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EXPRESSION OF CD68 ANTIGEN IN CHRONICALLY DISEASED HUMAN PALATINE TONSIL

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Based on the pathohistological examination of tonsillar tissue, chronic tonsillitis can be classified as chronic hypertrophic tonsillitis (CHT) and recurrent tonsillitis (RT). CD68 is a glycoprotein ubiquitously expressed on the cells of the monocyte-macrophage lineage, as well as on the dendritic cells. Macrophages and dendritic cells are major initiators, effectors, and regulators of immune response in the palatine tonsil. The aim of this paper was to examine microanatomical distribution of CD68-immunopositive cells and to determine their numerical areal density in morphological compartments of palatine tonsils with CHT and RT, in order to show the possible differences in antigen-presentation potential between these two pathological conditions. As a material we used tonsils taken after tonsillectomy, from patients of both sexes, aged 10-29 years: six tonsils with RT and nine tonsils with CHT. The quantification of CD68-immunopositive cells by "ImageJ" software was performed on 5 μm thick serial paraffin tissue slices, which were stained immunohistochemically, by using monoclonal anti-CD68 antibody. The results of morphometrical analysis showed presence of CD68-immunopositive cells in all morphological compartments of tonsils with RT and CHT, being higher in number in RT compared with CHT. Statistically significant difference in numerical areal density of the CD68-immunopositive cells was found in the germinal centers of lymphoid follicles (RT > CHT), and interfollicular lymphoid tissue (CHT > RT). The difference in the number of CD68-immunopositive cells might imply the different mechanisms involved in the infiltration of tonsillar tissue with CD68-immunopositive cells, as well as the different antigen-presenting potential in these two conditions.

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Key words: *CD68 antigen, morphometry, chronic tonsillitis, macrophage, dendritic cell*

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Introduction

Palatine tonsil is the organ of the immune system that significantly contributes to the local and general immunity, due to its specific anatomic location and histological structure. As a part of Waldeyer's tonsillar ring, it is responsible to initiate both the cellular and humoral immune response against the antigens entering the organism trough the oral cavity (1, 2). The parenchyma of palatine tonsils contains both T- and B-lymphocytes, as well as antigen presenting cells, which are specifically distributed into four morphological compartments: crypt epithelium, subepithelial lymphatic tissue, lymphoid follicles (germinal center and mantle zone) and interfollicular lymphoid tissue (3-5). Lymphoid follicles are structurally and functionally divided into mantle zone and germinal center. The mantle zone contains mostly small B memory lymphocytes, while in the germinal center there are large dividing B lymphoblasts (centroblasts) and their differentiated non-dividing forms called centrocytes, specific subset of T helper cells, germinal center dendritic cells and follicular dendritic cells (4, 6-8).

Chronic inflammations are the most common pathological conditions of the palatine tonsil. However, the exact pathogenetic mechanisms leading to the chronic inflammation of the palatine tonsils are not yet elucidated (3, 9). Based on the pathohistological examination of tonsillar tissue, chronic tonsillitis can be classified as chronic hypertrophic tonsillitis (CHT) and recurrent tonsillitis (RT). CHT is characterized by enlarged palatine tonsils and hypertrophy and hyperplasia of lymphoid follicles, while in RT palatine tonsils contain lymphoid follicles with active germinal centers, fibrosis in interfollicular lymphoid tissue and thin crypt epithelium (10).

CD68 is glycoprotein expressed on the cells of the monocyte-macrophage lineage, as well as on dendritic cells. Unlike macrophages that are part of the innate immune system, dendritic cells are components of the acquired immunity and have the ability of its modulation (11). The main function of macrophages in germinal centers is the phagocytosis of cellular remains after the division of B lymphoblasts and plasma cells, and of those localized in subepithelial tissue is the antigen presentation to T-lymphocytes (2, 12). The sole role of dendritic cells is the antigen presentation to T-lymphocytes (11).

After penetrating the crypt epithelium the antigens reach the subepithelial tissue and interfollicular lymphoid tissue where they are being caught and processed by macrophages and dendritic cells, and subsequently presented via MHC II molecules to CD4+ T-lymphocytes (4, 13). T helper lymphocytes stimulate the divisions of germinal center B cells which give rise to two populations: antibody-expressing B memory cells and antibody-producing plasma cells (14). Dendritic cells also have the ability to activate naïve T cells and to initiate, coordinate, and regulate adaptive immune responses (15, 16).

