Morphological and Immunological Analysis of the Oral Mucosa in Tobacco Smoking and Odontopreparation

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Tobacco smoking causes pronounced structural reorganization of the gingival mucosa and its atrophic changes. Odontopreparation stimulates destructive processes in the gingival mucosa promoting activation of local immunity (stimulation of immunoglobulin synthesis). Significant differences in the local humoral immunity (IgG dysimmunoglobulinemia) and cytokine spectrum in nonsmoking and tobacco-smoking patients were detected. Subcompensated level of immune resistance in tobacco smokers prompts referring them to a group at a high risk of chronic pathological processes of the oral cavity.

Key Words: *oral mucosa; chronic inflammation; odontopreparation; tobacco smoking; local immunity*

The prevalence of periodontal diseases reaches 98% [5,11], which explains the increased volume of dental care rendered to the population, including orthodontic care. The inevitable odontopreparation (OP) is fraught with development of inflammatory processes in the oral cavity [1], their intensity depending on many exo- and endoecological factors (tobacco smoking, nutrition, microflora, *etc.*), age, and adequacy of local and total systems immune reactions [3,4,10,15].

The role of local immunity, in addition to the production of immunoglobulins aimed at elimination of foreign antigens, consists in regulation of tissue homeostasis of the oral mucosa at the expense of production of a wide spectrum of cytokines by immunocompetent cells [10]. Some cytokines, *e.g.* IL-1, are regarded as pathogenetic markers of severe forms of chronic periodontitis [13, 14]. On the other hand, prediction of the course of the pathological process in the oral cavity and evaluation of the treatment efficiency should be based on dynamic evaluation of the levels of many cytokines (and their ratios) and analysis of correlations between these changes and the course of destructive and regenerative processes. Analysis of the effects of the most prevalent untoward exogenous factors, for example, tobacco smoking, on the structural and functional reorganization of the oral mucosa also provides important data.

We carried out a morphological analysis of gingival biopsy specimens and studied local oral immunity values during tooth preparation in tobacco smoking and nonsmoking patients.

MATERIALS AND METHODS

Clinical morphological analysis of the oral mucosa was carried out in men in need of orthodontic treatment aged 25-56 years (mean age 36.5±1.1 years) directly before orthodontic treatment and 14-16 days after OP. The patients were divided into 3

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groups: 1) common group (n=20) including tobacco smoking and nonsmoking patients; 2) nonsmokers (n=12); and 3) tobacco smokers (n=8). Odontopreparation of vital teeth (premolars and molars with permanent position in the dentition) was carried out by the common method.

Fragments of the gingival mucosa (2 mm^3) were collected for morphological analysis in accordance with medical indications under conduction anesthesia. Gingival biopsy specimens were fixed in 10% neutral formalin and the tissue was treated by standard methods before embedding the specimens into paraffin. Paraffin sections were stained with hematoxylin and eosin and by the Romanowsky—Giemsa method. Tissue specimens for electron microscopy were postfixed (after fixation in 4% paraformaldehyde) in 1% OsO_4 and after standard treatment were embedded in epon-araldite mixture. Semithin (1 μ) sections were stained with Azur II. Ultrathin sections were contrasted with ethanol solution of uranyl acetate and lead citrate and examined under a JEM 1010 electron microscope.

Oral fluid for immunological analysis was collected in the morning after overnight fasting by the method recommended by the International Association of Dentists [2]. The concentrations of secretory immunoglobulins (sIgA, IgG_1, IgG_2, IgG_3, and IgG_4), IL-2, IL-4, IL-6, TNF-α, and IFN-γ in the oral fluid were measured by enzyme-linked immunosorbent assay using Polignost test systems.

Statistical analysis was carried out using standard methods of variant statistics. The statistical hypothesis of equality of the group means was verified by Student's t test.

RESULTS

The structure of changes detected in the buccal and gingival mucosa of tobacco smoking and nonsmoking patients indicates the negative effect of tobacco smoking on the health status. Oral hygiene indexes were significantly higher in smoking patients in comparison with non-smokers: the papillary/marginal/alveolar (PMA) index was 3.5 times higher, simplified oral hygiene index (OHI-S) 4.8 times higher. Slight gingivitis was detected in 50% smokers, medium in 12.5%, and severe in 37.5% (with PMA index no higher than 70%). In the nonsmoker group mild gingivitis was detected in 83.3% and medium in 16.7%. The PMA index increased after OP more abruptly in nonsmoking patients (3-fold) than in tobacco smokers (1.7 times).

