlation analysis established the presence of a statistically significant strong positive relationship ($r=0.83$; $p<0.05$) between the time interval and change in the proportion of degranulated MC.



a *b* *c*

Figure 1. Morphofunctional features of mast cells in intact animals

P.G. Unna method. Microscope: Leica DM4000B. Magnification: ×900: a – non-degranulated mast cells (1) located near the vessel; b – non-degranulated (1) and little degranulated mast cell (2); c – little degranulated mast cell (1).

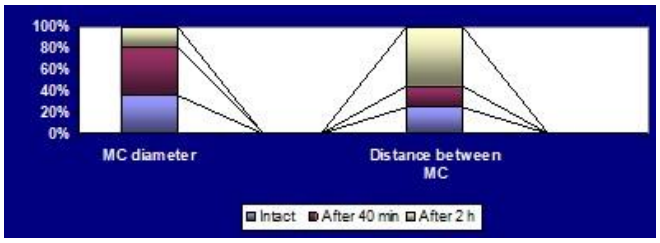


Figure 2. Diameter of mast cells and distance between mast cells in intact animals and after bone marrow allotransplantation, μm

MC – mast cells

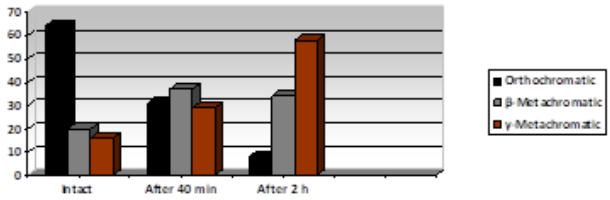
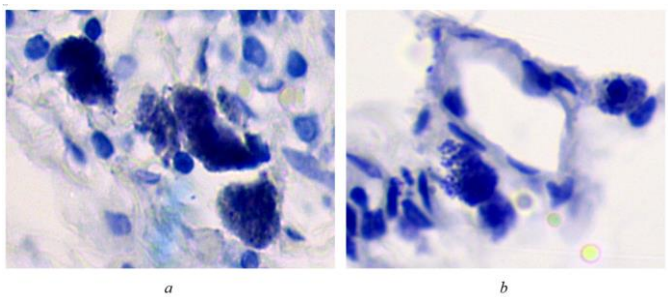


Figure 3. The degree of mast cell sulphation (%) in intact animals and after bone marrow allotransplantation



a *b*

Figure 4. β-metachromatic mast cells 40 min after bone marrow allotransplantation

P.G. Unna method. Microscope: Leica DM4000B. Magnification: ×900: a – little degranulated mast cells; b – degranulated mast cells.

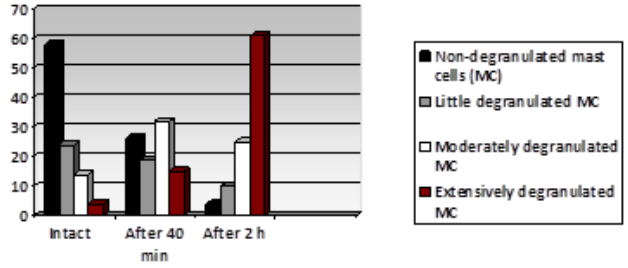


Figure 5. Mast cells with varying degrees of degranulation (%) in intact animals and after bone marrow allotransplantation

Table. Mast cells with varying degrees of degranulation in intact animals and after bone marrow allotransplantation, M±σ, %

Degranulation degree	Intact animals	Forty minutes after bone marrow allotransplantation	Two hours after bone marrow allotransplantation
Non-degranulated mast cells	58±2.4	26±1.7*	4±1.2*
Little degranulation	24±1.9	19±1.1*	10±1.1*
Moderate degranulation	14±0.8	32±0.9*	25±1.9*
Extensive degranulation	4±0.3	15±0.9*	61±1.6*

* differences with the intact group are statistically significant ($p<0.05$).

Discussion

Thus, an increase in the percentage of MC with an active degree of degranulation indicates their active participation in the cellular antigen introduction. It is noteworthy that MC in our study were determined as insufficiently mature, which was indicated by a change in their size. The introduction of foreign bone marrow led to an increase in the synthesis of highly sulfated glycosaminoglycans, the presence of which was directly proportional to the degree of MC metachromasia. An increase in the proportion of degranulated MC was established, and their ratio increased with the duration of the experiment. The predominant cells were actively degranulated MC. Obviously, MC responded to the introduction of a foreign antigen by degranulation and subsequent disintegration. As is known, MC have an ability to regulate the content of biogenic amines in the intercellular space via the synthesis of many active substances, such as serotonin, catecholamines, histamine, nitric oxide, heparin, and proteolytic enzymes [1, 2, 4–6]. It is worth noting that the contacts of MC with each other, as well as their location near the vessels, are of undeniable importance for implementing the functional potential of cells, which explicitly implies an existence of a regulatory system of the local homeostasis development.

Conclusion

We discovered an increase in the lymph node MC activity against the background of a foreign bone marrow introduction. We also revealed a change in their tinctorial properties with an increase in the proportion of

metachromatic MC and in the degree of their degranulation. The change in MC tinctorial properties coincided with the change in their morphometric characteristics. All of the above significantly modify the functional profile of MC, which indicates their active participation when a foreign antigen is introduced.

Conflict of interest: None declared.

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