Cell Characteristics of the Lymphoid Nodules in the Peripheral Organs of Immunogenesis

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Dehydration for 3, 6, and 10 days leads to morphological changes in the small intestinal and splenic lymphoid nodules: the number of lymphoid nodules with germinative centers reduces on days 6 and 10; the percentage of blasts and mitoses decreases starting from day 3; the distance between nodular edges and the blood and lymph vessel structures increases.

Key Words: *lymphoid nodules; albino rat; small intestine; spleen*

Studies of the morphology of peripheral immune organs exposed to ecological and hydrological factors has attracted special attention of scientists in recent years [5,9,10]. Among the important organs of immunogenesis are solitary and group lymphoid nodules in the small intestine and spleen. The morphology of these nodules has not been studied over the course of dehydration.

The food processing in the duodenum and small intestine is particularly intense. The digestion is over by the end of the first meter of the small intestine, though its length is 3 to 5 meters. Cavitary, parietal, membrane digestion, hydrolysis, secretion, absorption, *etc.*, take place in the small intestine [6]. The quality of water consumed is an ecological problem all over the world, because of its direct effect on the vital activity of living organisms. The inner environment homeostasis is supported by many systems, including water factors and the lymph system [2-6,8,10,13].

We studied the impact of dehydration for the lymph nodule morphology in the spleen and small intestine in albino rats.

MATERIALS AND METHODS

The study was carried out on adult male albino rats (n=40; 180-200 g). Four series of experiments, 10 rats per point of study (control, dehydration days 3, 6, and 10) were carried out. Dehydration was induced by dry fodder (oats) without access to water. The animals were sacrificed by nembutal narcosis. All manipulations on animals were carried out in accordance with the Helsinki International Declaration on Humane Attitude to Animals. The preparations were fixed in 8-10% neutral formalin and processed in ascending alcohols.

Similar portions of the small intestine were collected in albino rats for reliable comparison of local morphology under the effect of dehydration: the middle part of the jejunum and terminal part of the ileum. In the spleen, the lymphoid nodules were selected.

The material was stained with hematoxylin and eosin, azur-nitrofungin-fuchsin, after Romanowski–Giemsa, van Gieson and Kurnik, with silver nitrate after Foot; collagen fibrils were stained after Mallory. The cell composition of the lymphoid structures in the small intestine and spleen was evaluated per unit of a histological section area (880 μ^2) using A. A. Glagolev's morphometric lattice. Microtopography and morphology were studied under an MBR-1 microscope with a 10x ocular, objectives 8, 40, and 90.

The data were statistically processed by common methods using computer analysis. The differences were considered significant at $p \leq 0.05$.

RESULTS

Analysis of the data (Tables 1, 2) showed the progress of changes in the nodular sizes (shrinkage) with

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prolongation of dehydration; cell proportions changed, the reticular fibril loops in the lymphoid nodule stroma increased, and the percentage of lymphoblasts and cell mitosis decreased. The content of macrophages in the lymphoid nodules increased by 15-20% during the first 3 days of dehydration. Starting from day 6 the levels of macrophages and mast cells dropped.

The proportions of minor, medium, and large lymphocytes changed with prolongation of dehydration. The areas of germinative centers in the lymphoid nodules decreased in dehydration, the integrity of lymphoid cells per μ^2 decreased.

Comparative study of the morphology and cytology of lymphoid nodules in the small intestine and spleen during dehydration showed some common regularities. The counts of lymphoid cells with germinative centers decreased sharply on days 6 and 10 of dehydration. The connective tissue *vs.* lymphoid tissue area in the lymphoid nodules increased. The percentage of blasts and mitoses decreased starting from dehydration day 3; on days 6-10 they disappeared. The content of large lymphocytes decreased 2-4-fold on days 6-10 of dehydration.

