

ATYPICAL MICROORGANISMS' ROLE IN THE PROCESSES OF MORPHOLOGICAL AND IMMUNOLOGICAL SHIFTS DURING PERSISTENT FOCAL TONSILS INFECTIONS

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Abstract

The chronic tonsillitis as a frequent expression of the mentioned pathology is considered to be the most common one for the adult cohorts of population. Anyway, an increased attention to this problem is connected not only with its extremely high coverage, but also with fact that chronic tonsillitis is both an inflammatory disease of the pharynx mucosa associated lymphoid structures and a serious microbial-allergic attack onto the whole organism, resulting in the organism's innate resistance specific and non-specific factors inhibition and also in some severe complications, which are potent to bring to disability and even to death of the patients (rheumatic and renal diseases, paratonsillar and retropharyngeal abscesses, tonsillogenic sepsis).

Multiple studies of persistent pharyngeal infection show that the mixed flora are produced from the lacunes and tonsils' surface, wherein together with the so called typical microflora are observed atypical microorganisms, such as Chlamydia, Mycoplasma and E. coli. However, their role in the mechanism of formation and course of the regional infection process till has not been fully studied so far. Urgency of the problem is obviously increasing with respect of the fact that Chlamydia, Mycoplasma and E. coli persistence in non-typical eco-niches of an organism may be predetermined by processes of conditionally-pathogenic microorganisms' bacterial translocation from the evolutionary fixed eco-niches.

The goal of the study was to reveal the Mycoplasma hominis and E. coli role in the processes of regional immunopathogenic disorders formation in chronic tonsillitis. Based upon obtained data, we can conclude that the relatively prolonged persistence of "atypical" microorganisms (in our case these are M. hominis and E. coli) in the tonsils tissues could be accompanied with tissue alteration changes of the lymphoid apparatus, whose mechanism is probably connected with direct cytotoxic effect on the target-cells of membrane fractions of E. coli (LPS) and M. hominis.

KEYWORDS: chronic tonsillitis, palatine tonsils, atypical microorganisms, immunomorphological shifts

INTRODUCTION

In the recent decades a huge number of investigations have been dedicated to the chronic focal infections' different expressions, amongst which tonsil pathology is outstanding [Bikova VP, 2004, 2006; Bredun A, 2007].

Chronic tonsillitis as a frequent expression of this pathology is considered to be the most common one for the adult cohorts of population [Barlett J, 2001; Palchun V et al., 2006]. Anyway, an increased atten-

tion to this problem is connected not only with its extremely high prevalence, but also with the fact that chronic tonsillitis is both an inflammatory disease of lymphoid structures associated with pharynx mucosa, and a serious microbial-allergic attack on the entire organism, resulting in inhibition of specific and non-specific factors of an organism's innate resistance, and also in some severe complications, potentially leading to disability and even death of patients (rheumatic and renal diseases, paratonsillar and retropharyngeal abscesses, tonsilogenic sepsis) [Palchun V et al., 2001, alchun V, 2006].

After an infection agent invades an organism, the humoral and cellular links of defense are activated to destroy the pathogen in normal anti-inflammatory

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immunity. But why sometimes an organism is unable to eliminate it? What is the reason for the immune process misbalance, manifested in immune response attenuation, decreased formation of antibody-producing cells, and development of autoimmunization, leading to chronization of the process? Answers to these very serious issues are beyond the scope of otolaryngology.

Multiple studies of persistent pharyngeal infection show that the mixed flora are produced from the lacunes and tonsils' surface, wherein together with the so called typical microflora are observed atypical microorganisms, such as *Chlamydia*, *Mycoplasma* and *E. coli* [Huminer D et al., 1994; Lindroos R, 2000; Kovaleva LM, 2001; Normann E et al., 2001; Nelson C, 2002; Polyakova T, Polyakova, EP, 2004; Loganathan A et al., 2006; Samsigina G, 2006; Bredun A, 2007; Kapustina T et al., 2007, 2008; Kryukov A et al., 2008]. However, their role in the mechanism of formation and course of the regional infection process has not been fully studied so far.

Urgency of the problem is obviously increasing with respect of the fact that *Chlamydia*, *Mycoplasma* and *E.Coli* persistence in the non-typical eco-niches of an organism may be predetermined by processes of the conditionally-pathogenic microorganisms' bacterial translocation from the evolutionary fixed eco-niches, first of all, from the distal parts of the gastro-intestinal tract. The issue of microbial translocation proliferation processes regarding *Mycoplasma*, which are commensals of the mammal being urogenital tract, including man's, is open-ended up to date.

