Immunomorphology and Immunocorrection in Calves with Experimental Enterobacteriosis

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Acute dystrophic and inflammatory changes in the gastrointestinal and immune organs and immunodeficiency were detected in calves infected with an association of *Enterobacteriaceae.* Correction with immunomodulators (T- and B-activins) and lactobacterin was effective.

Key Words: *enterobacteriosis; pathomorphology; immunodeficiency; T- and B-activins; lactobacterin; immunocorrection*

Acute gastrointestinal diseases of young animals, particularly of newborn calves, rank first among the causes of animal death [2,9]. Their etiological structure is as a rule complex. Stress, disorders in the normal and opportunistic gastrointestinal and environmental microflora, impaired resistance and immunobiological reactivity of calves and their mothers are the main causes of these diseases.

Nonspecific resistance and immunobiological reactivity, the role of opportunistic microflora and the efficacy of therapy and prevention of gastrointestinal infections with the bioactive drugs T- and B-activins and lactobacterin (LB) are now intensively studied [2-5,8]. However, their individual and complex effects on the organism, morphofunctional status, and immunomorphological reactions in animals are little studied.

We investigated the pathomorphological and histochemical changes in immune organs of calves infected with an association of enterobacteria and the effects of T- and B-activins and LB on these changes.

MATERIALS AND METHODS

Experiments were carried out on calves in vivarium of the Skryabin Academy. Newborn calves were divided into controls (n=3), groups infected with an association of *Enterobacteriaceae* (n=3), and infected after LB (n=4) or LB+T- and B-activin (n=3) protection. Calves were infected with food with a culture containing *E. coli EPEC, Salmonella typhimurium, Citrobacter freudi, Proteus vulgaris,* and *Klebsiella pneumoniae* in a dose of 2×10^7 microbial bodies.

LB was administered from the moment of birth in a dose of 1 tablet three times a day (each tablet contained 10^8 microbial bodies of *L. fermentum,* TOTC, and *L. plantarum* 8P-A.

T- (1 ml) and B-activin (3 ml) were injected subcutaneously into the neck 5 days before infection. Specimens of organs (1×1×0.5 cm) collected 3-4 days after infection were fixed in 10% neutral formalin and Carnoy's fluid. Histological sections were stained with Hematoxylin-Eosin. Lipids were histochemically visualized with Sudan III; glycogen and other neutral and acid glycosaminoglycanes by Shabadash's test, ribonucleoproteins by Brachet's test, deoxyribonucleoproteins by Feulgen—Rosenbeck's test, and tissue basophils (labrocytes) by panoptic Pappenheim's method. Morphometrical data were processed by variation and unifactorial dispersion analyses.

RESULTS

Newborn calves infected with association of *Enterobacteriaceae* developed acute gastrointestinal disease with pathognomonic clinicomorphological symptoms of enterobacteriosis within 3-4 days. Pathomorphological changes were typical of acute catarrhal gastroenteritis with pronounced vascular reaction and leukocyte-macrophage infiltration. In the abomasum and

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small intestine, numerous bacterial cells and their agglomerations with signs of adhesion were seen on the mucosal surface, in enterocytes with destroyed microvilli, and, especially, in desquamated cell debris.

Pronounced signs of accidental transformation of the second degree were detected in the thymus [6]. Along with capillary plethora and acute serous edema of the stroma, the cortical zone was depleted of T lymphocytes due to their release into the circulation or apoptosis; the phagocytic activity of macrophages was increased, and products of cell degradation (lymphocytes and Hassal's bodies) were seen in them.

Considerable changes were observed in the gut-associated lymphoid tissue, lymph nodes, and spleen. In the lymphoid system of intestine involved in catarrhal inflammation, leukocyte and lymphocyte infiltration and proliferation of histiocytes in the vascular adventitium of the mucosa and submucosa were associated with slight hyperplasia of lymphatic follicles (Peyer's patches and solitary follicles), sometimes with their fusion, and enhanced degranulation of mast cells, particularly of their subepithelial population.

