Structural Modification of the Gastrointestinal Epithelium during Immune-Dependent Granulomatosis

D. L. Nepomnyashchikh, V. A. Burkovskaya*, S. V. Aidagulova, M. A. Bakarev, O. A. Postnikova, V. I. Kapustina, Zh. V. Nokhrina, and E. V. Vinogradova

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 150, No. 10, pp. 467-471, October, 2010
Original article submitted July 5, 2009

We present results of pathomorphological study of the mucosa epithelium in various parts of the gastrointestinal tract in Crohn's disease. Particular attention was paid to structural transformation of epithelial cells. Barrier dysfunction of the gastrointestinal mucosa was shown to be one of the major pathogenetic factors for Crohn's disease.

Key Words: *Crohn's disease; epithelium of the stomach and intestine; pathomorphology*

The epithelial layer determines functional activity of gastrointestinal tract. Apart from secretion and absorption, the epithelium serves as a barrier with selective permeability for nutrient substances and macromolecules. This barrier permits specific influence of microorganisms on the immune system of the mucous membrane. Changes in barrier function impair transport of antigens from the intestinal lumen. The state serves as a pathogenetic factor for chronic inflammatory diseases of the intestine (*e.g.*, ulcerative colitis and Crohn's disease). These diseases are characterized by an abnormal immune response to intestinal microorganisms [5-7]. The majority of studies with granulomatosis (*e.g.*, Crohn's disease) were directed toward evaluating the presence and type of granulomas. These data are of diagnostic significance. Studying the epithelial barrier can provide new data on the pathogenesis of granulomatosis, which suggests the interaction of the internal and external media of the organism [1,3,9].

Here we studied the structural changes in epitheliocytes in various parts of the gastrointestinal system during Crohn's disease.

Institute of Regional Pathology and Pathomorphology, Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk; *Siberian State Medical University, Ministry of Health and Social Development of the Russian Federation, Tomsk, Russia. ***Address for correspondence:*** pathol@soramn.ru. D. L. Nepomnyashchikh

MATERIALS AND METHODS

We examined 24 patients with Crohn's disease (15 men and 9 women, 16-68 years). An endoscopic examination showed that damage to the large intestine exists in 12 patients. The other patients were characterized by significant changes in various parts of the intestine and stomach. The clinical severity of pathological changes was shown to vary from mild to severe dysfunction. Remission of the disease was revealed in 3 patients. Biopsy was preceded by therapeutic treatments of different duration (depending on the severity of disease). The standard therapy included glucocorticosteroids and 5-aminosalicylic acid.

Biopsy specimens (n=80) were taken from various parts of the gastrointestinal tract (fundal and pyloric parts of the stomach, descending portion and bulb of the duodenum, ileum, transverse colon, sigmoid colon, and rectum). The number of samples to verify the clinical diagnosis varied from 3 to 10 (according to the results of an endoscopic examination). Liver samples were taken from 6 patients with symptoms of hepatic dysfunction. Biopsy specimens were fixed in 4% paraformaldehyde. Paraffin sections were stained with hematoxylin-eosin. This treatment was performed in combination with the Pearl's method and van Gieson staining. The elastic fibers were repeatedly

0007-4888/11/1504-0490

stained with Weigert's resorcin-fuchsine. The PAS reaction was conducted. Semithin sections were stained Schiff's reagent and azure II. Ultrathin sections were contrasted with uranyl acetate and lead citrate. Light microscopy was performed using a Leica DM 4000B microscope and Leica DFC 320 digital camera. Ultrastructural study was performed under a JEM 1010 electron microscope (accelerating potential 80 kV).

RESULTS

The most significant changes were found in the mucosa of the stomach, ileum, sigmoid colon, and rectum. An endoscopic examination revealed the presence of chronic erosions in the pylorus of 2 patients. Two patients had ulcerative lesions of the sigmoid colon. Ulcerative lesions and erosions of the rectum were found in 50% patients.

