HISTOMORPHOLOGIC CHANGES IN LYMPH NODES WITH LYMPHOTROPIC IMMUNOSTIMULATION IN EXPERIMENT

Article history:
Received 9 February 2019
Received in revised form 5 March 2019
Accepted 10 March 2019

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ABSTRACT — The research deals with the study of histomorphologic changes in the lymph nodes of rats arising from the lymphotropic and intramuscular administration of acidic peptidoglycan. The authors investigated histologic changes and applied the morphometric method including the determination of the average size of the lymph nodes, the size of the germinal zones, the density of the lymph nodules. It is stated that the restructuring of the lymph nodes during lymphotropic administration occurs at different times in comparison with its intramuscular administration. The lymphotropic method provides a relatively earlier and prolonged onset of an immunostimulating effect. This made it possible to conclude that the method of administering immunomodulators should differentiate depending on the phase and form of the disease progression.

KEYWORDS — lymph nodes, lymphotropic administration, immunostimulation, immunodeficiency, peptidoglycan, murein.

INTRODUCTION

Currently, doctors in their practice increasingly confront with a state of secondary immunodeficiency which is caused by the influence of many external and internal factors [1, 2, 3]. These factors include malnutrition, helminth infection, chronic infectious diseases, injuries or surgeries, endocrine dysfunction, intoxication of various origins, etc. Doctors are equipped now with a huge number of immunostimulating drugs that are included in the treatment of diseases [4, 5, 6]. One of the groups of such drugs are those based on murein, acid peptidoglycan (APG) — a component of the cell wall of bacteria or some algae. Despite the fact that these drugs have been used for several decades, histomorphologic changes in the immune system in response to this type of immunostimulation have not been studied enough. One of the methods of *targeted* drug delivery to the lymphatic system is the lymphotropic method. It allows to create a high and long-lasting concentration of drugs in the lymphatic system [7, 8]. The research objective was to experimentally study the nature and timing of histomorphologic changes in the lymph nodes during lymphotropic immunostimulation using APG.

MATERIAL AND METHODS

The study was conducted in a certified laboratory in 20 male rats of the Wistar line at puberty, weighing 150–170 g. All the studies were carried out in accordance with the European Convention for the Protection of Vertebrate Animals used for experiments or for other scientific purposes (ETS N 123). The research has been granted a permission of the Astrakhan SMU Bioethics Committee (the protocol N 3 of 31.10.2011). The animals were divided into 2 groups — observational and experimental, 10 animals each. Immunostimulation modeling, in groups, was performed by administration of APG with a molecular weight of 1000–40 000 kDa in a dose of 0,3 U. In the observational group, the drug was administered intramuscularly, in the experimental group — lymphotropicaly on the day 1, 2, 3, 8, 9, 10 of the experiment. Hyaluronidase was used as a lymphotropic substance in a dose of 0,07 U. Withdrawal from the experiment was carried out on the day 1, 3, 7, 14 and 30. The sampling, fixation of lymph nodes and the manufacture of paraffin blocks were carried out according to the standard technique of working with lymphoid organs. The samples were stained with hematoxylin and eosin, and azure II-eosin staining was used to identify the cells. Details of the histological structure were studied using a hardware-software complex including a Leica MZ 12,5 stereomicroscope, Pixera television cameras, and a Leica DM-1000 microscope using the Morpholog computer program. Linear lymph node indicators were recorded by creating an *object-distance* between two points. The cross-sectional areas of the structural components of the lymph nodes (cortical, medullary substance and paracortical zone) were determined by the method of *masking*. Morphometric calculation was performed in six fields of view of each slice, six slices from each object being analyzed. Statistical data processing was performed using variation statistics with Statistica 6.0 software.

