Ultrastructure of Gingival Epithelium in Chronic Gingivitis

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We studied ultrastructural reorganization of the gingival mucosa in chronic gingivitis. It was found that chronic inflammation leads to significant intracellular reorganization of epitheliocytes in the basal and prickle cell layers of gingival epithelium and their pronounced structural and functional heterogeneity. The main ultrastructural alterations of epitheliocytes in the basal and prickle cell layers include pronounced vacuolization of the perinuclear zone (partial necrosis), formation of thick tonofilament bundles, focal lysis and sequestration of glycogen, and destruction and reduction of intracellular junctions in some cases accompanied by acantholytic alterations. Chronic inflammation in the gingival mucosa induced extensive remodeling of the lamina propria manifested in multiplication of the basement membrane and obturation of blood vessels with collagen fibrils.

Key Words: *chronic gingivitis; gingiva; ultrastructure; stereology*

Chronic gingivitis (CG) is the most common form of gum disease. Gingivites have various etiology and pathogenesis; local factors, oral microflora (and especially that of dental plagues), and adverse effects (*e.g.,* smoking, xenobiotics) play an important role [4,8, 10,12,14]. Microorganisms of plaques located on the tooth surface (in gingival sulcus and interdental spaces) trigger inflammatory processes in the periodontal tissues contributing to progression of pathological process and periodontal pocket formation [2,3,7].

Prediction and early diagnosis of periodontal and oral mucosa diseases can be based on clinical symptoms and information obtained by visual inspection. However, clinical manifestations of inflammation in the periodontal tissues, *e.g.* gingival tissues, do not always correlate with the severity of pathological alterations. The absence of data on the nature and expression of gingival remodeling, in turn, does not allow choosing appropriate treatment in chronic inflammatory diseases of the oral cavity.

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Development of successful therapies for the treatment of periodontal diseases requires analysis of a range of morphological changes in the gingival mucosa and detection of structural markers of alteration and regeneration.

The aim of the work was to study ultrastructural reorganization of the gum in CG and identify the main morphological markers of chronic inflammation.

MATERIALS AND METHODS

Biopsy specimens of human gum for electron microscopy were obtained in 22 patients (mean age 35.8±2.4 years) with CG according to strict medical indications under anesthesia (Sol. Septanest 1.8 ml with epinephrine 1:100,000). A fragment of gingival mucosa (~1 mm^3) was cut off from the region of the interdental papilla after tooth extraction. The specimens were fixed in 4% paraformaldehyde, post-fixed in 1% OsO_4, and then after dehydration embedded in Epon and Araldite mixture. Ultrathin sections were obtained on LKB III and Leica ULTRACUT EM UC7 ultratomes (Leica) and contrasted with uranyl acetate and lead citrate. Ultrathin sections were examined under a JEM1400 electron microscope (Jeol) at accelerating

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voltage of 80 kV. Images were obtained with digital Veleta camera and iTEM software (Olympus).

Ultrastructural stereological analysis of epitheliocytes was performed using iTEM software at a final magnification of 20,000. Volume densities of mitochondria, granular endoplasmic reticulum, tonofilaments, glycogen granules, and cytoplasm were evaluated.

Statistical treatment of the results was performed using Student's *t* test. Differences were considered significant if the achieved significance level did not exceed the critical significance level ($p<0.05$).

RESULTS

In patients with CG, significant inflammation of the gum characterized by severe erythema, swelling, and bleeding was seen. In the examined gingival specimens, catarrhal sclerosing type of chronic inflammation predominated, other types (sclerosing and ulcer-

