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DISTRIBUTION OF S100 -POSITIVE CELLS IN THE STRUCTURES OF THE SPLEEN IN THE SIMULATION OF CHRONIC NORMOBARIC HYPOXIC HYPOXIA IN THE EXPERIMENT

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ABSTRACT — The aim of the research was to study the behavior of S100-positive cells in the structures of the spleen of laboratory animals in normal conditions and against the background of chronic hypoxic hypoxia. S100-positive cells are professional antigen-presenting cells and at the same time create a microenvironment for B-lymphocytes in the corresponding B-dependent areas of the spleen. Along with this, the expression of markers such as Ki67 and p53 was defined as determining the relationship between the processes of cell regeneration and apoptosis. Chronic isolated normobaric hypoxic hypoxia was simulated on 86 white outbred male rats in special priming chambers with controlled air supply for four hours a day, five days a week for 4 months. The analysis of the data showed that as the duration of the chronic experiment increased, the expression of the p53 marker increased mainly in the B-dependent zones, while the expression of Ki67 in the analogous zones decreased. Also, as the period of chronic hypoxia increased, a significant increase in the expression of the S100 marker in the lymphoid nodules of the white pulp of the spleen was noted. In parallel with this, there is an increase in the relative volume of the T-dependent zone and a decrease in the marginal zone of the white pulp. Thus, an increase in the volume fraction of stromal components along with an increase in the expression of an apoptosis marker in lymphoid nodules of the white pulp of the spleen may indicate the formation of tension in humoral immunity and subsequent depletion of compensatory-adaptive cellular elements of the corresponding zones by the end of the experiment.

KEYWORDS — hypoxic hypoxia, spleen, macrophages, S100 marker, apoptosis, proliferation, white pulp.

INTRODUCTION

The immunological foundations of the study of the mechanisms and cellular foundations of the adaptation process in the conditions of the formation of acute and chronic hypoxia have become one of the main tasks of ecological immunology, the subject of which is the study of changes in the body's immunoreactivity under the influence of environmental factors [3, 5, 6]. The information available today about the effect of prolonged exposure to hypoxia on the state of the immune system is ambiguous [4]. This is most likely due to the lack of standardized models of hypoxic exposure, as well as the difference in methodological approaches for the quantitative and qualitative characteristics of the parameters of the body's immune status and, possibly, insufficient coverage of this issue.

The hematopoietic system, which combines myeloid and lymphoid tissues, is the supplier of all types of cellular elements of the body's immune defense and is recognized as one of the most sensitive systems to the action of external damaging factors. The processes of proliferation and subsequent differentiation of immunocompetent cells require a specific hematopoietic microenvironment with special structural, anatomical and functional characteristics. The stromal components of hematopoietic tissue are capable not only of recognizing and retaining antigen, but also providing the factors necessary for the regulation of proliferation, differentiation, maturation, and in some cases the death of immunocompetent cells. The stroma of the hematopoietic organs consists of various cell populations that make a specific contribution to the organization of the hematopoietic microenvironment. So, there are two populations of dendritic cells - follicular and interdigitating, located, respectively, in the B- and T-dependent compartments of the white pulp of the spleen. It is believed that they have a different origin: follicular cells are cellular elements of stromal origin, and interdigitating ones are of bone marrow origin [7]. Follicular dendritic cells form a three-dimensional network in the cells of which B-lymphocytes are located. They are believed to be responsible for the survival of B-lymphocytes and stimulate the process of their proliferation and differentiation. The S100 protein, which is a group of calcium-binding proteins with low molecular weight, acts as a marker for these cells [2].