Microscopy showed that the majority of patients have typical granulomas of different size in the stomach (Fig. 1, *a*-*c*). Conglomerates of granulomas were sometimes found. Typical granulomas were not identified in the duodenum. Lymphoid aggregates and follicles were found in the duodenum. Biopsy examination of the ileum revealed the formation of large elongated giant granulomas. They were characterized by the presence of perifocal polymorphic infiltrates and completely substituted for structural components of the lamina propria of the mucosa (Fig. 1, *d*, *e*). Small lymphoid aggregates were also found. The absence of Pirogov–Langhans' giant cells in granulomas probably serves as a sign of drug-induced pathomorphosis. Morphological signs of toxic exposure were revealed during liver biopsy examination (severe intracellular cholestasis, particularly in hepatocytes of the pericentral lobular area, and lipid infiltration of hepatocytes).

The irregular and sometimes dense cellular infiltration was diffuse and perifocal. It was found around the granulomas and mainly consisted of lymphocytes and considerable number of plasma cells. Some areas of infiltration also included a considerable number of eosinophils. Plasma cells differed in hyperplasia of the cytoplasmic reticulum, varying density of the cytoplasmic matrix, and different content or degeneration of mitochondria (Fig. 1, *f*). Cellular infiltration in biopsy specimens of the rectum was characterized by the presence of a considerable number of neutrophils. It was associated with ulcerative lesions. Transepithelial leukopedesis and subepithelial and perivascular edema of the proper membrane were observed in the majority of gastrointestinal samples. In some samples, we revealed a tendency to the development of fibrosis (*e.g.*, periglandular fibrosis) and deformation or atrophy of the glands.

The surface epithelium was modified in biopsy specimens of the gastric and intestinal mucosa. It was characterized by degeneration and atrophy (Fig. 2, *a*). Focal dysplasia and "nonstandard" deformation of epitheliocytes were associated with changes in the cytoarchitectonics and plastic supply of the mucosa due to excessive growth of granulomas and high density of cellular infiltration. Disintegration of the epithelial layer was accompanied by destruction of tight junctions and widening of the intercellular space (Fig. 2, *b*, *c*). Slight deformation of the epithelium and atrophic modification were found in the pylorus and ileum. The surface epithelium in the fundal and pyloric portions of the stomach was characterized by a significant decrease in biosynthetic functions, reduction of the mitochondria, ribosomes, and cytoplasmic reticulum, change in the cytoskeleton, and absence of mucus-containing secretory granules (Fig. 2, *d*, *e*). The fundal and pyloric glands were characterized by atrophy and complete reduction, respectively. Duodenal enterocytes had normal structure, but included the focuses of pronounced polymorphism and small areas of regeneration (typical of grade I dysplasia). Ulcerative and erosive lesions of the sigmoid colon (Fig. 2, *f*) and rectum were associated with severe atrophy and desquamation of the surface epithelium (perifocal area).

Complex interaction between the genetic, microbiological, and environmental factors can be followed by long-term activation of the immune system in the mucosa, which results in Crohn's disease. The majority of therapeutic approaches are directed toward the modification or suppression of an abnormal immune response. Published data show that the intestinal microbiota has an important role in the pathophysiology of Crohn's disease [14]. The epithelial layer provides normal function of the digestive tract. The increased permeability of this layer for abdominal antigens can result in the development of inflammatory processes and injury to the mucosa.

According to the modern concept, the pathogenesis of Crohn's disease depends on the following three factors: presence of bacterial antigens and adjuvants in the intestine; dysregulation of the immune system, which provokes the immune response against these antigens; and barrier dysfunction of the mucosa, which contributes to the interaction of bacterial antigens and intestinal adjuvants with the cells of innate and acquired immunity and development of the immune response [6].

Clinical and experimental studies showed that normal function of the epithelial barrier is of considerable significance. Epithelial cells of the large intestine form the first line of defense against enteral antigens and bacteria [8]. The gastrointestinal tract mucosa