Nevertheless, it was ascertained that the host-bacterium interaction at the primary stage of regional infectious process starts with the isthmus mucosa epithelium surface [Principi N, Esposito S, 2002] including the innate and adaptive immunity reactions [Bernstein J et al., 1999; Palchun VT et al., 2007], in the literature accessible to us, there are just single facts of intercellular and microbial-cellular interactions occurring on the surface, in the epithelium, in the crypts and the tonsils' lymphoid tissue of the patients suffering from chronic tonsillitis. That is why provision of investigations directed at revealing of the atypical microorganisms' role in the chronic tonsillitis pathogenesis is rather expedient.

The goal of the study was to reveal the *Mycoplasma hominis* and *E. coli* role in the processes of regional immunopathogenic disorders formation in

chronic tonsillitis.

MATERIALS AND METHODS

The surgical material from ENT department of the Yerevan Arabkir Public Children Clinic was used in this study. The object of study were tonsils of 103 patients (55 females and 48 males), who underwent the tonsillectomy after ineffective conservative treatment. Chronic tonsillitis was diagnosed based on the classification of I.B. Soldatov (1997), according to which all the patients were divided into 2 groups. The first group comprised 58 patients with decompensated chronic tonsillitis with 2-3 angina relapses per year. The second group included 45 patients having associated damage of internal organs and systems with 4 angina relapses per year. Diagnosis of the associated diseases of internal organs and systems was determined by a therapist.

It should be particularly noted, that while the patients were being divided into groups, not a number of relapses, but exactly morphological shifts in the tonsils' lymphoid tissue was taken into account in chronic tonsillitis. The age content as per study groups is brought in the Table 1.

The first group was composed of the patients suffering from chronic tonsillitis and exhibiting hyperplastic processes in the tonsils' lymphoid tissue, as revealed by a morphological examination of the biopsy material. The second group was composed of the patients with dominant hypoplastic processes in morphological analysis of the tonsils' lymphoid tissue on the background of fibroplastic processes activation. The control group was composed of 20 practically healthy people of 20-50 years of age, who died from trauma, whose medical history indicated no tonsils-related complications. The tonsils biopsy samples were divided in

TABLE 1.
Distribution of the patients suffering from chronic tonsillitis depending on age and relapse frequency

Age group (years)	Decompensated chronic tonsillitis with angina relapses (per year)	
	1-2	4 and more
3-8	23	12
9-14	18	11
15-35	17	22
Total	58	45

two parts for morphological examination. The first part was used to prepare paraffin sections, stained with hematoxylin-eosine, using Van-Guison method and with toluidine blue. The other half of the biopsy and autopsy material was used to prepare cryostat sections for immunomorphological analysis for determining populations of B-lymphocytes, CD₄, CD₈, endotoxin (lipopolysaccharides) of *E. coli* and *M. hominis*.

To detect the *E. coli* LPS, the "O"-escherichious rabbit serum – the product of bacterial preparations company of the I.I. Mechnikov Central Research Institute of Vaccines and Sera – were used. To detect *M. hominis* antigens, indirect reaction of immunofluorescence together with Mycohomofluoscreen diagnosticum product of Niarmedic Company (Russia) was used. In the preparation evaluation the fluorescence color was taken into consideration. *M. hominis* should emit bright-green fluorescence. Yellow and greenish-yellow fluorescence in the preparation processing with both sera was considered an artifact. Beyond the immunomorphological method of *M. hominis* detection we also performed a tonsils tissue bacteriological analysis with the implementation of the special selective medium from for culturing *M. hominis*, using the essay-kit Mycoplasma-50 – product of Pasteur Research Institute of Epidemiology and Microbiology (St.-Petersburg, Russia). Besides the above-mentioned immunohistochemical examinations, we also performed the quantitative immunofluorescence analysis for CD₄, CD₈, the B-lymphocytes populations in tonsils of the control, as well as the first and second study groups. To this end, the tonsils tissues were subjected to homogenization for further sowing of B-lymphocytes populations from them by means of centrifuge tubes saturated with polyethylene and histopaque-1077, Accuspin Histopaque -1077 System, product of Sigma (USA). In the B-lymphocytes populations' detection the direct reaction of immunofluorescence was performed by means of the labeled FITC serum against the human IgG, production of Sigma (USA). CD₄ and CD₈ were also detected by the direct immunofluorescence using the labeled FITC monoclonal antihuman antibodies against CD₄ and CD₈, production of Sigma (USA).

