Regeneration of Colorectal Epithelium in Diverticulosis

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Excessive poorly differentiated epitheliocytes were detected in the crypts and subepithelial regions of the colorectal mucosa during the regeneration process in the majority of patients with diverticular disease. The compensatory reaction of the sigmoid mucosa decreased, which was seen from rarely detected cryptic hyperplasia. Disorders in the epitheliocyte proliferation and differentiation were paralleled by changes in tissue levels of proinflammatory cytokines (elevation of TNF-α and IFN-γ and reduction of IL-1β and IL-8) and increase of IL-4, regulating lymphocyte activation.

Key Words: *diverticular disease; colorectal mucosa; epithelial proliferation; tissue proinflammatory cytokines*

Colorectal diverticula are most often located in the sigmoid compartment. They may be caused by neuronal dysplasia of the nerve plexuses, disorders in the colorectal motoricity, loss of wall elasticity, local vascular disorders, dysbacteriasis [8,12]. Asymptomatic complications often develop. The diverticula are fraught with complications: hemorrhages, perforations, inflammations [11,14]. The knowledge of the pathogenetic mechanisms of destructive changes in the intestinal wall and the processes regulating the compensatory and adaptive reactions is insufficient for effective prevention of complications. Destruction of intercellular matrix, resulting in the formation of low-molecular peptides with regulatory effects even in ultralow levels, plays an important part in inflammation [1]. Cytokines produced by macrophages, lymphocytes, and stromal cells are important regulators of tissue homeostasis. Imbalance of the pro- to anti-inflammatory cytokines (IL-1, TNF-α, IL-4, IL-10) and transforming growth factor-β was detected in the large intestine in chronic processes [9]. These cytokines function as intracellular and cell-cell signal systems inducing apoptosis or protecting the cells from it [6,15]. Intense production of cytokines leads to stimulation of the regulatory peptides accumulating in the pericryptic fibroblasts of the colorectal mucosa and modulating the epitheliocyte proliferation [10]. Hence, the function of the epithelium is to a great measure determined by the immune system with the complex of IL and growth factors and the regulatory peptides. Activation of immune reactions with increase of the levels of IgG, IgA, IgM, antineutrophilic cytoplasmic antibodies, and antibodies to protein stimulating epitheliocyte permeability was detected in complicated diverticulosis [2,5].

We studied the epithelium regeneration and the effects of pro-inflammatory cytokines on the proliferative process in the sigmoid mucosa in uneventful diverticulosis (DVC).

MATERIALS AND METHODS

Twenty-five individuals with colorectal DVC (mean age 57.6±9.4 years), patients of the Institute of Gastroenterology, were examined: 13 with first diagnosed pathology and 12 with more than 8-year history of colorectal DVC. The disease was diagnosed by clinical, laboratory, and instrumental methods. Morphological and immunological studies of the colorectal mucosa were carried out on biopsy specimens from the diverticulum edge. The reference group consisted of 25 patients with irritable bowel syndrome (IBS) in

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whom the sigmoid mucosa was analyzed with diagnostic purpose. Tissue specimens were fixed in 2% glutaraldehyde in 0.1 M cacodylate buffer and in 1% osmium tetroxide. After dehydration the tissue specimens were embedded in epon and araldite mixture. Semithin and ultrathin sections of the specimens were examined in a JEM-1200 EX electron microscope.

The levels of pro-inflammatory (IL-1β, IL-8, TNF-α, IFN-γ) and anti-inflammatory cytokines (IL-4) were measured in homogenized biopsy specimens of the colorectal mucosa. The immune response deviation index (DI) was calculated as the IFN-γ/IL-4 proportion [3]. Colorectal tissue was homogenized in 0.5 M Tris-HCl buffer (pH 7.3) and centrifuged. The cytokines were measured in the supernatant (1 pg/g tissue) by ELISA using Cytokine and Protein Contour commercial kits.

The results were statistically processed by Statistica 6.0 software; confidence intervals for the compared means were calculated [7].