The percentage of destroyed cells in the small intestinal and splenic lymphoid nodules increased 1.4-3 times. These cells were particularly incident in the germinative centers of the small intestinal and splenic lymphoid nodules, where their levels increased 2.4-3.5 times. Dehydration for 6 and 10 days led to a reduction (2.4 times) of the counts of plasmoblasts, mature and immature plasmocytes in the spleen and their disappearance on day 6 in the small intestinal lymphoid nodules without germinative centers, but not in the nodules with germinative centers.

Cell density in the nodules per unit of area decreased 1.2-1.5 times starting from day 3 of dehydration and later.

Dehydration involved microtopographic and morphometric parameters of the lymphoid nodules in the small intestine and spleen. Solitary lymphoid nodules shrank by 11-12% on day 3 of dehydration, by 20-30% on day 6, and by 34-38% on day 10.

The distance between the lymphoid nodule edge and enteric epitheliocytes in the small intestine increased 1.2-2.2 times with prolongation of dehydration. The same regularity was found for the splenic nodules and blood capillaries.

Plasma cells are essential for the morphology, immunology, and physiology of the small intestine. We studied them over the course of dehydration in comparison with the controls. Cell counts decreased 1.2 times by day 3 of dehydration, 3-fold by day 6, and 5-fold on day 10. The counts of mature plasma cells decreased 1.2-2 times, that is, the synthesis of cells supporting the immunity was inhibited. The time course of plasma cells in the lymphoid structures of

TABLE 1. Cytological Composition of Solitary Lymphoid Nodules in the Ileum of Albino Rats during Dehydration ($X \pm Sx$)

Cells	Nodules without germinative centers				Nodules with germinative centers			
	control	day 3	day 6	day 10	control	day 3	day 6	day 10
Large ymphocytes	10.4±0.4	7.5±0.2	6.9±0.6	4.9±0.4	17.10±0.87	15.4±0.6	13.4±0.3	12.1±0.2
Medium lymphocytes	18.3±1.2	16.4±1.1	15.6±0.4	13.0±0.2	26.20±1.32	24.2±0.6	20.2±0.6	18.0±0.2
Minor lymphocytes	42.74±1.10	57.2±0.3	36.6±4.2	33.2±0.4	37.56±1.31	35.5±9.8	32.3±0.5	30±0.8
Mitoses	0.30±0.02	0.10±0.01	0.10±0.01	-	2.06±0.15	1.85±0.20	0.40±0.02	-
Immature plasmocytes	1.6±0.2	1.1±0.4	0.5±0.1	0.30±0.01	1.50±0.07	1.3±0.2	0.5±0.1	0.20±0.01
Mature plasmocytes	1.7±0.3	2.2±0.6	1.4±0.4	-	0.21±0.01	0.2±0.1	0.20±0.01	0.10±0.01
Macrophages	1.6±0.2	2.9±0.5	1.10±0.01	0.40±0.01	1.30±0.01	1.6±0.2	0.7±0.1	0.20±0.01
Mast	0.50±0.03	0.8±0.2	0.4±0.1	-	1.95±0.20	2.2±0.3	0.9±0.1	0.30±0.01
Reticular	7.4±0.4	7.2±0.3	6.5±0.2	6.1±0.3	11.92±0.55	11.2±0.3	9.80±0.04	9.6±0.2
Destructive	14.40±0.09	4.3±0.3	31.2±0.4	42.4±0.2	0.2±0.1	0.6±0.2	22.2±0.5	29.5±1.5
Cell density per unit of area	37.4	34.2	31.5	25.8	37.90	35.60	30.2	28.5

TABLE 2. Cytological Composition of Solitary Lymphoid Nodules in the Spleen of Albino Rats during Dehydration ($X \pm Sx$)