While the above-mentioned immunomorphological studies were being conducted, we observed all the necessary stages of the processing of lympho-

cyclic populations, presented in methodical outlines that are designed for each reagent (antiserum).

The quantitative analysis (count of B-lymphocytic populations, CD₄ and CD₈ subpopulations) was conducted by means of histostereometric grid Kova Glasstic Slide, product of Hycor Company (UK).

All the immunomorphological studies were performed using luminescent microscope Boeco (Germany). The statistical analysis was done using Student's criteria with SPSS for Windows (version 13).

RESULTS AND DISCUSSION

As the morphological study results have showed, we detected some structural shifts that can be interpreted as lymphoid tissue hyperplasia in the tonsils' immunocompetent lymphoid tissue of the study group I patient population (Fig. 1a). These processes were manifested by follicle hypertrophy signs (up to huge forms), as well as of their reactive centers represented by the macrophages line cells, reticular cells and the blast forms of lymphocytes.

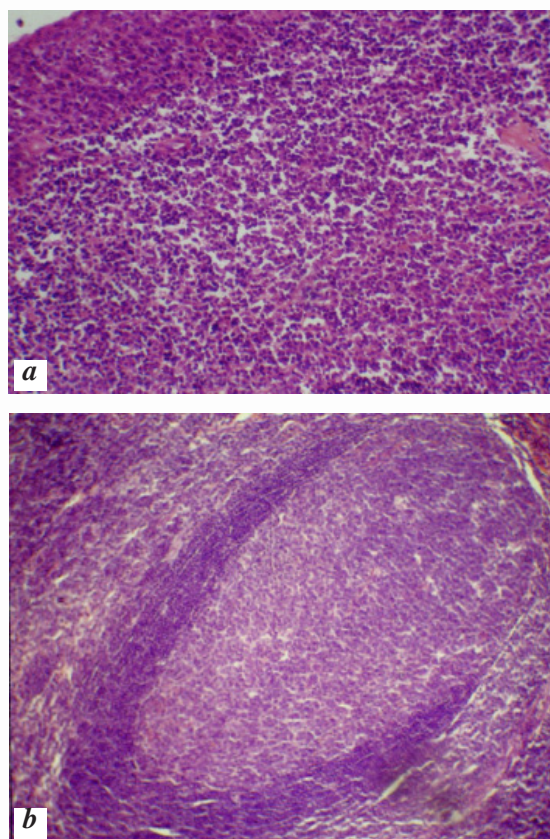


FIGURE 1. Structural shifts in the tonsils. 1st study group. Hematoxylin-eosin. Ob. 10, oc. 10.

- a) Hyperplastic processes in the subepithelial parts and the T- dependent zone
- b) A "huge" follicle with an expressed reactive center and a thickened mantle and marginal zones.

In the marginal parts, there were signs of enlargement of their borders, in the presence of thick circularly arranged strands, represented mainly by lymphocytes and lymphoblasts (Fig. 1b). There were also signs of significant activation of the lymphocytic line cells in the superficial parts of tonsils – in the crypts' subjacent parts, which was expressed by significant increase in the lymphocytes and the plasmatic cells number. Hyperplastic processes in the perifollicular zone were manifested as a hypertrophy of the cellular strands formed with the involvement of small lymphocytes and lymphoblasts.

The tonsils of the 2nd group patients, suffering from chronic tonsillitis, exhibited dominating hypoplastic activities of the lymphoid tissue (Fig. 2).

This phenomenon was manifested by decrease in the lymphoid follicles number in the mucosa *lamina propria*. The reactive centers were absent in some follicles. The mantle zone of such follicles was represented by a narrow limbus of loosely arranged lymphocytes or was even completely absent. The perifollicular zone was characterized by relatively narrowed borders and decreased content of the lymphocytic line cells. Lymphocytes in this zone were arranged loosely in the reticular stroma and were represented in the form of singular cells. The fibroplastic processes dominated everywhere and were expressed by fibrosis and sclerosis. The integrity of the tonsils' isthmus surface was impaired (Fig. 3).

In some discrete parts, the epithelium lining the free surface of tonsils was either strictly thinned-down, or fragmented due to the connective tissue fibrosing strands' spread into the impaired parts. The subepithelial parts looked significantly thickened because of the connective tissue overgrowth. The rough connective tissue strands were revealed in the mucosa profound parts – in the interfollicular lymphoid tissue. The connective tissue strands deriving from the tonsils membrane were also thickened (Fig. 4).