RESULTS

Multicellular blocks of small peculiar epitheliocytes connected to each other by axons (Fig. 1, *a*) were found in the diverticulum edge in the colorectal mucosa of 15 patients. The basal epithelial membrane was incomplete or lacked in sites outside the crypts where these cells accumulated. A specific feature of these epitheliocytes was their small size, polygonal shape, large nucleus with finely dispersed chromatin. There were also cells with karyopyknosis and "dark" hyperchromatic cells connected to similar and common epitheliocytes. Ultrastructural analysis of unchanged cells showed solitary mitochondria and free ribosomes in the cytoplasm and long processes of the plasma membrane, connecting the cells to each other (Fig. 1, *b*). The structural characteristics of these cells indicated their poor differentiation. Epitheliocyte hyperplasia was found along the entire length of the crypts, which was confirmed by the presence in the basal mem-

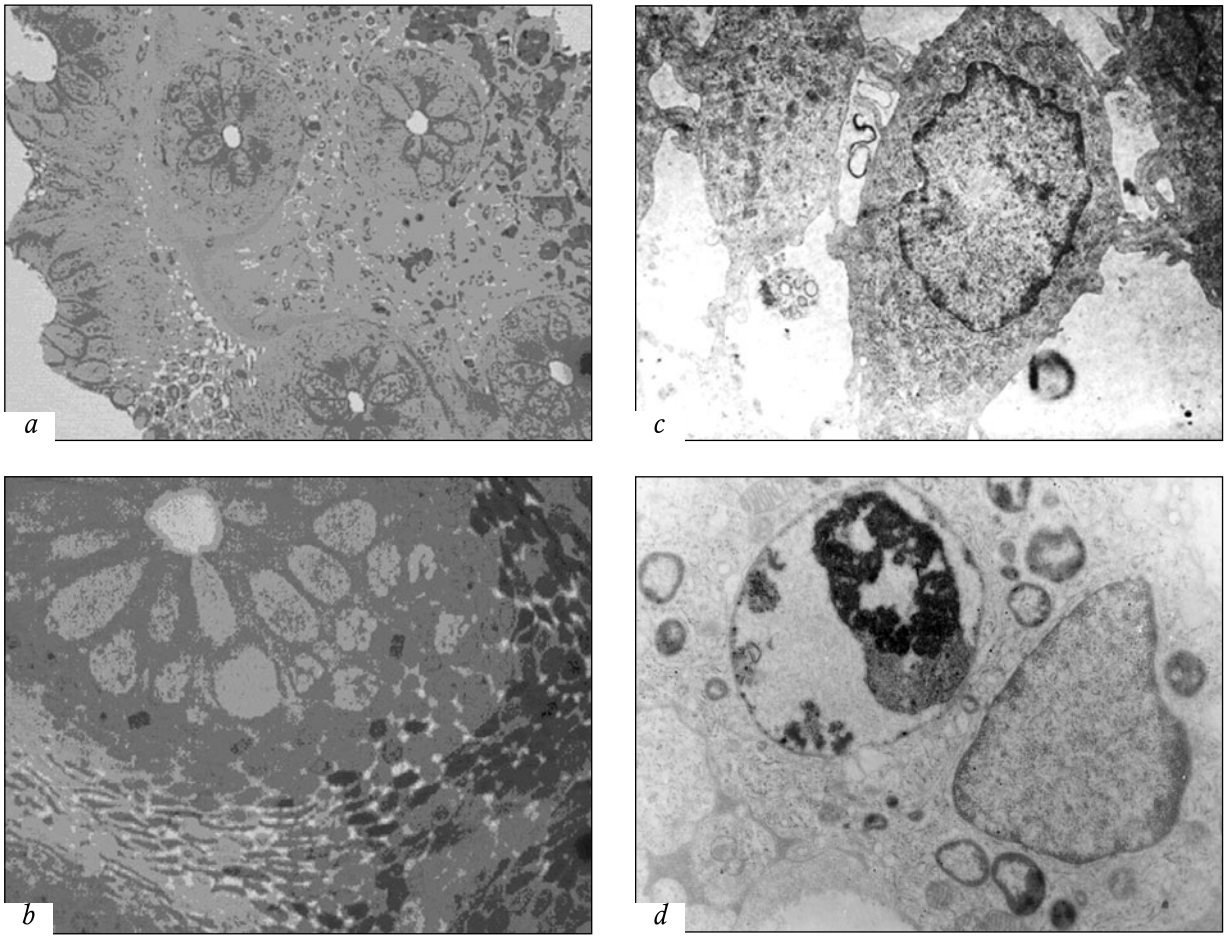

Fig. 1. Sigmoid mucosal epitheliocyte proliferation near diverticulum. *a*, *b*) semithin section, Toluidine Blue staining; *c*, *d*) ultrathin section. *a*) multicellular block of poorly differentiated epitheliocytes in the upper compartments of crypts: crypt contours are retained, basal membrane not detected, hyperadhesion of macrophages, plasma cells, and extravasal erythrocytes (×240); *b*) poorly differentiated epitheliocytes are released from crypts and form a block of cells connected by processes: "dark" and "clear" cells (×600); *c*) a poorly differentiated epitheliocyte with processes of cell-cell connections (×12,600); *d*) active macrophage with lysosomes containing compact incorporations (×12,600).