MATERIALS AND METHODS

Modeling of chronic normobaric hypoxic hypoxia was performed on 86 white outbred male rats. The animals were kept in the appropriate rooms of the vivarium, where they were cared for in accordance with the rules and regulations for handling laboratory animals [8]. The experiment was carried out in accordance with the standards of the Declaration of Helsinki (2000). Removal of animals from the experiment was carried out by decapitation under chloroform anesthesia in accordance with the Rules of laboratory practice in the Russian Federation (order of the Ministry of Health of the Russian Federation of June 19, 2003 No. 267), in accordance with the International Recommendations for Biomedical Research Using Animals (1985), and also in accordance with the regulations of the law "On the protection of animals from cruelty" [1]. The model of normobaric hypoxia was reproduced using special priming chambers manufactured at the F.F. Erisman Moscow Institute of Occupational Diseases and Occupational Hygiene (Russia) with a volume of 200 liters with a controlled composition of the air-gas mixture. An "Ankat" gas analyzer was used as a control device. The animals were kept under experimental conditions for four hours a day for five days a week. In the experimental group, four subgroups were formed in accordance with the duration of the experiment (30, 60, 90 and 120 days). A control group was also allocated (n = 14). Animals of the control group were placed in the chamber in a similar time regime, but with the usual air composition. We studied histological sections, stained with hematoxylin and eosin, according to Van Gieson for connective tissue. For immunohistochemical studies, paraffin histological sections were prepared on a LEICA RM 2255 microtome (Germany) with a thickness of 4 μm and stained using a Leica Microsystems Bond™ immunohistostainer (Germany). The following panel of monoclonal antibodies was used as primary: Ki-67 (Ready-to-Use, clone Mib 1, Daco, Denmark); P53 (Ready-to-Use, clone 7JUL, Leica Biosystems Bond™, Germany); S100 (Ready-to-Use, clone 4C4.9, Diagnostic Biosystems, The Netherlands).

An indirect streptavidin-biotin detection system Leica BOND (Novocastra™, Germany) was used for staining. The specificity of the reaction was assessed by staining sections without primary antibodies. The study and visualization of the preparations was carried out using a Zeiss Axio Scope A1 microscope (Germany) and a Leica Aperio CS2 digital slide scanner with specialized software for controlling the settings and image capture.

To assess the results of the immunohistochemical reaction, the expression indices of S100 and P53 were calculated as a percentage per 1000 cells (or their nuclei for Ki-67) in 10 randomly selected fields of view (magnification of the microscope ×400). Moderate and pronounced immunohistochemical staining was taken into account.

RESULTS AND DISCUSSION

The analyze of preparations from the control group stained with hematoxylin and eosin in animals showed that the spleen has a characteristic structure of the parenchymal organ, covered with a capsule of dense fibrous connective tissue, in which smooth myocytes, individual or lying in groups, are observed. Connective tissue trabeculae extend from the capsule into the organ, and two morphofunctional compartments are clearly delimited from each other — white and red pulp, the ratio of which relative to each other was 26, 39 ± 1.02% and 73.61 ± 0.86%. Clear boundaries are determined between the compartments; in the compartments themselves, a different density of arrangement of cellular elements is revealed. In the composition of the white pulp, two main areas of B-dependent zones are revealed — the germinal center and the mantle of the lymphoid nodule; as well as the periarterial lymphoid sheath — the T-dependent zone. A marginal zone is located along the periphery of the white pulp, which passes into the red pulp and differs from the latter also in the density of the population of cellular elements. The relative area of the B-dependent zone was about a third of the entire area of the white pulp — 32.84 ± 0.92%, half of the area was occupied by the marginal zone — about 51.36 ± 1.04%, and the rest fell on the T-dependent zone — about 15.8 ± 0.82%. In the red pulp of the spleen of the control group of animals, a significant number of blood vessels with a relatively straight course are determined. In the lumen of the vessels, isolated or small groups of shaped elements are revealed.

Immunohistochemical analysis of the S100 marker content in the structural components of the spleen in the control group of animals showed the predominant localization of the studied marker in the white pulp in the B-dependent zone; we did not find S100-positive cells in the T-dependent periarterial sheath (Fig. 1a).

The analyze of preparations stained with hematoxylin and eosin obtained from the group of animals of stimulated hypoxic normobaric hypoxia revealed certain changes. When the time of the experiment was prolonged, the organ capsule thickened and became fragmented with symptoms of edema. On the part of the trabeculae arising from the stroma of the organ, similar changes were found — thickening due to the phenomena of collagen formation and edema.