As the study results showed, an antigen against the LPS of *E. coli* and of *M. hominis* was detected exactly in the cases when the hypoplastic activities in tonsils were dominating (Fig. 5 a,b).

As a rule, a specific fluorescence, which was of fine-granular and/or homogenous nature and indicated the presence of LPS of *E. coli* and *M. homi-*

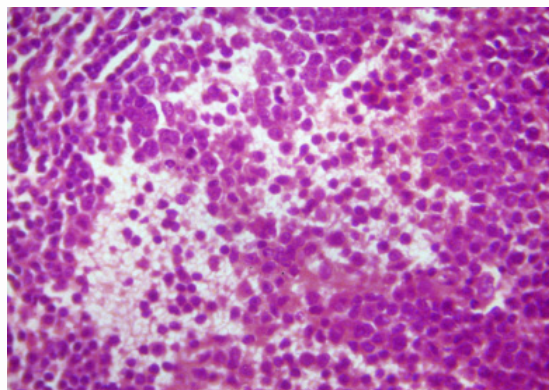


FIGURE 2. Structural changes in the tonsil lymphoid tissue. 2nd study group. Hematoxylin-eosin. Ob. 10, oc. 10. Hypoplastic processes in the reactive center of an inperforate follicle. A loose arrangement of the intact reticulocytes and macrophages on the local vacuolization of their cytoplasm, appearance of optically light regions in the presence of amorphous eosinophilic material.

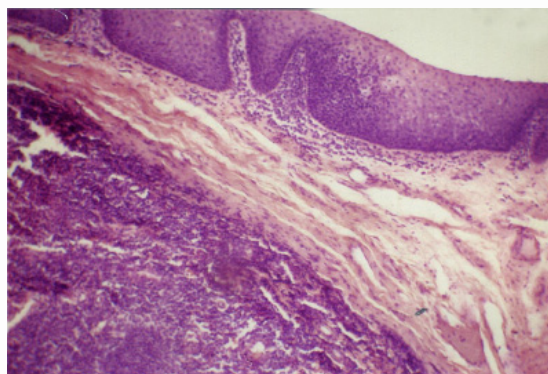


FIGURE 3. Structural changes in the tonsils. 2nd study group. Hematoxylin-eosin. Ob. 10, oc. 10. The epithelium lining the free surface of tonsils is thickened in places, but in some areas it is strictly thinned-down. In impaired parts of the epithelial tissue there are linearly arranged strands of collagen fibers.

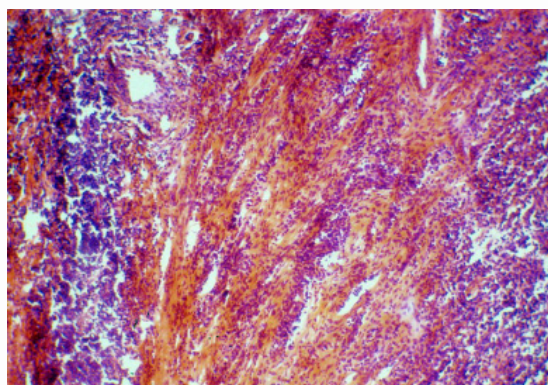


FIGURE 4. Structural changes in the tonsils lymphoid tissue. 2nd study group. Hematoxylin-eosin. Ob. 10, oc. 10. Focal fibrosis of T-dependent zone. Hypoplasia of T-dependent zone lymphoid tissue in the presence of perifocal fibrosis.

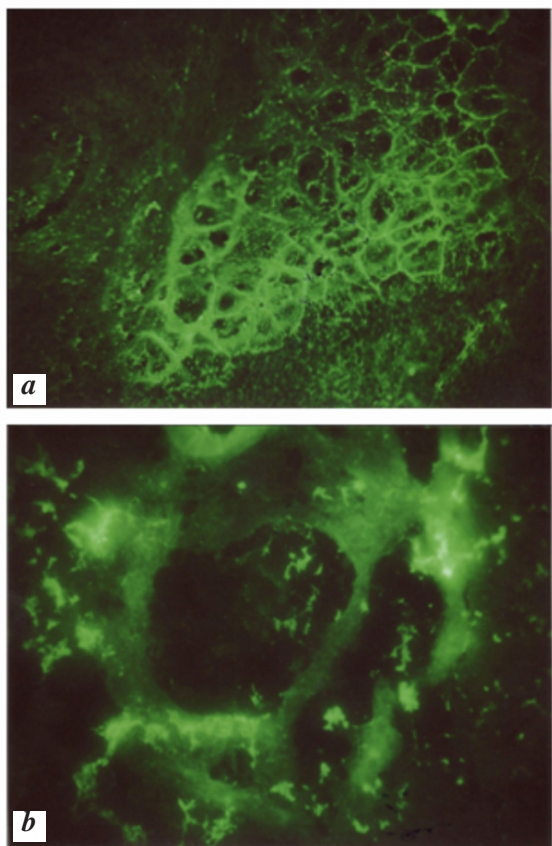


FIGURE 5. Cryostatic slide of tonsils. 2nd study group. Luminescent microscopy.

