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Vladimir Petrovic[1], Verica Avramovic[1]
Dragan Mihailovic[2], Miroljub Todorovic[3]

[1]Institute for Histology and Embryology of the Faculty of Medicine in Nis, Serbia
[2]Institute for Pathology, Clinical Center Nis, Serbia
[3]Clinic for Otorhinolaryngology, Clinical center of Podgorica, Montenegro

QUANTIFICATION OF TUMOR NECROSIS FACTOR-ALPHA-PRODUCING CELLS IN DIFFERENT TYPES OF CHRONIC TONSILLITIS

SUMMARY

Tumor necrosis factor-alpha (TNF-α) is the main proinflammatory cytokine of Th1 immune response. The aim of the study was to show the possible differences in the intensity of the Th1 immune response in chronic hypertrophic tonsillitis (CHT) and reccurent tonsillitis (RT) by quantifying the numerical areal density of the TNF-α-producing cells in tonsillar tissue.

As a material we used tonsils which were taken after tonsilectomy, from patients of both sexes, aged 10-29 years: five tonsils with RT and six tonsils with CHT. The quantification of the TNF-α-producing cells was performed on 5μm thick serial paraffin tissue slices, which were stained, by using LSAB+/HRP immunohistochemical method, on TNF-α. For quantification we used Image J software.

The numerical areal density of the TNF-α-producing cells show statistically significant differences in crypt epithelium, subepithelial lymphoid tissue and interfollicular region of the tonsils with RT and CHT. There is not statistically significant difference of TNF-α-producing cells in lymphoid follicles between the groups.

The results show that there is a significant difference in the production of TNF-α in tonsils with RT and CHT. This difference is probabably conditioned by the different pathogenetic mechanisms in the development of RT and CHT and point at the difference of Th1 immune response in CHT and RT.

Key words: **chronic tonsillitis, TNF-α-producing cells, morphometry**

INTRODUCTION

The palatine tonsil, as the organ of the immune system, significantly contributes to the systemic and local immunity due to its specific anatomic localization and histological structure. The main function of the palatine tonsil is to initialize the immune response against the airborne and alimentary antigens. Both the cellular and the humoral immune response occur in the palatine tonsil (1) due to specific distribution of T and B lymphocytes in the morphological compartments of the palatine tonsil: crypt epithelium, subepithelial lymphoid tissue, lymphoid follicles and interfollicular lymphoid tissue.

The chronic inflammations of the palatine tonsil are common pathological conditions. The forms of the chronic tonsillitis are chronic hypertrophic tonsillitis (CHT) which is characterized by augmented palatine tonsils and hypertrophy and hyperplasia of the lymphoid follicles and recurrent tonsillitis (RT) whose main features are smaller number of lymphoid follicles with active germinal centers, presence of the fibrosis in extrafollicular lymphoid tissue and thin and damaged crypt epithelium (2).

Corresponding author. Vladimir Petrovic • Phone: 069 69 29 79 • E-mail: vlada@medfak.ni.ac.rs

Numerous data show that macrophages, dendritic cells and T lymphocytes present in the palatine tonsil secrete tumor necrosis factor – alpha (TNF-α). TNF-α is the main proinflammatory cytokine of the local Th1 immune response, whose role is to initialize the activation of the cascade of the cytokines and to increase the permeability of the blood vessels, which ultimately leads to extravasation of macrophages and leucocytes in the place of infection (3).

The aim of this paper was to show the possible differences in the intensity of Th1 immune response in CHT and RT by quantifying the numerical areal density of TNF-α-producing cells in the different morphological compartments of the chronically deseased palatine tonsils.

MATERIAL AND METHODS

The material consisted of tonsils taken after tonsillectomy from patients of both gender: five tonsils with RT obtained from the patients aged 10-29 years and six tonsils with CHT obtained from the patients aged 18-22 years.