Cells	Nodules without germinative centers				Nodules with germinative centers			
	control	day 3	day 6	day 10	control	day 3	day 6	day 10
Large lymphocytes	5.80±0.61	4.30±0.30	2.83±0.31	5.32±0.60	12.52±0.93	16.12±1.27	7.42±0.81	15.12±1.61
Blasts	1.38±0.20	0.57±0.06	–	0.94±0.37	6.44±0.51	4.78±0.36	0.44±0.05	3.81±0.40
Minor lymphocytes	43.82±2.93	51.19±3.33	43.41±3.03	46.48±3.01	9.95±0.71	7.66±0.53	12.66±1.31	11.31±1.37
Mitoses	0.18±0.09	–	–	0.27±0.17	0.79±0.11	0.97±0.07	–	1.13±0.21
Immature plasmocytes	0.18±0.11	0.87±0.09	–	0.41±0.11	1.57±0.19	2.42±0.17	0.88±0.11	1.79±0.22
Macrophages	3.08±0.36	2.18±0.26	1.92±0.20	3.42±0.41	7.76±0.51	9.80±0.76	7.52±0.81	8.79±0.97
Destructive	10.86±0.62	9.90±0.07	19.50±2.01	11.05±4.38	11.83±1.00	18.54±1.21	28.42±2.09	15.89±1.61

intestinal walls demonstrated the most active lymphocyte transformation processes, presumably because of antigenic effects of the forming feces on the intestinal walls [1].

Dehydration led to a decrease in the total level of lymphoid cells in Peyer's patches and solitary lymphoid nodules. Comparison of the morphology and morphometric status of solitary and group lymphoid nodular structures demonstrated the changes in these parameters. The count of lymphoid nodules with germinative centers decreased, while the counts of nodules without germinative centers increased on dehydration days 6 and 10 in comparison with the control and day 3. The centers in the lymphoid nodules were poorly expressed and clarified on dehydration days 6 and 10. Similar changes in the splenic lymphoid nodule morphology in albino rats subjected to dehydration on days 3, 6, and 10 were described previously [3].

Our findings indicated that there were no one-piece muscle plate and lamina propria if the lymphoid nodules formed groups in the ileac walls of albino rats. The plates were terminated by Peyer's patches base. Solitary lymphoid nodules were often located in the thickness of the mucosa and round the intestinal crypts. In some preparations the nodules protruded by their domes into the lumen of the small intestine.

Our data confirmed the previous descriptions of the nodules histotopography [14,15]. However, our findings did not confirm that some crypts "passed" through nodules and that they had epithelial tubules. Presumably, they were parts of the intestinal crypts.

Solitary lymphoid nodules were present in the small intestinal mucosa and submucous layer, but unlike Peyer's patches, they were not closely connected to the epithelium. These lymphocyte formations contained T- and B-cells and macrophages. Their forms and functional characteristics remain little studied up to the present time.

Peyer's patches (structurally organized foci of lymphoid cells in the small intestinal mucosa and submucous layer) can be referred to peripheral lymphoid organs. The patches are surrounded by B cells without microvilli [13,15]. We think that these authors failed to present accurate description of Peyer's patches lymph bed. They have mentioned the lymph ducts in the thickness of Peyer's patches, but according to modern nomenclature, there are two lymph ducts: the thoracic and the right.

The functioning of lymphocytes together with other cells provides adequate immune response in the digestive system. Continuous exchange between the cells of lymphoid organs enables generalization of the immune reactions [4,5,9], particularly in the intestine and Peyer's patches, where lymphoid cells are involved in immunoglobulin production and secretion of intestinal hormones [11,12,15] and suffer under conditions of dehydration.

In the small intestine, M-cells of Peyer's patches participate in the macromolecule and microorganism capture, in antigen transportation to lymphocytes. The mantle zone above the germinative centers of lymphoid follicles has a chain of minor and medium-sized lymphocytes and solitary plasma cells. This chain runs from the germinative center towards the epithelial surface and lumen and shows the pathways of lymphocyte migration for contacts with the antigen [9].

Dendritic cells with their antigen receptors providing immunological memory [5,11] are closely connected to B cells.