- a) Kuns direct method using labeled FITC serum against *M. hominis*, the presence of *M. hominis* antigens on the surface and in perivascular spaces of newly-formed microvessels (areas of angiogenesis).
- b) Kuns indirect method using anti-LPS rabbit serum and labeled FITC donkey serum against rabbit IgG. Specific fluorescence of *E. coli* LPS in the lumen, wall and perivascular parts of the interfollicular lymphoid tissue.

nis, was detected extracellularly both in subepithelial parts and in deep mucosa parts. A specific fluorescence indicating the presence of both antigens in tonsils area was of diffuse nature.

In the interfollicular tissue the LPS *E. coli* and *M. hominis* antigens were detected in immediate vicinity of morphologically intact lymphocytes, sometimes on their surface, in microvessels' walls, in the area of sclerotized collagen fibers. A specific fluorescence in the lymphoid follicles was more significantly detected in the periphery of nodules in the mantle zone. In bacteriological analysis employing specific selective medium for *M. hominis* culturing, *M. hominis* amounted to 10^3 CFU in tonsils of the 2nd group.

It should be noted, that the LPS *E. coli* and *M. hominis* antigens presence was detected in the ton-

sils of the 1st study group. But a specific fluorescence was purely of focal nature and characterized by predominant localization in the subendothelial parts as single extracellularly arranged fluorescing granules.

A similar pattern was also observed in tonsils of the control group patients; the results of bacteriological study for detection of *M. hominis* in tonsils were found to be negative.

Based on the studies, we can conclude the following: in the cases when the hypoplastic processes were dominating in the tonsils of the chronic tonsillitis patients *E. coli* and *M. hominis* were persistent; at the same time, the presence of just single granules of specific fluorescence in the 1st group patients with predominantly hyperplastic processes of the lymphoid tissue in the tonsils rules out the fact of organ's contamination by *E. coli* and *M. hominis*.

Noteworthy, up to date, we still do not possess concrete data pertaining to cellular shifts in T- and B- dependent zones of tonsils lymphoid tissue in the chronic tonsillitis patients. First of all, that refers to quantitative data of the T- and B- lymphocytic populations, T- lymphocytic subpopulations, pattern and peculiarities of their spread in particular zones of tonsils lymphoid tissue. The poor data in available literature does not form a complete picture of immunological reorganization of the tonsils lymphoid tissue of chronic tonsillitis patients. True, single studies of descriptive nature demonstrate data of the B-lymphocytic populations increase [Vartazaryan N, 1981], which argues for preferential activation of humoral immunity reactions in tonsils. At the same time, polymorphic character of microorganisms, persisting in tonsils, their taxonomic features and peculiarities concerning their toxic potencies, selective mitogenic activity concerning B-lymphocytic populations, T-lymphocytic subpopulations allows us to rule out the unidirectional pattern of immunopathological disorders in each discrete case.

The results of our immunohistochemical analysis revealed significant increase in the B-lymphocytic populations of lymphocytes not only in the B- dependent zones of the 1st study group patients tonsils, but also in the interfollicular lymphoid tissue, i.e. in the T- dependent zones.

The patients of the 2nd study group exhibited an

entirely opposite pattern. The CD₄ and CD₈ immunoreactive lymphocytes were almost not revealed in the imperforate lymphoid follicles. Only in their marginal parts single CD₄ and CD₈ positive lymphocytes were revealed. Throughout the tonsils lymphoid tissue of the given group of patients the B-lymphocytic population number also significantly decreased. T-helper and T-killer subpopulations were detected only in the perifollicular zone, cytotoxic lymphocytes subpopulations being the dominating ones in the latter.

For the purpose of quantitative immunomorphological analysis, we purified lymphocytes from the tonsils lymphoid tissue, which were then treated with labeled FITC sera, respectively, against CD₄ and CD₈, followed by the morphometric analysis. Results of the studies are brought in the Table 2.