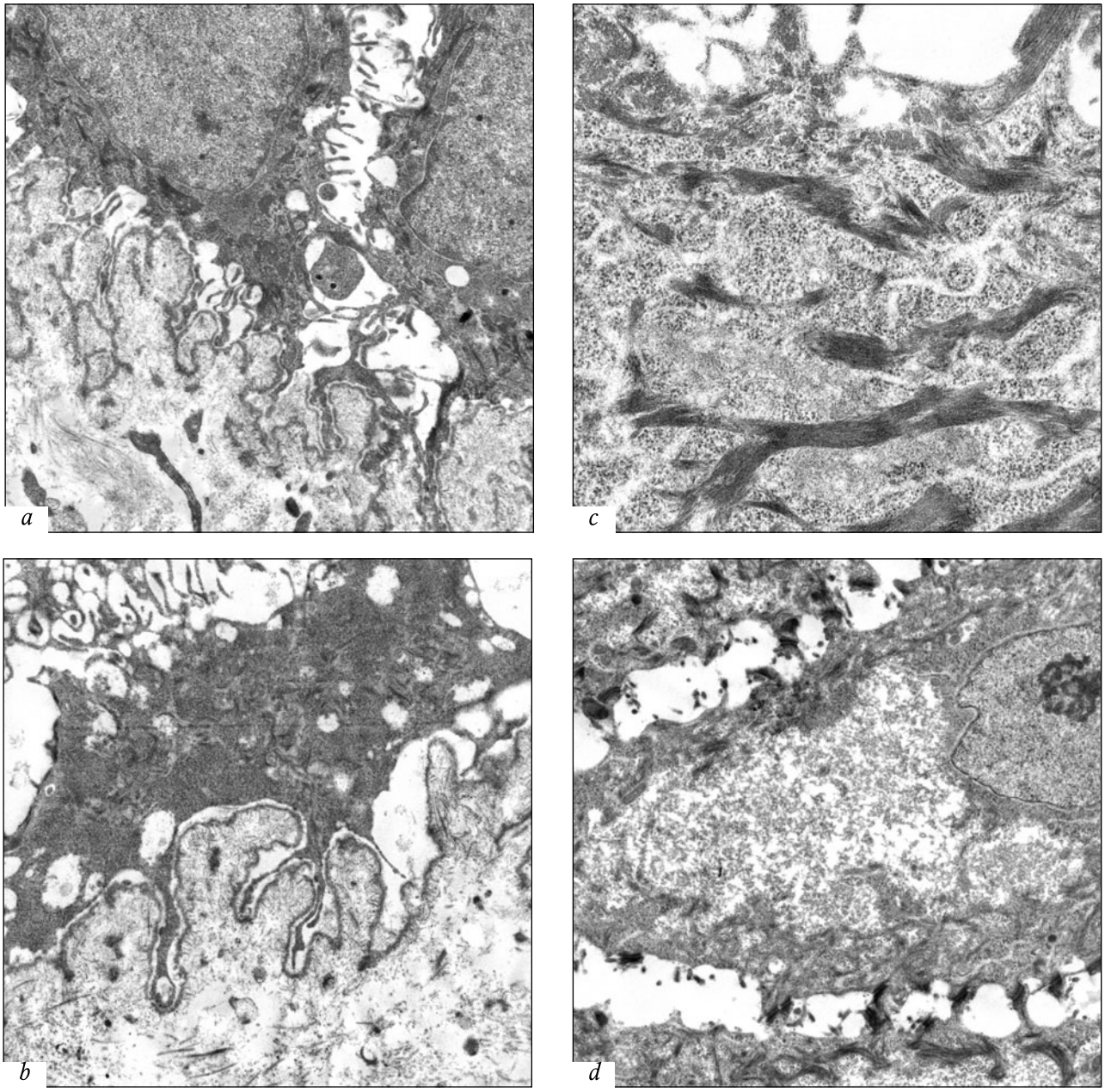

Fig. 1. Ultrastructural changes in the basal and prickle gingival epitheliocytes in CG. *a*) considerable expansion of intercellular spaces between basal epitheliocytes (×8000); *b*) basal epitheliocytes detach from the basement membrane; the remaining "pockets" are filled with fibers and ground substance of connective tissue (×15,000); *c*) massive deposition of glycogen granules and bundles of thickened tonofilaments in prickle epitheliocyte (×25,000); *d*) lysis of glycogen in the perinuclear area of prickle epitheliocyte, expansion of intercellular spaces (×8000).

ative necrotizing) were less common. Catarrhal sclerosing CG was characterized by pronounced structural and functional heterogeneity of epitheliocytes in the basal and prickle layers. Basal epitheliocytes were presented mainly by cells with moderately electron-dense cytoplasm containing numerous small mitochondria, single lamellae of the rough endoplasmic reticulum, and swolling (short) tonofilament bundles (Fig. 1, *a*). At the same time, cells with electron-dense cytoplasm, in some cases vacuolated, with poorly distinguishable ultrastructures were seen in the basal layer (Fig. 1, *b*). These cell forms separating from the basement membrane and losing contact with neighboring cells can be regarded as necrobiotic (apoptotic) cells.