The tonsils were fixated in 10% buffered formalin and routinely processed to the paraffin blocks. The paraffin blocks were cut on the Leica microtome and the obtained tissue slices were 5 μm thick. The tissue slices were stained with hematoxilin-eosin and immunohystochemically by using TNF-α monoclonal antibody (Santa Cruz Biotechnology, USA, Sc-7317) and LSAB+/HRP visualisation system (DAKO). We used three slices from each palatine tonsil for the analysis. The distance between the slices was 30 μm.

We determined the numerical areal density of TNF-α-producing cells (the average number of cells in $1mm^2$ of tissue) in the different morphological compartments of the palatine tonsil: (1) crypt epithelium, (2) subepithelial lymphoid tissue, (3) lymphoid follicles and (4) interfollicular lymphoid tissue. As a method we used image analysis and as a tool we used Image J software. The images of the compartments of tonsillar tissue were obtained on the microscope NU-2 (Carl Zeiss, Jena, Germany), on the objective x25, by using web camera MSI370i. In each group of palatine tonsils we examined 10 fields in each morphological compartment per tonsil.

The obtained values for numerical areal density were compared between the groups by using Mann-Whitney rank sum test.

RESULTS

The slices of the tonsillar tissue stained with hematoxilin-eosin were used to confirm the clinical diagnosis of the type of the tonsillitis *(Figure 1)*.

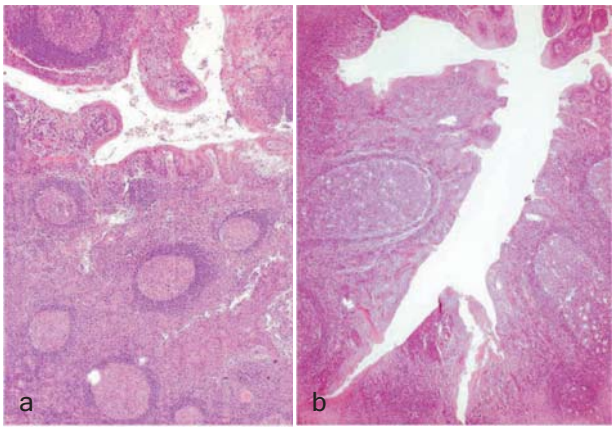

Figure 1. Palatine tonsils with a) reccurent tonsillitis; b) chronic hypertrophic tonsillitis, x 5, HE.

TNF-α-producing cells were found in all morphological compartments of palatine tonsils, in both CHT and RT. In CHT, in the subepithelial compartment TNF-α-producing cells form groups or cords, while in the lymphoid follicles they are mostly present in the germinal centers and are organized in clusters or can be seen as single cells. In the mantle zones there are rare, sporadic TNF-α-producing cells. The presence of these cells in the crypt epithelium is limited to its basal portions, where they can be seen as single cells or form minor groups. Also, TNF-α-producing cells are present in smaller number in the interfollicular region, where they form minor groups or are present as single cells *(Figure 2a)*.

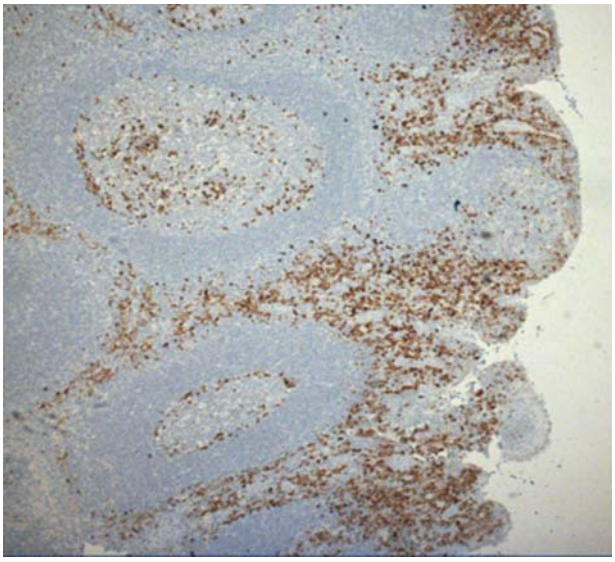

Figure 2a. Distribution of TNF-α-producing cells in chronic tonsillitis. TNF-α-producing cells are present in all morphological compartments. Recurrent tonsillitis: the majority of TNF-α-producing cells are present in the subepithelial lymphoid tissue and in the interfollicular region of the palatine tonsil