RESULTS END RESEARCH

Histological examination of lymph nodes in both groups showed an increase in the number and hyperplasia of the follicles in the subcortical zones, the formation of *light centers* with the proliferation of cellular elements. There was also noted a filling of the lumen of the sinuses with lymphoreticular cells. See fig. 1. Our attention was attracted to a pronounced proliferation of lymphoreticular cells. We registered the formation of follicles with designated *light centers* by the effect of lymphoblasts and hemocytoblasts. See fig. 2. In the group with lymphotropic administration of APG, on the 3rd day, there was a significant increase in the density of lymph nodules per surface unit of the sample, as well as the size of the germinal centers, which increased by 36% and 32%, respectively. Lymphatic nodules appeared not only in the cortical, but also in the medullary substance. See fig. 3. There was also a slight increase in the average size of the lymph nodes, which was within the margin of error. Lymphatic cells filled the lumen of the follicles. On the 30th day, the density of the lymph nodules and the size of the germinal centers decreased by about a third and amounted to 19% and 20%, respectively. In the observational group, an increase in the studied parameters was observed on the 7th day of the experiment. Lymph nodes' growth was found to be 25%, the germinal centers and density of lymph nodules increased by 31% and 28%, respectively. On the 30th day, the above changes almost came to initial values. See fig. 4.

DISCUSSION

Analyzing the obtained results, it can be stated that the introduction of APG causes stimulation of immunoregenerative processes in the lymph nodes. There were differences in the timing of the formation of an immunostimulating effect depending on the method of administration. With lymphotropic administration, the effect occurred earlier (on the 3rd day), and with intramuscular administration — on the 7th day. The duration of the immunostimulating effect after intramuscular administration was leveled by the 30th day, and in the experimental group it was preserved, which resulted in a more prolonged effect.

CONCLUSION

The lymphotropic administration of APG, in contrast to intramuscular administration, provides an earlier and more prolonged onset of an immunostimulating effect. The data obtained may allow the doctor to prescribe a rational treatment regimen, depending on the characteristics of the clinical course of the disease and to obtain the optimal effect.

REFERENCES

1. **Borodin Yu. I.** Guide to clinical lymphology. M.: MIA, 2010 (In Russ).
2. **Nicander L., Nafstad P., Landsverk T., Engebretsen R.H.** A study of modified lymphatics in the deep cortex of ruminant lymph nodes. J.Anat. 1991;178:203–212.
3. **Rukavishnikova S. A., Pushkin A. S., Akhmedov T. A., Prokhorenko I. O.** Basics of Clinical Immunology. Samara.: Sammi, 2015. (In Russ).
4. **Ki-Wook Kim, Joo-Hye Song.** Emerging Roles of Lymphatic Vasculature in Immunity. Immune Netw. 2017;17(1):68–76. Doi: 10.4110/in.2017.17.1.68.

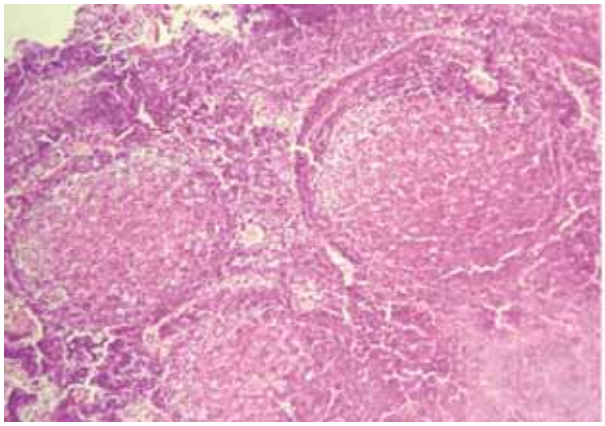

Fig. 1. *A large number of hyperplastic follicles in subcortical zones, «light centers» with proliferation of cellular elements. Lymphoreticular cells fill the lumen of the sinuses. Stained with hematoxylin-eosin ×100*