The aim of the paper was to examine microanatomical distribution of CD68-immunopositive cells and to determine their numerical areal density in morphological compartments of palatine tonsils with CHT and RT, in order to show the possible differences in the antigen-presentation potential between these two pathological conditions.

Materials and methods

The research was performed at the Department of Anatomy and Department of Histology of the Faculty of Medicine, University of Niš, and at the Clinic for Ear, Throat and Nose of the University Clinical Center of Niš, Serbia.

The material was obtained following the ethical guidelines and consisted of palatine tonsils taken after the tonsillectomies of patients of both genders: 5 tonsils with RT (patients aged 10-29 years) and 5 tonsils with CHT (patients aged 18-22 years).

The tonsils were fixated in 10% buffered formaldehyde and were routinely processed to paraffin blocks. The paraffin blocks were cut on Leica microtome in order to obtain 5μm thick tissue sections that were subsequently stained with hematoxylin-eosin and immunohistochemically by using antibody against CD68 antigen (GeneTex, GTX41865, 1:100). As a visualization system for immunohistochemistry was used EnVisionFLEX, High pH (Agilent).

The numerical areal density (N_A) is the parameter showing the average number of cells in mm^2 of the tissue. We were determining the numerical areal density of CD68-immunopositive cells in different morphological compartments of palatine tonsils with CHT nad RT: crypt epithelium and subepithelial lymphoid tissue, germinal centers of lymphoid follicles, mantle zones of lymphoid follicles and interfollicular lymphoid tissue. The values are obtained by using formula $N_A = (N/A)*1000000$ (N – number of cells on the examined visual field, A – area of the examined visual field in μm^2).

The images of the tonsillar tissue were obtained by using Olympus BX50 (Olympus, Japan) microscope equipped with Leica DFC 295 camera (Leica Microsystems, Germany). All images were taken under the magnification of the objective x40. For the numbering of cells and determining the area of the examined visual field we used Image J software. Fifty visual fields per the morphological compartment were examined in each group (CHT or RT), after the calibration of the images.

The obtained values for N_A were compared between the examined groups by using Mann-Whitney test.

Results

CD68 immunopositive cells were found to be present in all examined morphological compartments of palatine tonsils with CHT and RT (Figures 1, 2).

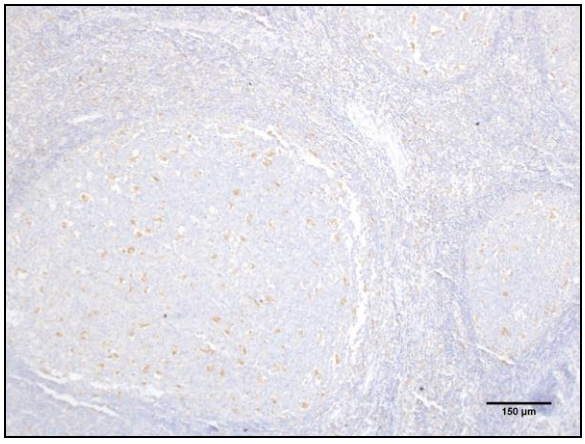

Figure 1. Distribution of CD68-immunopositive cells in the palatine tonsil with chronic hypertrophic tonsillitis x100

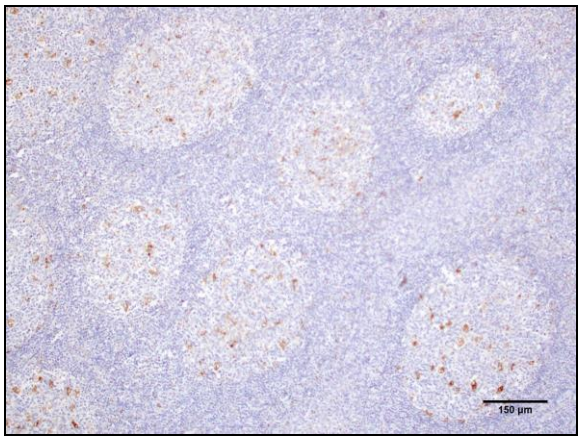

Figure 2. Distribution of CD68-immunopositive cells in the palatine tonsil with recurrent tonsillitis x100

The results of CD68-immunopositive cells numerical areal density in morphological compartments in palatine tonsils with CHT and RT are presented in Table 1.