Catarrhal changes in the oral mucosa and symptoms of atrophic gingivitis were more often observed in smokers. Analysis of gingival biopsy specimens from patients with catarrhal gingivitis showed acanthosis and papillomatous growth of the epithelium in the majority of cases (Fig. 1, *a*), the most prevalent common pathological reactions of multilamellar epithelium to untoward chronic exposure [8,9]. The main morphological sign was structural and functional heterogeneity of the basal and pricky layer epitheliocytes, which manifested by different tinctorial characteristics of cells. A significant part of epitheliocytes in the pricky and granular layers were characterized by degenerative changes and necrobiosis. The horny layer was retained in the majority of cases and had several rows of hornified cells. Its density was different at different sites; dyskeratosis was seen in some cases. Atrophic gingivitis was characterized by focal degeneration of the epithelial layer (Fig. 1, *b*) with retained papillomatous growth, in some cases significant.

Significant dilatation of extracellular spaces in the basal and pricky layers was one of the manifest and highly incident morphofunctional changes in the epithelial layer in patients with chronic inflammatory diseases of the oral cavity in need of orthodontic treatment (Fig. 1, *c*, *d*). These changes were most pronounced after OP. These structural transformations of the oral mucosa were described for different pathological processes and for different filling materials [7,12] and can result from penetration of transudation from the papillary layer into the epidermal basal and pricky layers. An appreciable dilatation of the extracellular spaces in the epidermal layer can be also due to suppression of regeneratory plastic processes in epitheliocytes, impaired biosynthesis of many important structural and functional proteins, for example, desmosome components. Electron microscopy of pricky layer epitheliocytes showed the appearance of vacuole-like dilatations in the perinuclear area, decreased number of keratohyalin granules and dense cell-cell contacts (Fig. 1, *d*). These ultrastructural changes were most pronounced in heavy smokers. Dilated extracellular spaces contained as a rule floccular substance and membranous structures; diapedesis of lymphocytes and macrophagal cells into the gingival mucosal epithelium was noted (Fig. 1, *c*).

Edema of different severity in the papillary layer of the lamina propria was observed before OP; interepithelial papillae were of different height. Perivascular infiltration was mononuclear in the majority of cases. Papillary layer edema in the gingival mucosa augmented after OP, inflammatory cell infiltration progressed and disseminated.

Morphological changes after OP were paralleled by changes in the local oral immunity parameters. The concentrations of sIgA in the oral fluid varied

