Original article

UDC: 616.322-002:611.018+576.3
doi:10.5633/amm.2020.0412

QUANTIFICATION OF CD11C-IMMUNOPOSITIVE CELLS IN DIFFERENT TYPES OF CHRONIC TONSILLITIS

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CD11c is a transmembrane protein, belonging to the β2 integrin subfamily. It is generally accepted as a marker of conventional dendritic cells, but can also be found on macrophages, neutrophils, and some B cells. The aim of this paper was to determine numerical areal density of CD11c-immunopositive cells in different morphological compartments of tonsillar tissue in recurrent tonsillitis (RT) and chronic hypertrophic tonsillitis (CHT). As a material we used tonsils which were taken after tonsillectomy, from patients of both sexes, aged 10-29 years: six tonsils with RT and nine tonsils with CHT. The quantification of the CD11c-immunopositive cells was performed on 5 μm thick serial paraffin tissue slices, which were stained immunohistochemically, by using mouse monoclonal anti-CD11c antibody. For quantification we used ImageJ software. Our results showed that CD11c-immunopositive cells were present in all morphological compartments of tonsils with RT and CHT. The higher value for numerical areal density of CD11c-immunopositive cells in RT showed statistically significant difference in crypt epithelium and subepithelial lymphoid tissue compared to CHT. There was not statistically significant difference of CD11c-immunopositive cells in lymphoid follicles and interfollicular regions between the groups. Crypt epithelium and subepithelial lymphoid tissue represent the first site of contact between antigens and tonsillar tissue, and are crucial for the initiation of the immune response. The higher number of CD11c-immunopositive cells in crypt epithelium and subepithelial lymphoid tissue in RT might be connected with more efficient immunological response of this morphological compartment, compared to CHT.

Acta Medica Medianae 2020;59(4):xx-xx.

Key words: *recurrent tonsillitis, chronic hypertrophic tonsillitis, CD11c, dendritic cells*

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Introduction

Palatine tonsils, as part of the mucosal immune system of oral cavity, are lymphoepithelial organs that have significant role in local and systemic immunity (1, 2). Due to their anatomical position, on the crossroad of digestive and respiratory system, they come in contact with airborne and alimentary antigens (3). The immune function of palatine tonsils is facilitated by its specific histological structure consisting of four well defined compartments: reticulated crypt epithelium, subepithelial tissue, lymphoid follicles and interfollicular region (1, 4). Interfollicular region is mainly composed of T cells, interdigitating cells (type of dendritic cells) and macrophages (2, 5). Lymphoid follicles are further structurally and functionally divided into mantle zone and germinal center. 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, both of which have undergone Ig switch recombination (6, 7). Beside them, germinal center contains specific subset of T helper cells, germinal center dendritic cells and follicular dendritic cells that should not be mistaken for conventional or plasmacytoid dendritic cells (2, 6, 8).

Upon the entry through the crypt epithelium, antigens reach the interfollicular region or secondary lymphoid follicles of palatine tonsil where they are being caught and processed by dendritic cells and macrophages, and subsequently presented to $CD4^+$ T helper lymphocytes (2, 9). T helpers lymphocytes then stimulate germinal center B cells that start to proliferate, and develop into antibody-expressing B

memory cells and antibody-producing plasma cells (10).

Dendritic cells (DC) are professional antigen-presenting cells with ability to activate naïve T cells and to initiate, coordinate, and regulate adaptive immune responses (11, 12). These cells are classified into two groups, as conventional DC (cDC) and plasmacytoid DC (pDC), both of which are found to be present in the parenchyma of human palatine tonsils (12). Conventional DC exibit strong expression of CD11c, and can be further classified as cDC type 1 and type 2 (13). cDC_1 present antigens to CD8+ T cells and promote cytotoxic T lymphocytes and Th1 cells, while cDC_2 present antigens via MHC II and promote Th1, Th2 and Th17 immune response (13-15). Plasmacytoid DC do not express CD11c as a surface marker and are included in the response to viral or bacterial infection, by producing high amounts of interferons and by recruiting natural killer T lymphocytes (16). In resting state, pDC are weak antigen-presenting cells, however, when they are activated, they gain the ability to present antigens directly to the T lymphocytes (13).