Numerical areal density of CD68-immunopositive cells shows statistical significance between the germinal centers and interfollicular lymphoid tissue of palatine tonsils with RT and CHT.

Table 1. Average values of numerical areal density (N_A) of CD68-immunopositive cells in morphological compartments of palatine tonsils with CHT and RT

Morphological compartment of palatine tonsil	CHT n = 5	RT n = 5	p
Crypt epithelium and subepithelial connective tissue	185.17 ± 47.1	227.54 ± 48.32	0.3
Germinal center	276.16 ± 85.18	477.58 ± 27.9	0.018
Mantle zone	179.72 ± 47.68	210.17 ± 45.72	0.106
Interfollicular lymphoid tissue	262.40 ± 36.84	197.67 ± 23.49	0.001

RT – recurrent tonsillitis,
CHT – chronic hypertrophic tonsillitis,
n – number of palatine tonsils per examined group

Discussion

Our results show that the CD68-immunopositive cells are distributed in all morphological compartments of the palatine tonsil, which is in accordance with the findings of the other authors (12, 17-19). Statistically significant difference in numerical areal density of these cells was found in germinal centers of lymphoid follicles and interfollicular lymphoid tissue, which differs from the reports of Gorfien et al. that found the difference in the number of these cells only in interfollicular areas in tonsils with RT and CHT (17). Stent et al. reported that CD68 antigen was expressed in all dendritic cell populations and macrophages in cell cultures obtained from human palatine tonsils after tonsillectomy. Interestingly, their findings suggest that S-100 antigen, usually associated with macrophages and dendritic cells, was not expressed in subpopulation of dendritic cells that were CD11c negative (20). Yamamoto et al. reported the presence of S-100-immunopositive cells in all morphological compartments of palatine tonsils with RT, tonsillar hyperplasia and tonsils with focal infection (19). They examined the numerical areal density of these cells in crypt epithelium and interfollicular lymphoid tissue, and found the statistically significant difference in the number of these cells in crypt epithelium of tonsils with tonsillar hyperplasia and tonsils with focal infection. Although these results cannot be directly compared with the results of our study, it is noteworthy to mention that they reported 611 ± 231 S-100-immunopositive cells by mm^2 in crypt epithelium in RT, while our findings suggest the number of 227.54 ± 48.32 CD68-immunopositive cells in mm^2 in both crypt epithelium and subepithelial connective tissue in tonsils with RT.

The previous studies reported that the number of macrophages increases in the diseased palatine tonsils, compared to the healthy ones (17). However, regardless the higher numbers, the increase in number of macrophages in superficial and crypt epithelium of the chronically diseased palatine tonsils is not followed by the increased expression of RFD7 antigen that is a characteristic of mature phagocyte cells, which might imply that these cells are still functionally immature and inactive. Also, the number of dendritic cells decreases in these compartments, as well as the expression of RFD1 antigen which is connected with functional activation and antigen-presenting potential of dendritic cells (17). The functional immaturity of macrophages and dendritic cells, possibly caused by chronic inflammation, might represent one of the reasons of local immunosuppression that occurs in the chronically inflamed tonsillar tissue (17, 21). T- and B- lymphocytes in palatine tonsils with CHT, although increased in number due to the bacterial load and hypertrophy of tonsillar tissue, are not adequately immunocompetent and the experiments performed in vitro showed that these lymphocytes are relatively unresponsive to the stimulation by antigens (12).

Chen et al. examined tonsils with the RT and the TH (tonsillar hyperplasia) by combining beta-galactosidase staining, connected with the cellular senescence, and immunohistochemical staining with CD68. Their results showed the increased number of senescent CD68-immunopositive cells in germinal centers and mantle zones in both examined groups (22). Macrophages and dendritic cells are the major initiators, effectors and regulators of immune system, and the senescence of these cells is characterized by increased inflammatory cytokine production and impairment of chemotaxis and phagocytosis (22, 23). The impairment of these three functions might be responsible for the hyperplasia of lymphoid follicles in TH and CHT, as well as for increased

overload of pathogens in palatine tonsils especially found in RT.