Intracellular reorganization of epitheliocytes in the basal and prickle cell layers was stereotypical and included considerable reduction of desmosomes, thickening of tonofilament bundles, large glycogen deposits (Fig. 1, *c*). Glycogen granules occupied a large part of the cytoplasm of prickle epitheliocytes (volume

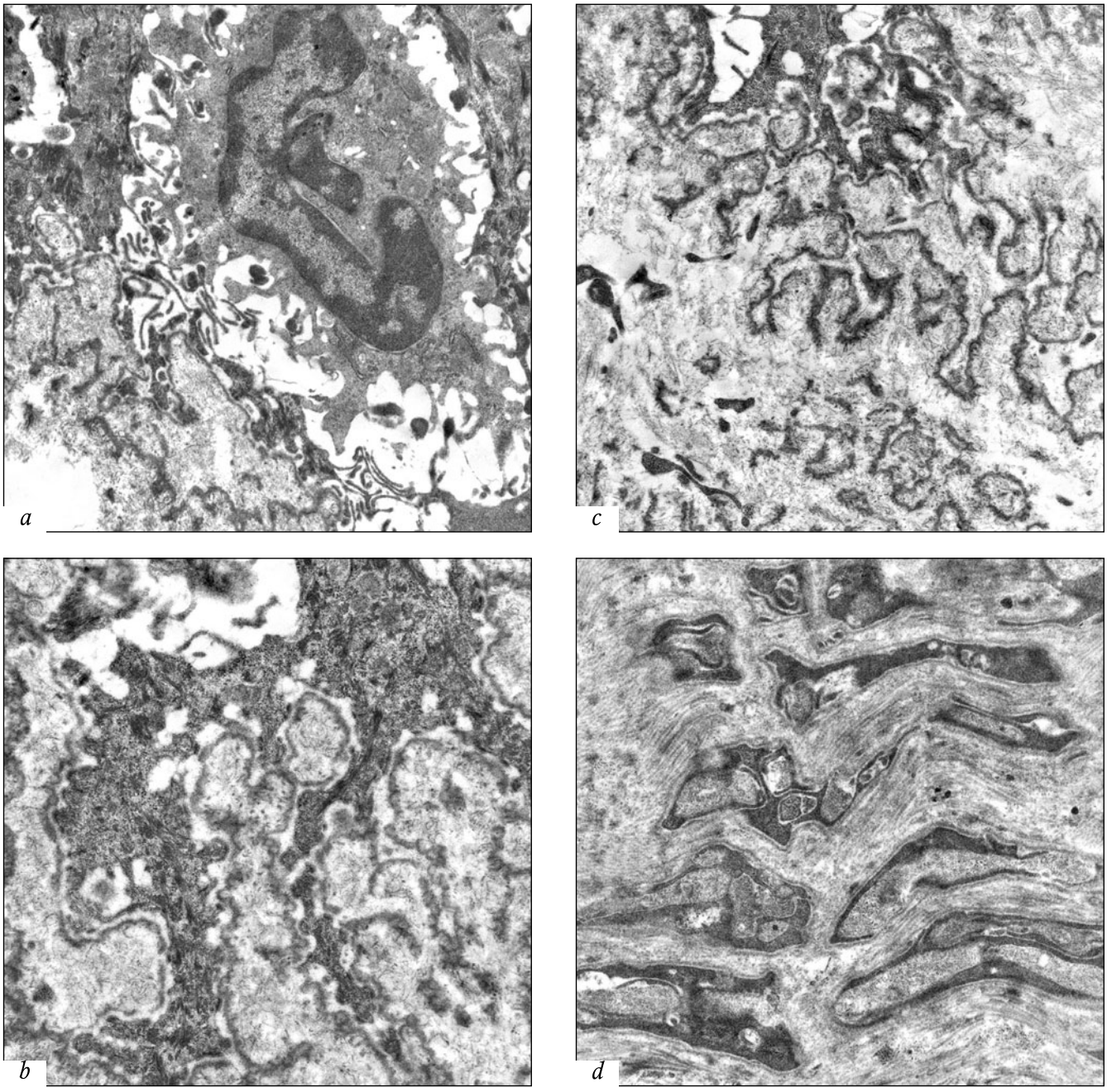

Fig. 2. Ultrastructural changes in the lamina propria of the gingival mucosa in CG. *a*) neutrophil migrates into basal layer of gingival epithelium (×8000); *b*) "multiplication" of the basement membrane (×15,000); *c*) multiplied basement membrane is immersed into the lamina propria (×10,000); *d*) capillaries of papillary layer of the lamina propria are obturated with collagen fibrils (×10,000).

density 32.2±3.4%) and were diffusely spread; small foci of sequestration and lysis more pronounced in the perinuclear area were observed (Fig. 1, *d*), which led to vacuole-like "devastations" of the cytoplasm.

In the cytoplasm of prickle epitheliocytes, moderately dilated cisterns of the Golgi apparatus, single mitochondria (volume density 1.4±0.4%), and extended lamellae of the rough endoplasmic reticulum (volume density 5.00±1.04%) were also observed. Tonofilaments formed thick bundles, which were located mainly under the plasmalemma and penetrated into the cytoplasmic outgrowths.

Intercellular spaces in the basal and prickle cell layers were unequally dilated, the number of cell-cell contacts (bridges) decreased (Fig. 1, *d*). Reduced number of cell-cell contacts and pronounced intercellular edema contributed to dissociation of the basal and prickle cell layers; in some cases acantholysis was observed. Some epitheliocytes formed atypical elongated outgrowths as compensatory response. Accumulations of floccular substance as well as lymphocytes and neutrophils were seen in the intercellular space. floccular masses, myelin-like structures, and erythrocytes were frequently observed between cells of the prickle layer. Among cells migrating into the basal and prickle layers, neutrophils and macrophages were the most frequent cell type (Fig. 2, *a*).