Bearing in mind that the lymphoid tissue of the palatine tonsil is constantly stimulated by airborne and alimentary antigens, the chronic inflammations of this organ are common pathological conditions. According to Surjan et al., there are two types of the chronic tonsillitis in adults: 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 (17).

Cd11c is a transmembrane protein, belonging to the β2 integrin subfamily. It is generally accepted as a marker of cDC, but can also be found on macrophages (especially in the digestive and respiratory system), neutrophils, some memory B cells, as well as in acute nonlymphocytic leukemia and some B cell chronic lymphocytic leukemias (18, 19).

In order to determine the possible differences in the number of antigen-presenting cells between CHT and RT, the aim of this paper was to quantify CD11c-immunopositive cells in different morphological compartments of chronically diseased human palatine tonsil.

Material and methods

Material and tissue processing

The material consisted of chronically diseased palatine tonsils, taken after tonsillectomies from patients of both genders: 9 palatine tonsils with CHT obtained from patients aged 18-22 years, and 6 palatine tonsils with RT obtained from patients aged 10-29 years. All palatine tonsils were fixated in 10% buffered formalin and were routinely processed through ascending series of alcohols (75%, 96%, 100%) and chloroform to paraffin blocks. The paraffin blocks were cut on Leica RM2255 microtome (Leica Micro-Systems, Reuil-Malmaison, France). 5 μm thick tissue slices of tonsillar tissue were adhered to microscopic slides and submitted to hematoxylin-eosin and immunohistochemical staining.

Immunohistochemistry

After deparaffination of tissue slices in thermostat at 64 ^{O}C and xylene, the rehydration was done in descending series of alcohols (100%, 96%, 75%) and distilled water. Heat antigen retrieval was performed for 30 minutes in citrate buffer and the endogenous peroxidase was blocked with 3% H_2O_2 for 10 minutes. For detection of CD11c antigen we used mouse monoclonal anti-CD11c antibody (Abcam, ab52632, 1:500) overnight at 4 ^{O}C. The secondary antibody was applied for 45 minutes and tissue slices were then stained with DAB (diaminobenzidine) and counterstained with Mayer hematoxylin. The secondary antibody, DAB and washing buffer, needed for rinses between the steps of immunohistochemical staining, were used from EnVisionFLEX, HighpH visualization system (Agilent, K8000/8002).

Morphometric and statistical analysis

Stained slides were examined using light microscope Olympus BX50 (Olympus, Japan) and images of different morphological compartments of palatine tonsil, under different magnifications, were captured in TIFF format with digital camera Leica DFC295 (Leica Microsystems, Germany). In order to quantify CD11c-immunopositive cells, we determined their numerical areal density (average number of cells in 1 mm^2 of tissue) in all morphological compartments of palatine tonsil:

a) crypt epithelium and subepithelial lymphatic tissue,

b) interfollicular region and

c) lymphoid follicles. As a tool, we used ImageJ software version 1.48v (Wayne Rasband, National Institute of Health, USA). In each group of palatine tonsils, we examined 30 fields (under magnification x40) 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 tonsillar tissue stained with hematoxylin-eosin were used to confirm the clinical diagnosis of RT or CHT.

CD11c-immunopositive cells were found in all morphological compartments of palatine tonsil, both in CHT and RT, and showed the same pattern of distribution in tonsillar parenchyma of both examined groups (Figure 1). They were mostly seen as diffusely dispersed single cells, with centrally located, large euchromatic nucleus and numerous cytoplasmatic processes. Their cytoplasmatic processes were in contact with surrounding lymphocytes, basement

membranes of the postcapillary venules or with the processes of adjacent CD11c-immunopositive cells thus forming a network (Figure 2). In interfollicular regions, CD11c-immunopositive cells were often seen grouped around the lymphoid follicles in a form of a ring. Lymphoid follicles contained CD11c-immunopositive cells predominantly in germinal centers, while they were lower in number in mantle zones.

Rarely, in interfollicular region and lymphoid follicles, CD11c-immunopositive cells exhibited the morphology of large oval cells containing large eu-chromatic centrally located nucleus and without cytoplasmatic processes.