In RT the most of TNF-α-producing cells are present in the subepithelial and interfollicular regions. In these compartments, TNF-α-producing cells are numerous and form cords, or can be seen as single cells. In the germinal centers they are single or form groups, and they are rare in mantle zones. On the border between the germinal centers and matle zones these cells form a ring. The thin crypt epithelium contains rare, single TNF-α-producing cells *(Figure 2b)*.

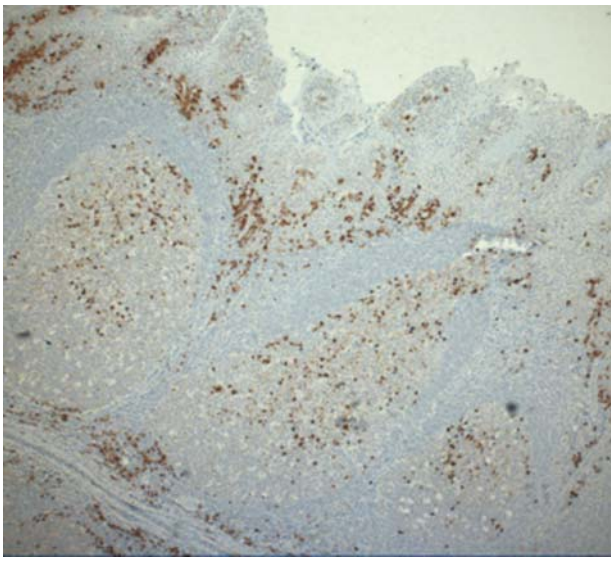

Figure 2b. Distribution of TNF-α-producing cells in chronic tonsillitis. TNF-α-producing cells are present in all morphological compartments. Chronic hypertrophic tonsillitis: the majority of TNF-α-producing cells are present in the subepithelial lymphoid tissue and in the lymphoid follicles, x 25, LSAB+/HRP.

The results of the quantification of TNF-α-producing cells in the morphological compartments of the palatine tonsils with CHT and RT are presented in the *Table 1*.

interfollicular lymphoid tissue of the palatine tonsils with CHT and RT, while we did not find statistically significant difference in the number of TNF-α-producing cells in the lymphoid follicles between the groups.

DISCUSSION

The results of the authors who have examined TNF-α-producing cells are different. Andersson and al. (4) found TNF-α-producing cells in all morpfological compartments of the palatine tonsil, except in the crypt epithelium, which is rather consistent with the results of our study. Agren et al. (5) and Hoeffaker et al. (6) found the presence of these cells only in germinal centers and interfollicular lymphoid tissue, while Rostaing et al. (7) claim in their paper that it is impossible to prove the presence of intracytoplasmatic cytokines in the paraffin tissue slices of tonsillar tissue.

By comparing the obtained values for the numerical areal density of TNF-α-producing cells between the groups, we found that there is statistically significant difference in the number of these cells in crypt epithelium, subepithelial lymphoid tissue and interfollicular lymphoid tissue. The most of TNF-α-producing cells are present in the subepithelial lymphoid tissue in both types of tonsillitis. The expression of the cytokines of Th1 immune response have been examined in numerous studies. However, in these papers the autors were using semiquantitative methods (4-7), while our approach was to quantify these cells, which is more valid for the interpretation of the results.