Ultrastructural changes in epitheliocytes of the granular layer were similar to those in the prickle layer. Epitheliocytes with electron-dense cytoplasm, reduced keratohyalin granules and intercellular contacts. Perinuclear vacuolization of the cytoplasm was less pronounced than in epitheliocytes of the prickle layer. However, increased autophagy response was recorded as formation of small residual cells. In horny layer, epitheliocytes with moderately electron-dense cytoplasm, pronounced lytic ultrastructural changes, and residual keratohyalin granules and cells with electron-dense (condensed) poorly structured cytoplasm were seen. In this layer, the loss of intercellular contacts needed for cell shedding was noted.

Ultrastructural reorganization of the basal layer of the epithelium and subepithelial zone of the gingival lamina propria in CG was characterized by large number of branched basal outgrowths formed by basal epitheliocytes and embedded in the mucosa lamina propria, and peculiar "multiplication" of the basement membrane (Fig. 2, *b*). "Devastated" invaginations of the basement membrane not in contact with the basal epitheliocyte membrane in the subepithelial zone of lamina propria (Fig. 2, *c*) reflect the process of elimination of basal cells caused by plastic process insuficiency, necrotic or apoptotic death, cell exfoliation/elimination (Fig. 1, *b*), and filling of the remaining "pockets" with fibers and ground substance of connective tissue. These pronounced alterations of the connective tissue framework of gingival epithelium can be regarded as a morphologic marker of CG.

Describing the ultrastructure of lamina propria of gingival mucosa, we should note the presence of a large number of plasma cells, lymphocytes and reactive fibroblasts (myofibroblasts), diffuse fibrosis in the papillary layer. In some cases, collagen fibers were collected in long massive bundles that formed layers oriented at an angle to each other. Lamina propria fibrosis was so pronounced that in some cases we observed obturation of the lumen of blood vessels and capillaries with bundles of collagen fibers and the ground substance (obliteration of capillaries; Fig. 2, *d*).