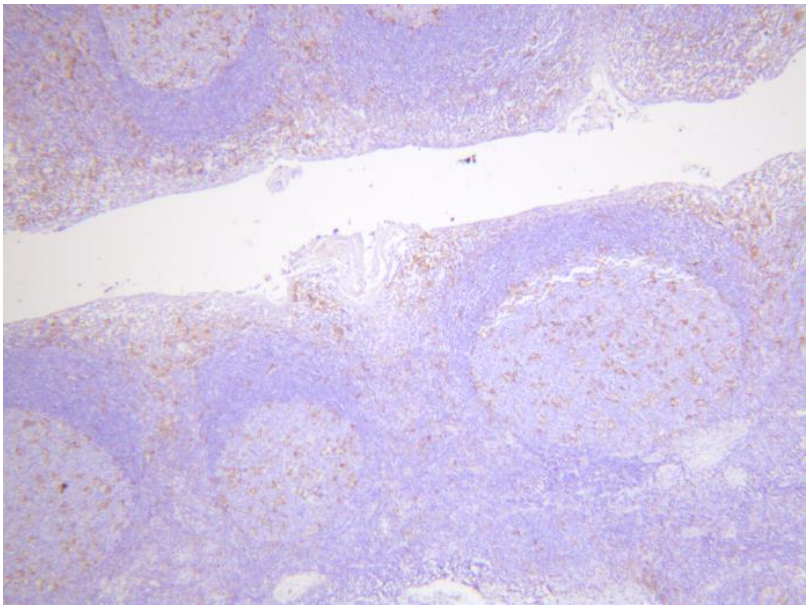

Figure 1. Expression of CD11c antigene in palatine tonsil with CHT. CD11c-immunopositive cells are present in all morphological compartments, x8.

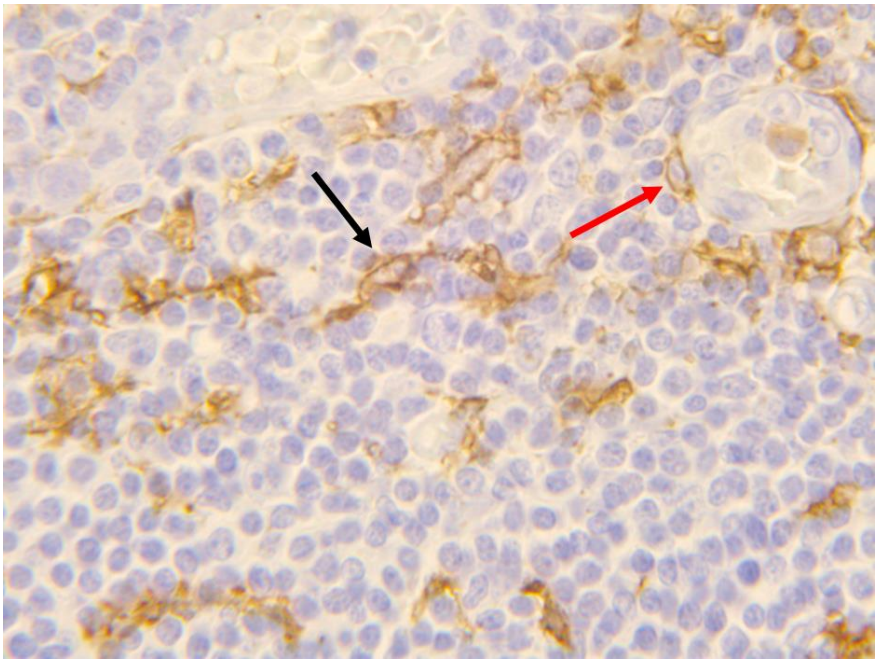

Figure 2. CD11c-immunopositive cells in interfollicular region in tonsil with RT. They are seen as single, dispersed cells between the lymphocytes and macrophages, with long cytoplasmatic processes (black arrow) or around the basement membrane of postcapillary venule (red arrow), x80.

The results for numerical areal density of CD11c-immunopositive cells in CHT and RT are presented in Table 1. The statistically significant difference in the number of CD11c-immunopositive cells was found in crypt epithelium and subepithelial lymphoid tissue, while we did not find statistically significant difference in the number of these cells in interfollicular region and lymphoid follicles between the groups.

Table 1. Value for numerical areal density of CD11c-immunopositive cells in morphological compartments of palatine tonsil with RT and CHT.