The lymphoid cells in the digestive mucosa include minor, medium, and large lymphocytes, macrophages, eosinophils, mast cells, and polymorphonucle-

ar leukocytes. The maximum density of lymphoid cells is characteristic of the small intestine, in which 1×10^6 lymphocytes and 40-60% inter-epithelial lymphocytes are present [9].

Comparison of experimental and control values showed shifts towards the increase in the distances between the lymphoid nodules, lymph and blood capillaries, and intestinal epitheliocytes as early as starting from day 3 of dehydration. Naturally, these morphological changes can deteriorate the absorption of the digestion products and the routes of lymphocyte and macrophage migration.

The lymphoid zones responsible for the formation of humoral immunity exhibited the greatest changes over the course of dehydration (on days 3, 6, and 10) [3]. The most pronounced shrinkage of plasma cells during dehydration was observed on days 6-10 in the small intestinal lymphoid tissue.

It has been reported that 70-80% plasma cells of the lamina propria of the intestinal mucosa contain 20-22% IgA and 4% IgM [15], and that plasma cells of the mucosa synthesizing IgA could be considered as the "first defense line". The differences in the rich and variegated microflora and antigens promote the development and differentiation of lymphoid accumulations of the gastrointestinal tract as a result of their continuous antigenic stimulation from the gastrointestinal lumen.

Plasma cells are located in the villous and nodular stroma and in the lamina propria of the small intestine. The bulk of plasmocytes are located under the intestinal epithelium, around the lymph and blood vessels. Plasmocytes are monocellular protein glands producing immunoglobulins.

Hence, the morphometric aspects of the lymphoid nodules cell composition, their densities per unit of area along the entire length of the digestive system under conditions of exposure to different hydrological factors (dehydration, rehydration, *etc.*) and correction of disorders thereof under conditions of various exposures remain little studied.

REFERENCES

1. G. G. Aminova, *Morfologiya*, No. 2, 10 (2008).
2. Yu. N. Borodin, I. A. Golubeva, and A. N. Mashak, *Ibid*., No. 4, 60-64 (2005).
3. D. E. Grigorenko, T. S. Guseinov, N. G. Omarov, and M. R. Sapin, *Vestn. Nov. Med. Tekhnol.*, **14**, No. 1, 173-175 (2007).
4. T. S. Guseinov and S. T. Guseinova, *Anatomy of Lymphoid Formations in the Small Intestine of Experimental Animals* [in Russian], Makhachkala (2008).
5. E. A. Ivanova, *Morfologich. Vedomosti*, Nos. 1-2, 55-57 (2008).
6. L. L. Kolesnikov, *Sphyncterology* [in Russian], Moscow (2008), P. 152.
7. V. I. Konenkov and Yu. I. Borodin, *Cellular Aspects of Lymphology*, *Abstracts of Reports at the 2nd Congress of Lymphologists of Russia* [in Russian], St. Petersburg (2005), pp. 150-151.
8. A. B. Panfilov and E. A. Perfilova, *Morfologich. Vedomosti*, No. 1-9, 196-107 (2008).
9. M. R. Sapin and D. B. Nikityuk, *The Immune System, Stress, and Immunodeficiency* [in Russian], Moscow (2000), p. 184.
10. P. Brandtzaeg, K. Valnes, H. Scott, *et al.*, *Scand. J. Gastroenterol.*, Suppl., **114**, 17-38 (1985).
11. D. A. Edwards and E. N. Rowlands, *Physiology of the Gastroduodenal Junction. Handbook of Physiology*, Washington (1986), sect. 6.4, pp. 1985-1989.
12. C. O. Elson, *Scand. J. Gastroenterol.*, Suppl., **114**, 1-15 (1985).
13. B. Hallerback and H. Glise, *Ibid.*, **220**, 60-62 (1996).
14. S. R. Hamilton, *The Gastrointestinal Tract in Health and Disease* [in Russian], Moscow (1989), p. 372.
15. I. M. Henderson, *Pathophysiology of the Digestive Organs* [in Russian], St. Petersburg (1997), p. 287.