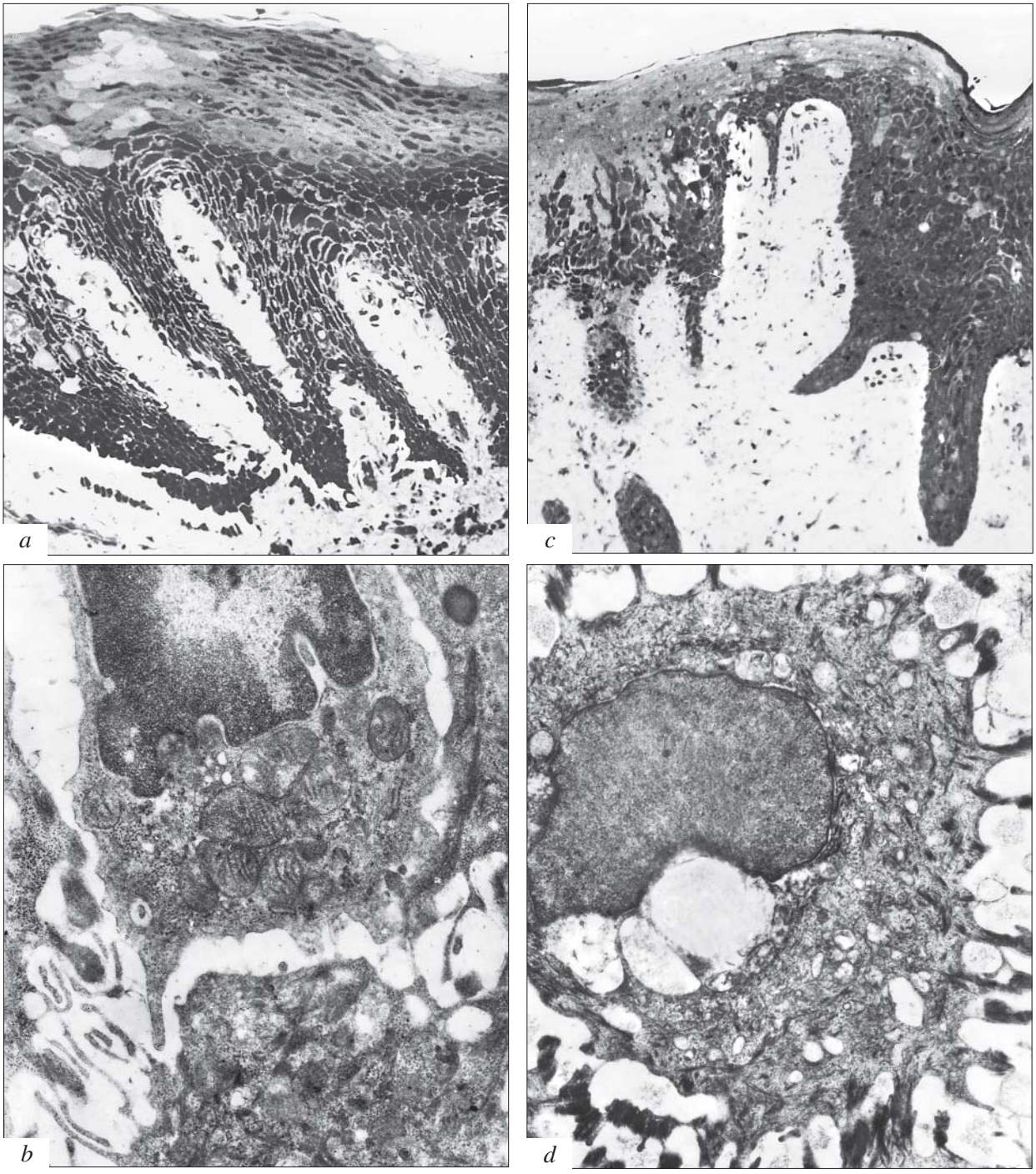

Fig. 1. Morphological changes in the gingival mucosa in chronic inflammation. *a*) acanthosis, degenerative changes in epitheliocytes and papillomatous growth; *b*) focal degeneration of epithelium; *c*) diapedesis of mononuclears into epidermal basal layer, pronounced dilatation of extracellular spaces, ×15,000; *d*) vacuole-like dilatation near the nucleus and decreased number of keratohyalin granules in a pricky layer epitheliocyte, ×6000. *a*, *b*: semithin sections. Azur II staining, ×200; *c*, *d*: electronograms.

from 40.72 to 58.60 μg/ml before OP in the common group and virtually did not change after OP (Table 1). In smokers, sIgA content in the oral fluid was reduced before and after OP.

The ratio of sIgA concentrations in the serum and oral fluid reduced in tobacco smokers (Table 1), indicating exhaustion of the adaptation reserves and stress of defense factors of local oral immunity.

The ratio of IgG subclasses in the oral fluid (by their concentrations) was as follows: $G_1>G_2>G_4>G_3$ in all groups, differed from that in the serum, and did not change after OP. The concentrations of

TABLE 1. Time Course of the Oral Fluid Humoral Immunity Parameters in Tobacco Smoking and Nonsmoking Patients under the Effect of OP (*M*±*m*)