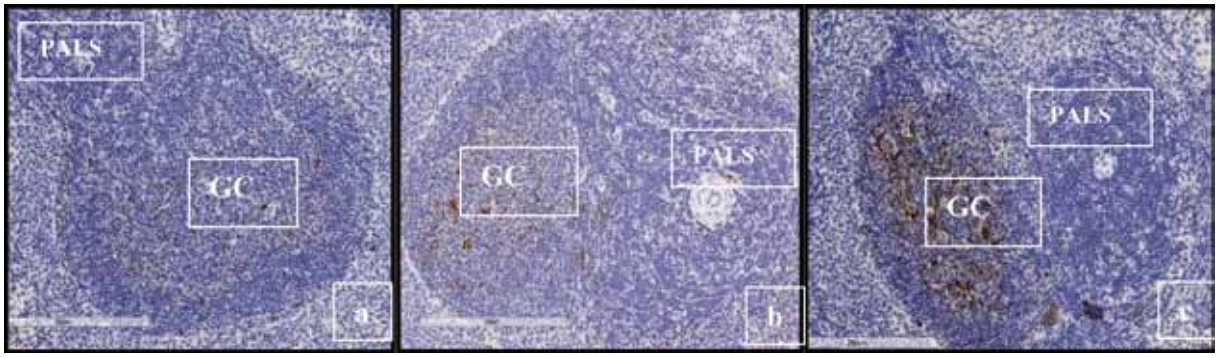

Fig. 1. *Distribution of S100-positive cells in the structures of the white pulp of the spleen in the control group (a), in the group after 60 days of the experiment (b) and after 120 days (c); GS — germinal center, PALS — periarterial lymphoid sheath. Immunohistochemical staining with antibodies to the S100 follicular dendritic cell marker. Indirect streptavidin-biotin detection system. Zoom ×400*

In the subgroup of animals exposed to isolated hypoxic hypoxia, there is an increase in the total volume of the white pulp by the end of the third month of the experiment to 36.03 ± 0.82, after which, by the end of 120 days of the experimental exposure, a decrease in the volume of the white pulp was revealed in comparison with the previous period and control and amounted to 23.4 ± 0.76% (Table 1).

the volume of the periarterial sheath gradually increased, while the marginal zone not only decreased in volume, but also the number of cellular elements in it decreased. So, by the 90th day of the experiment, the T-dependent zone increased by 6.52%, and by the 120th day, more than three times relative to the control values. Also, the volume of the marginal zone by the end of the 4th month of the experiment

Table 1. *The relative area of the structural components of the white pulp of the spleen in the control group and the group exposed to chronic normobaric hypoxic hypoxia*

Duration of the experiment	B-dependent zone	T-dependent zone	Marginal zone
Control	32,84 ± 0,92	15,8 ± 0,82	51,36 ± 1,04
30 days	39,6 ± 1,2*	17,44 ± 1,08	42,96 ± 0,83*
60 days	47,41 ± 0,95*	20,7 ± 0,74*	31,89 ± 0,7*
90 days	52,7 ± 1,07*	22,32 ± 0,82*	24,98 ± 0,88*
120 days	35,42 ± 0,74	48,63 ± 0,97*	15,95 ± 0,67

Note: ** — statistically significant differences compared with the control group (p <0.05)*

In the composition of the white pulp, changes were also determined from the side of its components. So, the main changes affected the lymphoid follicles: there was a gradual increase in their volume in the structure of the white pulp up to 90 days to 52.7 ± 1.07%, after which, by the 120th day of the experiment, a sharp decrease to 35.42 ± 0.74%. In the lymphoid nodule itself, the ratio of zones changed in favor of an increase in the volume of germinal centers and a relative decrease in cellular elements in the entire B-dependent zone.

As for the T-dependent and marginal zones, as the duration of the chronic hypoxic state increased, decreased by more than three times and amounted to 15.95 ± 0.67%.

The analyze of distribution of the marker of follicular dendritic cells S100 revealed that in the setting of chronic hypoxic hypoxia there was an increase in the number of S100-positive cells in the structures of the white pulp, especially by 90 and 120 days of the exposure. A statistically significant increase in S100-positive cells in the areas of the white pulp occurs in the germinal center of the lymphoid nodule. As for the proliferation marker Ki-67, we observed an increase in lymphoid nodules within two months of modeling chronic hypoxia, after which a significant decrease in

its expression by 120 days of the experiment in B-dependent zones. At the same time, the expression of the apoptosis marker p53, on the contrary, was detected mainly in the B-dependent zones of the white pulp, especially by 90th and 120th days of chronic development of normobaric hypoxia, which may indicate death processes, mainly among immunocompetent cells.

CONCLUSION

Thus, chronic normobaric hypoxic hypoxia that develops during a long time in the first months of exposure leads to activation of adaptive capabilities from the immunological components of the spleen. This is manifested by an increase in the relative volume of B-dependent zones and an increase in them not only S100-positive cells, but also the expression of the marker Ki67, however, by the end of the experiment, adaptation was disrupted and the expression of the proliferation marker decreased. In this case, the expression of markers of follicular dendritic cells and apoptosis, on the contrary, increases.

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