Morphological compartments of palatine tonsil	**RT**	**CHT**
	n = 6	n = 9
	X ± SD	X ± SD
Crypt epithelium and subepithelial lymphoid tissue	563.77 ± 65.34*	396.39 ± 104.65*
Interfollicular region	1012.97 ± 151.81	1042.79 ± 103.88
Lymphoid follicles	1137.77 ± 53.75	1161.23 ± 175.84

n – number of palatine tonsils per group, X – average value of numerical areal density, SD – standard deviation, *statistical significance, $p < 0,05$.

Discussion and conclusion

The presence of CD11c-immunopositive cells in human palatine tonsil is well documented in previous studies (12, 20-24). However, to our knowledge, there are no data concerning the morphological distribution and quantification of CD11c-immunopositive cells in human palatine tonsil. The studies of CD11c-immunopositivity were mainly limited to dendritic and some B cell populations, where this marker was used for isolation and identification of these cell types. Both cDC and pDC were found to be present in the tissue of human palatine tonsil, however, pDC are shown to be prevalent dendritic cell type in palatine tonsil (12, 24). Bearing in mind that CD11c is not a surface marker of pDC, it is clear that CD11c does not mark the whole dendritic cell population, but rather the cDC subtype.

The results of our study showed that CD11c-immunopositive cells were distributed in all morphological compartments of chronically diseased human palatine tonsils, with only crypt epithelium and subepithelial lymphoid tissue showing statistically significant difference in the number of these cells, in favor of tonsils with RT. Bearing in mind that almost all cells immunolabeled with CD11c antibody in our tissue slices showed dendritic morphology with the presence of cytoplasmatic processes, we can conclude that these cells correspond to conventional dendritic cells. Follicular dendritic cells in the lymphoid follicles of palatine tonsils, that display dendritic morphology similar to conventional DC, were shown to be CD11c negative in previous studies, and therefore were not taken in consideration (8, 25, 26). Rare, occasional CD11c-immunopositive cells were oval in shape with a centrally located nucleus, and without the presence of cytoplasmatic processes. Taking in consideration their morphology, we assume that these cells most probably correspond to macrophages and memory B cells.

Gorfien et al. examined the presence of dendritic cells in human healthy palatine tonsil, RT and CHT with anti-S100 (marker for cDC), CD1a (marker for cDC1) and RFD1 (marker for interdigitating cells) antibodies by using quantitative approach. Their results, like ours, showed the presence of dendritic cells in all morphological compartments of palatine tonsil. Statistically significant difference was only shown for CD1a immunopositive cells in crypt epithelium in RT/CHT compared to healthy tonsils (27, 28). However, while they found that S100-immunopositive dendritic cells did not show statistically significant difference between the groups, our results show the difference in the number of dendritic cells in crypt epithelium between RT and CHT. This difference might be due to the fact that we observed crypt epithelium and subepithelial lymphoid tissue as one morphological compartment.

The surface epithelium of palatine tonsils makes invaginations in the tonsillar parenchyma in the form of 10-30 tubular, branched crypts that can anastomose among themselves, and which enlarge the tonsillar surface up to 300 cm^2 (29). The crypt epithelium, also referred to as lymphoepithelium, is stratified squamous epithelium that, beside epithelial cells, contains T cells, immunoglobulin-expressing B cells, M-cells and dendritic cells (2). This epithelium represents the first site of contact between antigen and palatine tonsil, and has essential role in the initiation of the immune response (30, 31). Subepithelial tissue contains the cells that migrated from

interfollicular regions. Our results show that the number of CD11c immunopositive cells in crypt epithelium and subepithelial tissue is higher in RT compared to CHT. All CD11c-immunopositive cells in crypt epithelium and subepithelially showed dendritic morphology. However, recent studies of M-cells and Payer patches in the intestine, showed that there is a specific population of CCR6+CD11+ B cells found between the epithelium and lymphoid follicles, that is necessary for differentiation and maturation of M cells (32). Further research is needed to establish whether similar B cell population exists in palatine tonsils.