Thus, ultrastructural study of gingival biopsy specimens in CG showed that epitheliocytes of the prickle and basal layers undergo most pronounced changes. In these layers, we often observed considerable intercellular edema (spongiosis) accompanied by expansion of intercellular spaces, loss of intercellular bridges with formation of vesicles and gaps between the cells (acantholysis), which can be regarded here as a manifestation of dyskeratosis, that is, impaired synthesis of keratohyalin and desmosome proteins. These structural changes of oral mucosa described in various pathologies and after using different filling materials [7,9] can be due to leakage of the transudate from the papillary layer into the basal and prickle cell layers of the epithelium.

In prickle cell and basal layers of gingival epithelium of practically all patients, epitheliocytes were structurally and functionally heterogeneous: larger polygonal cells with clear cytoplasm ("light" cells) and small dark elongated or polygonal cells ("dark" cells). Structural and functional heterogeneity of gingival epitheliocytes, detected, in particular, in smokers, is an intrinsic manifestation of chronic inflammatory response [11]. An important characteristic of CG is remodeling of the lamina propria of the gingival mucosa manifested in "multiplication" of the basement membrane, diffuse fibrosis, and obstruction of capillaries. Alteration of blood vessels, in turn, stimulates further progression of gingivitis and periodontitis with perivascular hyaline deposition [13].

Pronounced lymphoplasmacytic infiltration of the lamina propria of the gingival mucosa is another important morphofunctional characteristic of the chronic periodontal diseases of various etiologies [1, 5,6]. Lymphoplasmacytic infiltration is often accompanied by lymphangiogenesis, which enhances the remodeling and fibrosis of the lamina propria. Some authors believe that lymphocytes, but not neutrophils and macrophages among inflammatory cells play a key role in the destruction of periodontal tissue [1]. Characteristics of local immunity in the oral cavity

CG include high concentrations of IL-2, IL-6. and TNF-α (proinflammatory cytokines) in the oral fluid and significantly reduced levels of IL-4 and IFN-γ (anti-inflammatory cytokines) [11]. This cytokine imbalance reflects persistence of antigens of bacterial or other nature in the gingival mucosa accompanied by the development of destructive and subcompensatory responses.

REFERENCES

1. V. L. Bykov, *Arkh. Patol*., No. 2, 51-55 (2005).
2. A. S. Grigoryan and A. I. Grudyanov, *Stomatologiya*, No. 1, 5-8 (2001).
3. A. S. Grigoryan and O. A. Frolova, *Stomatologiya*, No. 3, 11-17 (2006).
4. Z. V. Yesayan, *Stomatologiya*, No. 1, 58-64 (2005).
5. S. A. Zufarov, *Osnovnye Stomatologicheskie Zabolevaniya*, No. 5, 45-49 (2003).
6. I. V. Mayborodin, A. B. Voytovich, E. V. Kozlova, *et al*., *Morfologiya*, **135**, No. 2, 43-47 (2009).
7. L. M. Mikhaleva, T. G. Barkhina, and V. D. Shapovalov, *et al*., *Arkh. Anat*., No. 6, 15-20 (2001).
8. G. I. Oskolsky, L. M. Nepomnyashchikh, A. V. Jurkevich, *et al*., *Dalnevost. Med. Zh.,* No. 3, 130-133 (2011).
9. V. A. Shakhlamov, T. G. Solnyshkova, and N. V. Artamonova, *Byull. Eksp. Biol. Med.*, **141**, No. 1, 95-98 (2006).
10. V. A. Shkurupiy, K. O. Samoylov, and G. N. Vereshchagina, *Chronic Catarrhal Gingivitis, Hypertension, Connective Tissue Dysplasia (Pathomorphology and Treatment)* [in Russian], Novosibirsk (2001).
11. A. V. Shcheglov, G. I. Oskolsky, E. L. Lushnikova, and L. M. Nepomnyashchikh*, Byull. Eksp. Biol. Med.,* **142**, No. 11, 576-580 (2006).
12. D. N. Tatakis and L. Trombelli, *J. Clin. Periodontol*., **31**, No. 4, 229-238 (2004).
13. H. Zoellner, C. C. Chapple, and N. Hunter, *Microsc. Res. Tech*., **56**, No. 1, 15-31 (2002).
14. T. E. Van Dyke and D. Sheilesh, *J. Int. Acad. Periodontol*., **7**, No. 1, 3-7 (2005).