The crypt epithelium of the palatine tonsil is the first site of the contact with the antigen. The crypt epithelium is infiltrated mostly with Th lymphocytes, B lymphocytes and macrophages, and

Table 1. The average values of the numerical areal density of TNF-α producing cells in the morphological compartments of the palatine tonsils with CHT and RT

Morphological compartments of tonsils	CHT	RT	p
	n=6	n=5	
Crypt epithelium	98.83±48.10	332.88±181.54	0.001
Subepithelial lymphoid tissue	3043.66±1160.37	3832.99±798.07	0.001
Lymphoid follicles	1517.21±720.76	1260.21±661.06	0.074
Interfollicular lymphoid tissue	692.45±271.29	1883.27±653.47	0.001

The numerical areal density of TNF-α-producing cells is significantly different in crypt epithelium, subepithelial lymphoid tissue and

also plasma cells can be found (8, 9). Our results showed that the number of TNF-α-producing cells is higher in the tonsils with RT compared to the tonsils

with CHT. Having in mind that Th cells and macrophages are the main producers of TNF-α, we consider that TNF-α immunopositivity in this region originates from these cells.

The most of TNF-α-producing cells, in both CHT and RT, are located in the subepithelial region. Our results show that in this region TNF-α-producing cells are more numerous in RT, and that the difference is statistically significant. Crypt epithelium and subepithelial lymphoid tissue represent the unique morphofunctional compartment which reacts first with the antigens. Subepithelial T lymphocytes originate from the interfollicular lymphoid tissue and migrate to the subepithelial region in order to initialize the immune response after the invasion of the antigene (10), of which 80% are T helper lymphocytes (11). It has been reported in previous studies that Haemophilus Influenzae was isolated from the crypts of the palatine tonsils with CHT in five of six cases and in three of six cases with RT (5). Also, in RT, Streptococcus pyogenes infection is a common finding. M protein present in the bacterial wall of Streptococcus Pyogenes is shown to be a very powerful stimulator of macrophages to secrete TNF-α (12). It is also shown that in vitro stimulation of tonsillar cells with antigene leads to the intensive production of cytokines, including TNF-α (13). On the other hand, Haemophilus Infuenzae can also cause a significant proliferation of Th lymphocytes (5). The greater number of TNF-α-producing cells in RT could be explained by the fact that, unlike in CHT, the signs of inflammation are present in RT (2), and the main proinflammatory cytokine is TNF- α.

Previous studies showed a significant presence of immunoglobulin (Ig)-producing cells in the subepithelial region. The majority of these cells produce IgA and IgG (14). It is possible that TNF-α, as a proimflammatory cytokine, stimulates the production of other cytokines which are important for the differentiation and secretion of Ig-producing cells, thus being the bridge between the cellular and humoral immunity (15, 16).

We found that there is no statistically significant difference in the number of TNF-α-producing cells in the lymphoid follicles of the palatine tonsils with CHT and RT. The presence of TNF-α-producing cells in CHT is limited to the germinal centers and these cells are probably macrophages and a small number of Th lymphocytes. The main function of the macrophages in the germinal center of the lymphoid follicles is the apoptosis of incompetent centrocytes and centroblasts (1). The characteristic position of TNF-α-producing cells in the lymphoid follicles in RT, a ring-like formation around the germinal center, implies that Th lymphocytes and follicular dendritic cells, present in this area (1), are also significantly involved in the production of TNF-α, beside the macrophages.

It is reported in the earlier studies that there is the important presence of TGF-β-producing cells in the palatine tonsils. Agren et al. (5) report their presence mostly in the crypt epithelium and in the extrafollicular regions of the palatine tonsils with CHT and RT, while Andersson et al. (17) found the presence of these cells in the crypt epithelium and germinal centers of palatine tonsils with RT. The main function of TGF-β is the activation of B lymphocytes, inhibition of bcl-2 gene expression and the supression of bactericyde activity of macrophages (5). The significant presence of macrophages in the germinal centers could be explained by the increased apoptotic rate in this region, especially in RT (18). On the other hand, decreased bactericyde activity of macrophages allows the survival of the intracellular antigenes and explains the inability of adequate immune response.