In the regional mesenteric lymph nodes, acute hyperemia and diapedesis in capillaries, serous edema, germinal centers in follicles, and slight hyperplasia were observed; there were moderate focal activation of macrophagal cells and diffuse leukocyte infiltration in the lymphoid tissue and in the sinuses, particularly in the central and cerebral. Small necrotic foci surrounded by lymphoid tissue were seen. Slightly changed lymph nodes, particularly surface ones, were characterized by negligible capillary hyperemia, moderate focal accumulation of lymphoid and macrophagal cells, and minor reactive changes in the primary and secondary lymphoid follicles.

Histological and histochemical changes in the spleen were characterized by slight hyperplasia of the lymphoid, macrophagal, and plasma cells. Focal rarefaction and necrobiotic changes in spleen lymphoid tissue (T and B lymphocytes) were associated with slight hyperemia, serous edema of the stroma, leukocyte infiltration, and moderate hemosiderosis.

Protein, fatty, and carbohydrate dystrophy in the liver was paralleled by hyperplasia of the mononuclear-macrophagal cells and increased content of leukocytes and lymphocytes in the intralobular capillaries and intra- and interlobular connective tissue.

In LB-protected calves, the inflammatory changes in the gastrointestinal organs caused by enterobacterial infection were less pronounced and extensive. Defensive and adaptive reactions manifested by increased counts of parietal, gastrointestinal, mucous, mast cells, and lymphocytes diffusely infiltrating the mucosa and submucosa (signs of rapid immune reaction).

Combined treatment with LB and T- and B-activins markedly improved the parameters of the nonspecific resistance and inflammatory and regeneratory processes in tissues with limited involvement of the gastrointestinal organs and increased mitotic activity. The number of microbial bodies on the abomasum and small intestinal mucosa was lower in protected in comparison with nonprotected animals. The most marked changes were observed in the thymus (first-phase accidental transformation) [6], lymph nodes, spleen, and intraorgan lymphoid tissue with activation of lymphocytic, leukocytic, and macrophagal reaction, hyperplasia of Peyer's patches, pronounced formation of new secondary lymph follicles (lymphoid nodules), and increased content of pyroninophilic (T and B lymphoblasts and plasmoblasts) and granular forms of mast cells.

Increased secretory activity of diffuse endocrine cells plays an important role in these processes, specifically, enhanced mast cell reaction (particularly of the subepithelial population in the gastrointestinal organs), creating a new hormonal and metabolic environment and enriching the tissue microenvironment with bioactive monoamines (histamine, heparin, serotonin, etc.). These substances stimulate blood and lymph microcirculation and activate metabolic and immune processes in general (proliferation and differentiation of T and B lymphocytes, micro- and macrophages, development of cell and humoral immune reactions).

Other nonspecific and specific defense factors are activated, with increased production of deoxyribonucleoproteins and ribonucleoproteins in organs, hypersecretion of neutral and acid glycosaminoglycanes in the gastrointestinal tract and mucin in general, including secretory factor IgA, as a manifestation of inductive and productive reactions of local and general immunity [1,7]. The regeneratory processes are stimulated in the gastrointestinal organs of infected and treated calves, with the lesions decreasing and the mitotic activity of cells increasing.

Therefore, excessive antigenic stimulation induced dystrophic and inflammatory processes in the gastrointestinal, parenchymatous, and immune organs. This led to relative insufficiency of the immune system and immunodeficiency.

Opportunistic microorganisms belonging to *Enterobacteriaceae,* particularly in association, become (with consideration for their nature and quantity) pathogenic and cause an infectious process. Its pathogenetic basis is alimentary bacterial toxicosis and relative insufficiency of the nonspecific and specific defense mechanisms (immunodeficiency), which can be corrected by T- and B-activins and LB. Treatment with immunomodulators alleviated the dystrophic and inflammatory changes in organs of infected calves and sti-

mulated metabolic and immune processes (corrected immunodeficiency) providing prerequisites for reparative processes and improving the nonspecific defense factors and immunity.

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