Parameter	Before OP			After OP		
	common group	nonsmokers	smokers	common group	nonsmokers	smokers
sIgA, μg/ml	50.63±1.00	53.08±0.86	46.90±1.30^{+}	48.76±1.10	52.70±0.70	47.10±1.30^{+}
Serum sIgA/ oral sIgA	0.26±0.03	0.36±0.03	0.09±0.01^{+}	0.21±0.03	0.31±0.02	0.04±0.01^{*+}
IgG_1, mg/ml	1.05±0.03	1.10±0.04	0.97±0.01^{+}	1.14±0.03*	1.30±0.05*	0.93±0.02^{+}
IgG_2, mg/ml	0.84±0.06	0.89±0.03	0.77±0.02^{+}	0.88±0.05	0.95±0.02	0.78±0.02^{+}
IgG_3, mg/ml	0.19±0.01	0.17±0.02	0.21±0.01	0.19±0.01	0.18±0.02	0.21±0.01
IgG_4, mg/ml	0.26±0.05	0.21±0.03	0.32±0.01^{+}	0.27±0.06	0.19±0.03	0.39±0.02^{*+}
IL-2, pg/ml	544.59±63.85	334.71±28.78	859.40±46.00^{+}	316.04±71.33*	76.21±12.70*	687.80±57.70^{*+}
IL-4, pg/ml	20.57±6.27	16.29±1.39	27.00±1.80^{+}	65.67±9.90*	97.39±6.40*	18.10±5.19^{*+}
IL-6, pg/ml	68.96±4.30	60.32±4.73	81.93±5.73^{+}	66.62±3.22	63.9±3.2	70.56±6.40
TNF-α, pg/ml	394.26±30.07	391.30±47.30	398.63±29.32	664.34±41.12*	729.50±59.11	566.62±31.10^{*+}
IFN-γ, pg/ml	5.67±0.70	5.14±1.03	6.48±0.81	5.87±0.63	5.09±0.80	7.04±0.80

Note. $^{*}p<0.05$ compared to the corresponding parameter before treatment; $^{+}p<0.05$ compared to nonsmokers.

IgG_1, IgG_2, and IgG_4 in the oral fluid of tobacco smoking patients was significantly lower than in nonsmokers (Table 1). Repeated analysis showed that these differences persisted and progressed.

A specific feature of local immunity formation in the oral cavity (in contrast to systemic immunity) were high concentrations of IL-2, IL-6, TNF-α and significantly reduced concentrations of IL-4 and IFN-γ in the oral fluid. Comparison of IL-2, IL-4, and IL-6 concentrations in the oral fluid of tobacco smokers and nonsmokers before therapy showed lower levels in nonsmokers (Table 1). The concentrations of TNF-α and IFN-γ in the oral fluid of patients of all the studied groups were virtually the same.

Significant differences in the cytokine status of local immunity were retained in tobacco smoking and nonsmoking patients after OP. A decrease in IL-2 level in the oral fluid was characteristic of both groups, but its concentration remained higher in the group of tobacco smoking men (9-fold *vs.* nonsmokers; Table 1). The dynamics of concentrations of antagonistic cytokines (IL-4 and IL-6) was different in nonsmoking and tobacco smoking patients. The concentration of IL-6 in the oral fluid did not change much in nonsmoking patients, while the concentration of IL-4 increased almost 6-fold ($p<0.05$). In tobacco smokers, the concentrations of both cytokines decreased (Table 1). After OP the concentration of TNF-α increased 1.9 times in nonsmokers and 1.4 times in tobacco smokers.

The IL-2/IL-4 imbalance can lead to disturbances in intraimmune relations and development of immunopathological processes [6], as one of the main biological effects of IL-4 is activation of proliferation and differentiation of immunocompetent cells and regulation of biological effects of IL-2.

Hence, remodeling of gingival mucosa in patients in need of orthodontic treatment is determined by the severity and duration of inflammatory process, unfavorable effects of tobacco smoking products on cell populations, and destructive effect of the tooth preparation process. Chronic tobacco smoking causes the development of regeneratory plastic insufficiency of epidermocytes and atrophic changes in the gingival mucosal epithelial plast. This remodeling of the mucosa is concomitant with developing IgG dysimmunoglobulinemia, redistribution of antibodies produced in the body, and activation of proinflammatory cytokine biosynthesis in the oral cavity. In nonsmoking patients OP leads to more pronounced activation of local immunity, stimulates the production of antiinflammatory cytokines, which can be regarded as a compensatory reaction to increased antigenic challenge. In tobacco smokers OP results in more pronounced dysimmunoglobulinemia and redistribution of sIgA. Deficiency of antiinflammatory components (IL-4) can be regarded as a subcompensated level of immunological resistance, which prompts referring tobacco smokers to a group at a high risk of immunopathological conditions.

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