The studies of dendritic cells in human tonsil reported the presence of three subsets of DC: $CD11c^+$ $CD4^+$ $CD3^-$ cDC and $CD11c^-$ $CD4^+$ $CD3^-$ pDC, both localized in the interfollicular region and a subset of $CD11c^+$ $CD4^+$ $CD3^-$ cDC located within the germinal center, evenly distributed in both the dark and light zone, and in close contact with T cells. These CD11c-immunopositive cells had strong antigen-presenting ability and were also able to directly react with B lymphocytes in vitro (20, 24). $CD11c^+$ $CD4^+$ $CD3^-$ cDC exert their function by secreting chemokine ligand 18 (CCL-18) and chemokine ligand 13 (CXCL-13). CCL18 has the role in attracting naïve T cells ($CD45RA^+$), and CXCL13 is essential for homing of lymphocytes into secondary lymphoid organs and for the development of B-cell follicles (33).

Summers et al. identified five subsets of dendritic cells in human palatine tonsil. Three types expressed CD11c on their surface: HLA-DR^{hi} $CD11c^+$, HLA-DR^{mod} $CD11c^+$ $CD13^+$ and HLA-DR^{mod} $CD11c^+$ $CD13^-$, and all these cells were shown to have strong antigen-presenting potential. Two subtypes were Cd11c immunonegative and were lacking the ability of antigen presentation (23).

In conclusion, CD11c-immunopositive cells are present in all morphological compartments of palatine tonsils with RT and CHT. Statistically significant difference in the number of these cells was observed only in crypt epithelium and subepithelial lymphoid tissue. It was proposed that the chronic infections may structurally alter crypt epithelium and contribute to local immunosuppression (31). The higher number of CD11c-immunopositive cells in crypt epithelium in RT might be explained with preserved immunological function in this morphological compartment, compared to CHT. In order to define the role of these cells in RT and CHT, it is necessary to further research their functional maturation, activation and secretion profile.

Acknowledgments

This paper was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, under the project number 175061 and the Faculty of Medicine, University of Niš, Serbia under the project number 38/20.

Declarations of interest

None.

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Originalni rad **UDC: 616.322-002:611.018+576.3**
doi:10.5633/amm.2020.0412

KVANTIFIKACIJA CD11C-IMUNOPOZITIVNIH ĆELIJA U RAZLIČITIM TIPOVIMA HRONIČNOG TONZILITISA

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CD11c je transmembranski protein koji pripada β2 podfamiliji integrina. Smatra se markerom koncencionalnih dendritičnih ćelija, ali se takođe može eksprimirati na makrofagima, neutrofilima i nekim B limfocitima. Cilj rada bio je da se odredi numerička arealna gustina CD11c imunopozitivnih ćelija u različitim morfološkim odeljcima tonzile sa rekurentnim tonzilitisom (RT) i hroničnim hipertrofičnim tonzilitisom (HHT). Materijal su činile tonzile uzete nakon tonzilektomije bolesnika oba pola, starosti od 10-29 godina i to: šest tonszilasa RT i devet tonzilasa HHT. Kvantifikacija CD11c-imunopozitivnih ćelija vršena je na serijskim parafinskim presecima debljine 5 μm, koji su bojeni imunohistohemijski korišćenjem mišijeg monoklonalnog anti-CD11c antitela. Za kvantifikaciju korišćen je program Image J. Naši rezultati pokazuju da su CD11c imunopozitivne ćelije prisutne u svim morfološkim odeljcima tonzilasa RT i HHT. Veća vrednost numeričke arealne gustine CD11c-imunopozitivnih ćelija u RT pokazuje statistički značajnu razliku u odnosuna HHT. Nije pronađena statistički značajna razlika u broju ovih ćelija u limfnim folikulimai interfolikularnim regionima između grupa. Kriptični epiteli su bepitelno limfno tkivo predstavljaju prvo mesto kontakta između antigena i tkiva tonzile, i imaju ključnu ulogu u započinjanju imunološkog odgovora. Veći broj CD11c-imunopozitivnih ćelija u kriptičnom epitelu i subepitelnom limfnom tkivu tonzilasa RT ukazuje na efikasniji imunološki odgovor u ovom morfološkom odeljku, u odnosu na HHT.

Acta Medica Medianae 2020;59(4):xx-xx.

Ključne reči: *rekurentni tonzilitis, hronični hipertrofični tonzilitis, CD11c, dendritične ćelije*