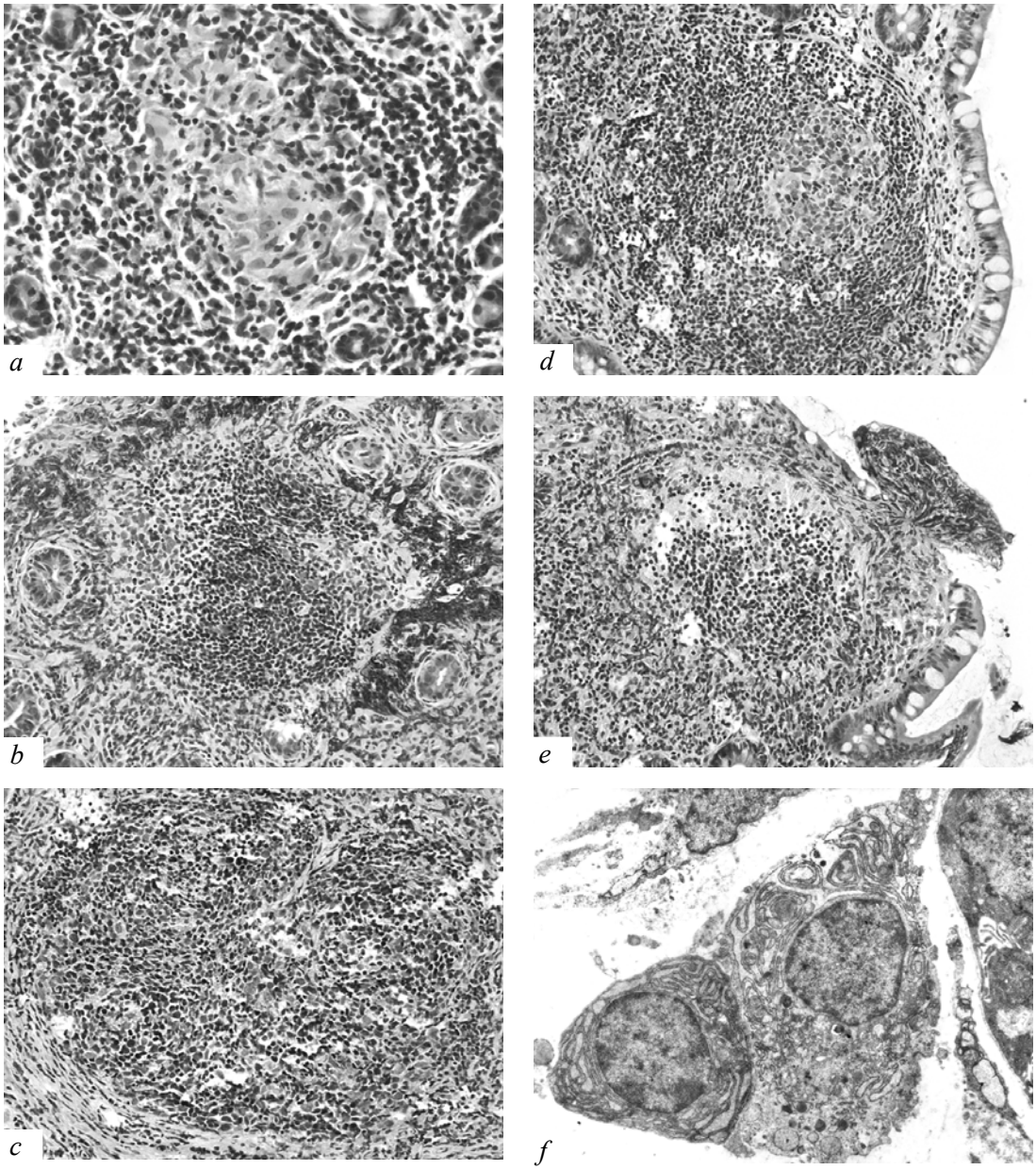

Fig. 1. Structural characteristics of granulomas during Crohn's disease. Biopsy specimens from the mucous membrane of the stomach (*a-c*, *f*) and ileum (*d*, *e*). Typical granuloma with light epithelioid cells in the central region (*a*, ×450); large granuloma and single perifocal pyloric glands (*b*, ×250); fibrosing granuloma (*c*, ×250); large subepithelial granulomas with destruction of the epithelium (*d*, *e*, ×250); plasma cells (*f*, ×2500). Hematoxylin and eosin staining (*a-e*); electron-diffraction pattern (*f*).

is composed by a layer of epithelial cells that are in close contact with each other. The epithelial barrier contributes to absorption of nutrient substances, but prevents accumulation of antigens or potentially toxic agents [11]. Under normal conditions, the epithelial layer prevents the transcellular (across epithelial cells) and paracellular (between cells) transport of antigens. Only small amounts of molecules can enter the mucosa and interact with the immune system of the intestinal mucosa. Functional activity of epithelial cells (*e.g.*, permeability) is modified under the influence of some neurotransmitters and immune mediators [13]. Long-term increase in paracellular permeability facilitates transport of antigens from the intestinal lumen through the mucosa, which can lead to the development of chronic inflammation in susceptible subjects [15]. By contract, transcellular permeability increases only in the acute period of inflammation.