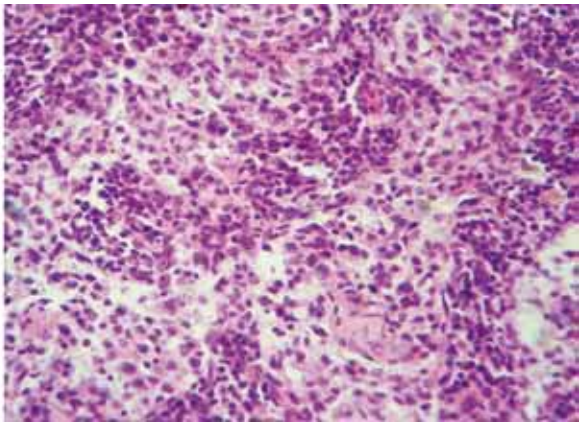

Fig. 2. *Proliferation of lymphoreticular cells. Lymphoblasts, hemocytoblasts form follicles with designated «light centers». Stained with hemotoxillin-eosin ×400*

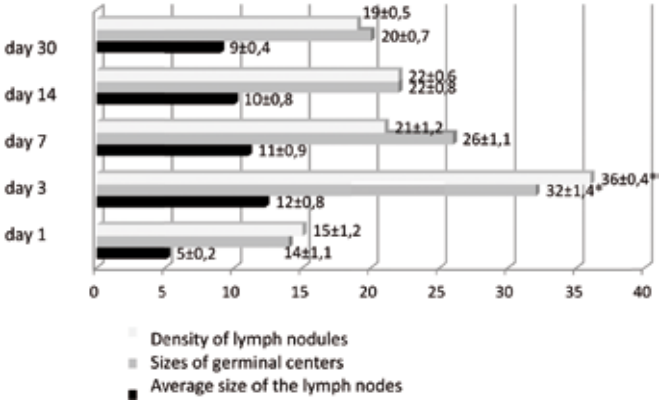

*Fig. 3. Dynamics of morphofunctional changes in lymph nodes with lymphotropic administration of HGP at different time of observation (%); * — statistically significant differences at p <0.05*

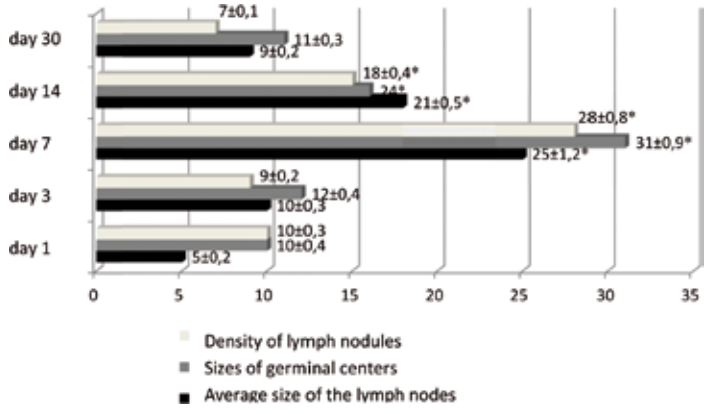

*Fig. 4. Dynamics of morphofunctional changes in lymph nodes with intramuscular administration of HGP at different time of observation (%); * — statistically significant differences at p <0.05*

5. **Randolph G.J., Ivanov S., Zinselmeyer B.H., Scallan J.P.** The Lymphatic System: Integral Roles in Immunity. Annu Rev Immunol. 2017;35:31–52. Doi: 10.1146/annurev-immunol-041015-055354.

6. **Srinivasan S., Vannberg F.O., Dixon J.B.** Lymphatic transport of exosomes as a rapid route of information dissemination to the lymph node. Sci Rep. 2016;6:24436. Doi: 10.1038/srep24436.

7. **Zeydlits A.A., Narov Y.E., Lubarsky M.S., Morozov V.V.** Implementation of lymphotropic technology in the oncology. Fundamental research. 2013;5(2):279–282. (In Russ).

8. **Dabuzov A.Sh., Omarova P.M., Atavova N.M.** Immune status in lymphotropic therapy in puerperas with organ-sparing operations in dynamics. Ural Medical Journal. 2018;3:58–61. (In Russ).