TABLE 1. Levels of Pro-Inflammatory and Anti-Inflammatory Cytokines (pg/g Tissue) in Colorectal Mucosa in DVC and IBS

Cytokines	Producer cells	IBS (*n*=15)		DVC (*n*=15)	
		M±*m*	range of concentrations	*M*±*m*	range of concentrations
IL-1β	Macrophages	21.2±0.5	19.5-22.5	31.6±5.6	21-42
IL-8	Macrophages	34.4±1.6	31.2-38.1	47.4±13.5	16-70
TNF-α	Macrophages	9.2±0.8	7.6-10.8	299.3±34.5*	230-368
IFN-γ	Th_1	32.2±7.8	16.6-47.8	248.8±16.1*	217-281
IL-4	Th_2	20.3±5.6	9.1-31.5	92.2±34.1*	25-152
DI (IFN-γ/IL-4)	Th_1/Th_2 N (0.9-1.2)	1.58±0.13		2.69±0.38	

Note. *n*: number of measurements in a group. *$p<0.05$ in comparison with IBS.

brane of small cells with bubble-like nuclei among differentiated colonocytes. Estimation of the counts of these cells in cross-sections of the crypts showed 15-20 small poorly differentiated cells per 6-8 goblet epitheliocytes (Fig. 1, *c*). Excessive proliferation of epithelial cells was caused by apoptosis suppression, leading to violation of the physiological balance of these processes [9]. Parallel presence of differentiated and undifferentiated epithelial cells along the entire length of the crypts indicated asymmetrical division of stem cells in the bottom. As a result of this division, one daughter cell retained characteristics of stem cell capable of further proliferation, while the other started either specific differentiation cycle with the formation of mucus-producing and absorbing epitheliocytes or apoptosis [13].

Proliferation of immunocompetent cells was detected in the multicellular blocks of poorly differentiated epitheliocytes. Numerous macrophages in the lamina propria were characterized by the formation of plasma membrane protrusions and large polymorphic lysosomes. The density of lysosomal inclusions was associated with hemosiderin formed as a result of ferritin metabolism (Fig. 1, *d*). Lymphocytes were a heterogeneous population of cells of different size, with large oval nuclei with finely dispersed chromatin. Dendritic cells with long branched axons, indicating high recognition of the antigen, were found in the few lymphoid infiltrations. The nuclear chromatin organization was impaired in many plasma cells. Changes in the structure of immunocompetent cells led to formation of small cell associations. Hyperadhesion was due to expression of antigen-recognizing receptors on macrophages and dendritic cells, this promoting the immune response stimulation [9].

Pro-inflammatory cytokines play an important role in the mechanisms responsible for cell-cell interactions and determining various stages of immune recognition of antigens and development of immune reactions. Changes in the levels of pro-inflammatory cytokines were detected in extracts of tissue from DVC patients with excessive proliferation of the colorectal epithelium (Table 1).

The levels of IL-1β and IL-8 were slightly elevated ($p<0.05$) in DVC. However, simultaneous production of IL-1β and chemoattractant IL-8 (preimmune inflammation mediators) promoted triggering of the initial steps of immune response, leading to stimulation of antigen-presenting cells, for example, macrophages, detected in the colorectal mucosa near the diverticulum. Macrophage stimulation was paralleled by hyperproduction of TNF-α, its concentration in DVC was significantly higher than in IBS (299.3±34.5 and 9.2±0.8 pg/g, respectively). Macrophage stimulation and induction of TNF-α synthesis in the colorectal tissue were associated with bacterial products (muramyldipeptide, proteases, LPS) forming protein complexes with macrophage CD14 receptors [8]. Intense production of TNF-α in the mucosa stimulated lymphocyte proliferation and differentiation in the lymphoid tissue associated with the intestine [13]. High concentration of IFN-γ in DVC *vs.* IBS detected in our study (248.8±16.1 and 32.2±7.8 pg/g, respectively) reflected hyperstimulation of Th1-dependent immunity determining the production of growth factors in the mucosa, regulating the epithelial cell proliferation and differentiation and apoptosis start [14,15]. Our studies showed that DVC with hyperproliferation of epitheliocytes was associated with changes in the pro-inflammatory cytokine proportion in the tissue, this reflecting the immune response dysregulation. This was confirmed by DI increase (2.7 times) in DVC in comparison with IBS. Hyperproduction of anti-inflammatory cytokine IL-4