Structural modifications of the surface epithelium of the stomach and intestinal mucosa (disintegration of the epithelial layer, destruction of tight junctions, and widening of the intercellular space; atrophy and

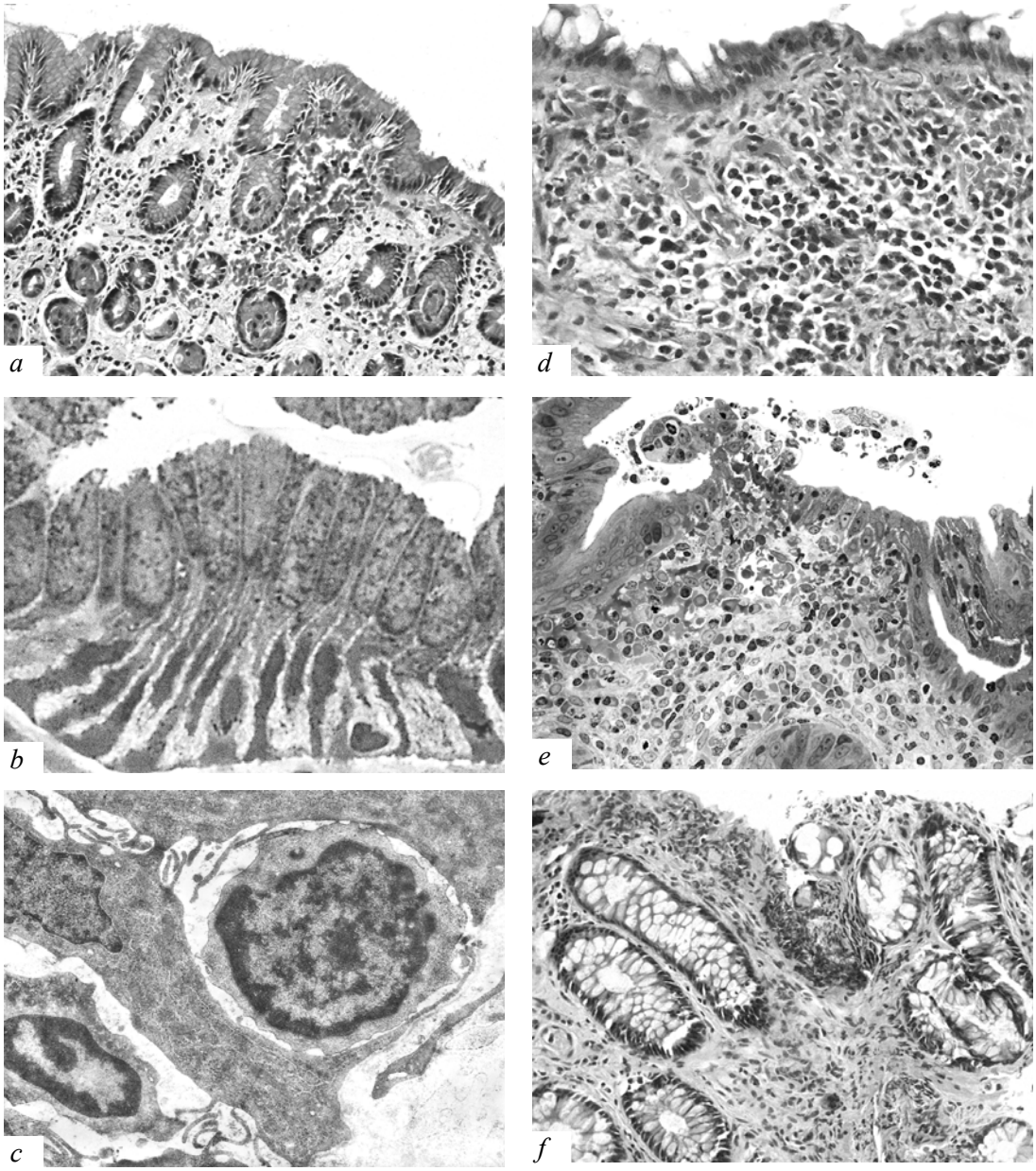

Fig. 2. Structural changes in the surface epithelium during Crohn's disease. Biopsy specimens from the mucous membrane of the gastric fundus (*a*-*e*) and sigmoid colon (*f*). Epithelium with high secretory function, atrophy of fundal glands, edema, and newly formed hemorrhages (*a*, ×250); disintegration of surface epitheliocytes (*b*, ×950); intercellular edema and interepithelial lymphocytes (*c*, ×4500); atrophy of the epithelium and pronounced cellular infiltration (*d*, ×450); leukopedesis and desquamation of the epithelium (*e*, ×650); peri-ulcer region, erosion and dense cellular infiltrates (*f*, ×250). Hematoxylin-eosin staining (*a*, *d*, *f*); semithin sections, staining with Schiff's reagent and azure II (*b*, *e*); electron-diffraction pattern (*c*).

reduction of biosynthetic and secretory functions of epitheliocytes; and changes in the cytoarchitectonics of the mucous membrane due to the excessive growth of granulomas) can serve as the criteria for barrier dysfunction of the gastrointestinal epithelium.

Complex interaction between genetic, microbiological, and ecological factors in Crohn's disease can lead to long-term activation of the immune system in the mucosa [2,4,10,12]. Changes in the type and number of colitis-inducing bacteria in the microenvironment of the intestinal lumen or prevention of the interaction of these bacteria with the immune system can form a basis for alternative therapy, which should increase the efficacy of modern medicinal methods.