The interfollicular regions are mostly populated with T cells. In the earlier studies it has been shown that 65% of T cells, present in this region, are T helper lymphocytes and 35% are cytotoxic T lymphocytes (19). In this region, macrophages and interdigitate cells are also present, which are shown to have the ability of production and secretion of TNF-α. This region of the palatine tonsil represents the site of interactions between T and B cells and antigen presenting cells (16). Our results show that TNF-α-producing cells are more numerous in the interfollicular region of tonsils with RT compared to the tonsils with CHT. This result differs from the result of Agren et al. (5) who found, by using semiquantitative analysis, the equal presence of TNF-α-producing cells in this region in both types of tonsillitis. Great number of TNF-α-producing cells in the interfollicular region of palatine tonsil with RT implies the possible key role of T cells in the initialization and the maintanance of the immune response.

CONCLUSION

The results of our study show that the production of TNF-α is significantly present in all the morphological compartments of the palatine tonsils with CHT and RT. The differences in the production of TNF-α imply the different intensity of Th1 immune response, which is most probably conditioned by the different pathogenetic mechanisms involved in the onset of the chronic tonsillitis.

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KVANTIFIKACIJA TUMORSKOG FAKTORA NEKROZE-alfa-PRODUKUJUĆIH ĆELIJA U RAZLIČITIM TIPOVIMA HRONIČNOG TONZILITISA

Vladimir Petrović[1], Verica Avramović[1], Dragan Mihailović[2], Miroljub Todorović[3]

[1]Institut za histologiju i embriologiju, Medicinski fakultet u Nišu, Srbija
[2]Institut za patologiju, Klinički centar Niš, Srbija
[3]Klinika za otorinolaringologiju, Klinički centar Podgorica, Crna Gora

SAŽETAK

Tumorski faktor nekroze-alfa (*TNF-α*) predstavlja glavni proinflamatorni citokin *Th1* imunog odgovora. Cilj rada bio je da se određivanjem numeričke arealne gustine *TNF-α*-produkujućih ćelija u morfološkim odeljcima hronično obolele palatinalne tonzile pokažu moguće razlike u intenzitetu Th1 imunog odgovora kod hroničnog hipertrofičnog tonzilitisa (HHT) i rekurentnog tonzilitisa (RT).

Materijal su činile tonzile uzete nakon tonzilektomije bolesnika oba pola, starosti od 10 do 29 godina i to: 5 tonzila za RT i 6 tonzila za HHT. Kvantifikacija *TNF-α*-produkujućih ćelija vršena je na serijskim parafinskim presecima debljine 5*μm* koji su bojeni imunohistohemijskom metodom *LSAB+/HRP* uz korišćenje monoklonskog antitela za *TNF-α*. Numerička arealna gustina *TNF-α*-produkujućih ćelija određivana je upotrebom programa *Image J*.

Numerička arealna gustina *TNF-α*-produkujućih ćelija je statistički značajno različita u kriptičnom epitelu, subepitelnom limfnom tkivu i interfolikularnom regionu tonzila sa RT u odnosu na HHT. U limfnim folikulima nije pronađena statistički značajna razlika u broju *TNF-α*-produkujućih ćelija između ispitivanih grupa.

Rezultati istraživanja pokazuju da postoji značajna razlika u produkciji *TNF-α* u tonzilama sa RT i HHT. Ova razlika uslovljena je, najverovatnije, raličitim patogenetskim mehanizmom nastajanja hroničnog tonzilitisa i ukazuje na različit *Th1* imuni odgovor u HHT i RT.

Ključne reči: **hronični tonzilitis, *TNF-α*-produkujuće ćelije, morfometrija**