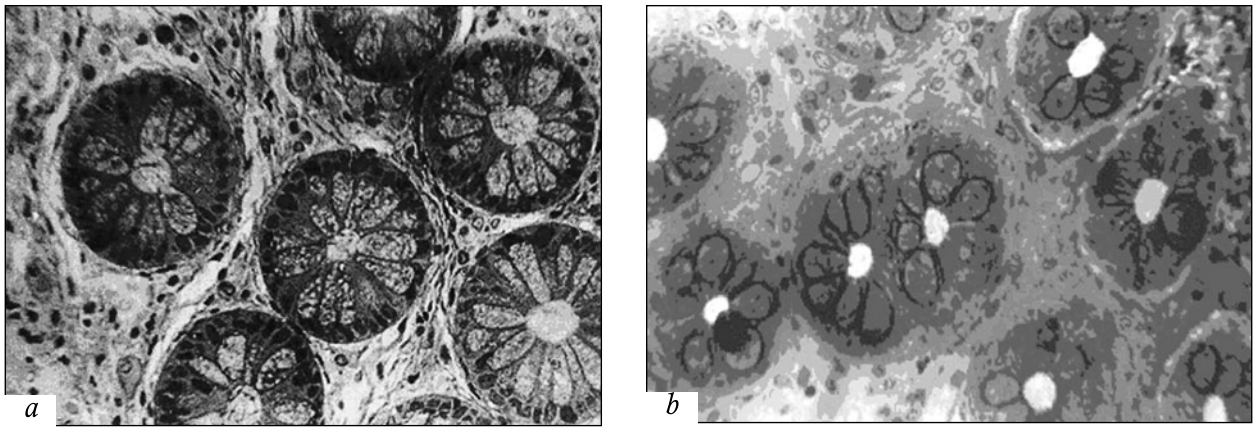

Fig. 2. Crypt proliferation in the sigmoid near the diverticulum (semithin section, toluidine blue staining; ×600). *a*) increase in the number of crypts, shrinkage of space between the crypts, dilatation of pericryptic capillaries; *b*) double crypt with a common basal membrane.

also indicated disorders in the regulation of immune reaction in DVC.

In contrast to patients with excessive proliferation of epitheliocytes, 6 patients developed crypt hyperplasia. Complex structures consisting of 2-3 small crypt-like formations enveloped in a common basal membrane and having a blurred lumen were found in the deep compartments of the mucosa among transverse sections of the crypts (Fig. 2, *a*). Closer to the mucosal surface they separated into individual crypts with the basal membranes of their own and wide lumens (Fig. 2, *b*). The number of daughter crypts was 2-3-fold higher in DVC in comparison with the control (7-15 *vs.* 3-6, respectively). A greater density of the crypts in the large intestine is regarded as a manifestation of the regenerative and adaptive process [4]. The spaces between the crypts in such regions were narrow, the counts of stromal and immunocompetent cells were reduced. The blood vessels were moderately dilated and contained solitary blood cells.

No appreciable changes in the mucosa adjacent to the diverticula were detected in 4 patients. Solitary macrophages contained compact incorporations. Lymphocyte and plasma cell structure was unchanged. No proliferative changes in the epithelium or macrophage activation were detected in the colorectal mucosa of IBS patients (reference group).

Hence, a peculiar regenerative process was detected in the sigmoid diverticular area. A specific feature of this process was inhibition of the normal regenerative adaptive process, presented by crypt hyperplasia (organ-specific proliferation) paralleled by epithelial proliferation not associated with specific differentiation of colonocytes (organ-nonspecific proliferation). A stable pool of poorly differentiated epitheliocytes connected to each other by processes, cells retaining the characteristics of the epithelium, formed in the latter case. Disorders in epithelial cell proliferation and differentiation were paralleled by disorders of different kind in the levels of pro-inflammatory cytokines (increase of TNF-α and IFN-γ and reduction of IL-1β and IL-8 levels) and elevation of the anti-inflammatory IL-4 level. The production of cytokines in tissue can be assumed to be induced by LPS, because dysbacteriasis is one of the pathogenetic factors of colorectal DVC.

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