REFERENCES

1. L. I. Aruin, L. L. Kapuller, and V. A. Isakov, *Morphological Diagnostics of Diseases of the Stomach and Intestine* [in Russian], Moscow (1998).

2. E. A. Belousova, *Farmateka*, No. 13, 2-10 (2004).
3. A. I. Strukov and O. Ya. Kaufman, *Granulomatous Inflammation and Granulomatous Diseases* [in Russian], Moscow (1989).
4. G. Bastida, P. Nos, M. Aguas, *et al*., *Aliment. Pharmacol. Ther*., **22**, No. 9, 775-782 (2005).
5. H. L. Cash, C. V. Whitham, C. L. Behrendt, and L. V. Hooper, *Science*, **313**, 1126-1130 (2006).
6. M. Chichlowski and L. P. Hale, *Am. J. Physiol. Gastrointest. Liver Physiol*., **295**, No. 6, G1139-G1149 (2008).
7. M. C. Collado, E. Isolauri, and S. Salminen, *FEMS Microbiol. Lett*., **285**, No. 1, 58-64 (2008).
8. S. Dahan, F. Roth-Walter, P. Arnaboldi, *et al*., *Immunol. Rev*., **215**, 243-253 (2007).
9. K. Flanagan, Z. Modrusan, J. Cornelius, *et al*., *J. Immunol*., **180**, No. 6, 3874-3881 (2008).
10. J. P. Gisbert, M. Luna, Y. Gonzalez-Lama, *et al*., *Inflamm. Bowel Dis*., **13**, No. 9, 1106-1114 (2007).
11. M. Porras, M. T. Martin, P. C. Yang, *et al*., *Ibid*., **12**, No. 9, 843-852 (2006).
12. R. Saich and R. Chapman, *World J. Gastroenterol*., **14**, No. 3, 331-337 (2008).
13. K. A. Sharkey and G. M. Mawe, *Curr. Opin. Pharmacol*., **2**, No. 6, 669-677 (2002).
14. H. Sokol, C. Lay, P. Seksik, and G. W. Tannock, *Inflamm. Bowel Dis*., **14**, No. 6, 858-867 (2008).
15. R. J. Xavier and D. K. Podolsky, *Nature*, **448**, 427-434 (2007).