The crypt epithelium and subepithelial connective tissue represent the main site of entry and contact of different antigens with M cells, macrophages and dendritic cells of the palatine tonsil (18). The previous studies showed the decreased ability of M cells to uptake the antigens, as well as the functional immaturity of macrophages and dendritic cells in this morphological compartment in hronic tonsillitis (12, 17, 21, 22, 24). Although the reasons for this local immuno-suppression are not yet completely elucidated, there is an increased number of evidences that some bacteria *(Pseudomonas aeruginosa, Streptococcus pneumoniae*), viruses (Epstein-Barr), bacterial bio-films and recurrent infections cause the cellular senescence probably via cellular oxidative stress, and lead to the morphological changes of the crypt epithelium (hyperkeratosis, cryptitis) (17, 22, 25-27). Lymphocytes and antigen presenting cells re-present the axis of the immune response in palatine tonsils and every change in their functional activity impairs the immunological potential of this organ. Bearing in mind the importance of epithelial com-partment for the function of the palatine tonsil, the future studies should focus on the possibly surface- and crypt epithelium-related mechanisms involved in the impairment of lymphocyte functions and in-adequate activation of macrophages and antigen presenting cells.

Conclusion

The results of our study showed that CD68-immunopositive cells were present in all morphological compartments of the tonsils with RT and CHT. Numerical areal density of the CD68-immunopositive cells was significantly higher in the germinal centers of lymphoid follicles with the RT compared to the CHT, and in the interfollicular lymphoid tissue in the CHT compared to the RT. The difference in the number of CD68-immunopositive cells might imply the different mechanisms involved in the infiltration of tonsillar tissue with CD68-immunopositive cells, as well as the different anti-gen-presenting potential in these two conditions.

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EKSPRESIJA ANTIGENA CD68 U HRONIČNO OBOLELOM HUMANOM NEPČANOM KRAJNIKU

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Hronični tonzilitis se prema patološkom nalazu tkiva krajnika može podeliti na hronični hipertrofični (HHT) i rekurentni tonzilitis (RT). CD68 predstavlja ubikvitarni glikoprotein, koji se eksprimira na ćelijama monocitno-makrofagne linije i dendritičnim ćelijama. Makrofagi i dendritične ćelije glavni su inicijatori, efektori i regulatori imunog odgovora u nepčanom krajniku. Cilj ovog istraživanja bio je da se istraži mikroanatomska distribucija ćelija pozitivnih na CD68, kao i da se odredi njihova numerička arealna gustina u morfološkim odeljcima nepčanih krajnika sa HHT i RT, da bi se odredile moguće razlike u antigenoj prezentaciji između ova dva stanja. Za materijal su korišćeni krajnici dobijeni nakon tonzilektomije, od bolesnika oba pola, starosti između 10 i 29 godina. Šest krajnika bilo je sa RT, a devet sa HHT. Kvantifikacija ćelija pozitivnih na CD68 rađena je putem programa "ImageJ", na parafinskim isečcima debljine 5 μm, koji su imunohistohemijski bojeni monoklonalnim anti-telom na CD68. Rezultati morfometrijske analize pokazali su prisustvo ćelija pozitivnih na CD68 u svim morfološkim odeljcima krajnika sa HHT i RT, pri čemu ih je više bilo u potonjoj grupi. Statistički značajno veća razlika u numeričkoj arealnoj gustini ćelija imunopozitivnih na CD68 nađeno je u germinativnim centrima limfnih folikula (RT > CHT) i interfolikularnom limfnom tkivu (CHT > RT). Ova razlika u broju imunopozitivnih ćelija na CD68 može ukazati na postojanje različitih mehanizama, koji utiču na infiltraciju tkiva krajnika ovim ćelijama, kao i na različit potencijal za prezentaciju antigena u ova dva stanja.

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Ključne reči: *antigen CD68, morfometrija, hronični tonzilitis, makrofag, dendritična ćelija*