As the table demonstrates, in the tonsils lymphoid tissue of 1st study group patients the number of B-lymphocytic population number decreased and became 1.2 times lower than the control indices. The least indices of B-lymphocytes were recorded in the 2nd studied group patients, which were lower the control ones 1.7 times. In the chronic tonsillitis patients of the 1st group tonsils lymphoid tissue exhibited a significant increase of T-helper lymphocyte subpopulations, whose number was 1.5 times higher than the control level's. Cytotoxic subpopulations (CD₈) number was also increasing in the given study group patients. T-helper and T-killer subpopulations count in the 2nd study group resulted in an entirely opposite pattern. The number of T-helper and T-killer lymphocytes in the tonsils lymphoid tissue significantly

decreased and became lower than the control indices 5.3 and 1.5 times, respectively.

While comparing the results of the quantitative immunomorphological analysis, we also determined the ratio of CD₄ and CD₈. Thus, in the 1st group patients the CD₄/CD₈ ratio apparently increased, i.e. in the lymphoid tissue the T-helper subpopulations of lymphocytes were dominant, despite an increase of both killer and helper cells (Table 2). At the same time, in the control group T-helper subpopulations were also dominant, but numbers of both CD₄ and CD₈ were much lower than those in the 1st study group patients.

In the 2nd group patients an entirely opposite pattern was observed. Thus, in the tonsils lymphoid tissue the T-killer subpopulations of lymphocytes dominated, whose content was 1.8 times higher than the indices characterizing T-helper populations. The CD₄/CD₈ index value was 0.5. At the same time, as the table demonstrates, in the tonsils lymphoid tissue of 2nd study group patients the number of B-lymphocytes, T-helpers and T-killers obviously decreased (compared to the respective indices of the control and 1st groups of patients).

Conclusion

Based on our findings, we can conclude that the relatively prolonged persistence of atypical microorganisms (in this case: *M. hominis* and *E. coli*) in the tonsils tissues could be accompanied by tissue alteration changes of the lymphoid apparatus, the mechanism of which is probably connected with direct cytotoxic effect on target cells of membrane fractions of *E. coli* (LPS) and *M. hominis*.

In this context, in our view, the main role should

TABLE 2.
Content of the B-lymphocytic populations and T-lymphocytic subpopulations in tonsils of the chronic tonsillitis suffering patients

Study groups	B-lymphocytes	T-helpers (CD ₄)	T-killers (CD ₈)	Index CD ₄ /CD ₈
Control group	25.2 ± 1.70	38.4 ± 1.70	20.2 ± 1.28	1.9
1 st study group	39.4 ± 2.15 <i>p</i> <0.0005	58.2 ± 2.23 <i>p</i> <0.0005	34.7 ± 1.60 <i>p</i> <0.0005	1.7
2 nd study group	14.5 ± 1.48 0.005< <i>p</i> <0.0005	7.2 ± 2.39 <i>p</i> <0.0005	13.4 ± 2.76 0.025< <i>p</i> <0.05	0.5

NOTE: *p* – 1st and 2nd study groups against the control group.

also be reserved to the tonsils lymphoid tissue degree of bacterization with *E. coli* and *M. hominis*, because in the case of the lower degree of their colonization, their immunomodulatory mechanisms are more likely come into action, selectively directed at activating discrete lymphocytic populations and subpopulations. In the case of high degree colonization, their membrane fractions trigger cytotoxic effect on the tonsils lymphoid tissue cells due to both microorganisms' permanent synthesis and degradation.

Our findings, regarding immunopathological shifts in tonsils of the patients suffering from chronic tonsillitis, should be interpreted exactly from these positions. Thus, when hyperplastic processes in tonsils lymphoid tissue were dominant, a significant activation of the T-killer subpopula-

tions and especially T-helper ones took place on the background of its rather low degree of colonization. It is not unlikely that in this particular case an obvious activation of the T-helpers followed by further proliferation of the B-lymphocytic populations is a result of the *E. coli* LPS immediate triggering effect on the given T-lymphocytes subpopulations (CD₄), since such mechanism of the LPS modulating effect is considered to be reliable [Gautreaux M et al., 1994; Zimmer S et al., 1996]. At the same time, when the hypoplastic processes transpired in tonsils lymphoid tissue of the patients suffering from chronic tonsillitis in the presence of its diffuse bacterization with *E. coli* and *M. hominis*, their cytotoxic effect was most distinct.

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