



THE NUMERICAL DENSITY OF IMMUNOGLOBULIN PRODUCING CELLS IN DISEASED PALATINE TONSILS

Verica Avramović¹, Predrag Vlahović², Milan Stanković³

Running title: *Immunoglobulin producing cells in palatine tonsil*

¹ *Institute of Histology and Embryology, Faculty of Medicine, Niš,* ² *Institute of Nephrology and Hemodialysis, Clinical Centre, Niš,* ³ *Clinic of Otorhinolaryngology, Clinical Centre, Niš, Yugoslavia*

Summary. *The number of immunoglobulin (Ig)-producing cells per mm³ of tonsillar tissue, i.e., numerical density and their distribution in tonsils from patients with idiopathic tonsillar hyperplasia (ITH), chronic hyperplastic tonsillitis (CHT) and recurrent tonsillitis (RT) were analysed. The numerical density was determined on serial sections of paraffin embedded tonsillar tissue stained by peroxidase-antiperoxidase (PAP) method. The number of IgA- and IgG-producing cells was significantly decreased in ITH, increased in RT, and unchanged in CHT in comparison with control group. In ITH and CHT numerical density of IgM-producing cells was increased whereas no difference in RT was registered. The obtained results could provide information relevant to the immunological competence of diseased tonsils.*

Key words: *Human palatine tonsil, stereology, immunoglobulin producing cells, tonsillitis*

Introduction

Palatine tonsil is a mucosa associated lymphoid tissue performing regional immune function because of its exposure to both alimentary and airborne pathogens. There have been many studies dealing with the role of the palatine tonsil in the immune system (1 - 4). Some of them are morphometrical reports on local B-cell system associated with tonsillar disease (5, 6), but there is no data focused on stereological analysis of Ig-producing cells in diseased tonsils. Considering that number of Ig-producing cells per mm³ of tonsillar tissue, i.e., numerical density (N_V) could be a possible indicator of the humoral immune defence and immunological competence, the aim of this study was to quantify Ig-producing cells (IgA, IgG and IgM) in diseased tonsils.

Materials and Methods

Palatine tonsils were obtained from the patients who had undergone tonsillectomy for idiopathic tonsillar hyperplasia (ITH)-nine patients aged between 3 and 6 years, chronic hyperplastic tonsillitis (CHT)-teen patients aged between 18 and 22 years and recurrent tonsillitis (RT)-teen patients aged between 10 and 29 years. Tonsils were classified on the basis of clinical

diagnosis according to Surjan et al. (1978) and submitted to histological analysis of paraffin sections stained by haematoxylin and eosin (5). At the time of surgery, all the patients had been clinically free of infection or antibiotic administration. The tonsils from three accidentally passed away healthy persons were used as a control.

Preparation of tissue for microscopy

The blocks of tonsillar tissue were fixed in 10% neutral buffered formalin and prepared for light microscopy using conventional technique. From each tonsil 2 μ m thick serial sections were made at 16 μ m distance. The 1st, 10th and 19th sections were used to determine N_V of IgA-producing cells; the 2nd, 11th and 20th sections were used to determine N_V of IgG-producing cells; the 3rd, 12th and 21st sections were used to determine N_V of IgM-producing cells. For immunohistochemical detection of Ig-producing cells peroxidase-antiperoxidase (PAP) method (7) was performed. Cytoplasmic IgA, IgG and IgM in Ig-producing cells were demonstrated using specific rabbit antihuman IgA, IgG and IgM monoclonal antibodies (DAKO, Denmark) diluted in PBS 1:2000, 1:1500 and 1:1500, respectively. Appropriate positive controls were performed.

Stereological analysis of Ig-producing cells

Numerical density of Ig-producing cells was determined by the point-counting method using an objective x 63, lattice M 42 (8) and calculated from the following equation (9):

$$N_v = \frac{N_A}{(GO + \bar{D})}$$

where N_A = number of I_g -producing cell per mm^2 of section area; GO = objective depth of focus; \bar{D} = the mean I_g -producing cells diameter.

$$GO = \frac{\lambda}{(n \cdot NA^2)}$$

where λ = wavelength (550 nm), n = breaking up coefficient (for air = 1), NA = objective numerical aperture.

For the 95% confidence limits and relative standard error under 0.1, 200 fields for each class of Ig-producing cells on three sections from each tonsil were inspected. Systematic field sampling was performed in referent space consisted of crypt epithelium, lymphoid follicles and extrafollicular areas. The real Ig-producing cells diameter (D) was calculated from the equation (9):

$$D = \frac{4}{\pi} \times \bar{D}$$

where \bar{D} = mean diameter of Ig-producing cells determined on 100 Ig-producing cells; $\pi = 3.14$.

Statistical significance was estimated using Student's t-test for unpaired values.

Results

The distribution of Ig-producing cells was similar in all studied groups. IgA-producing cells were predominantly associated with the crypt epithelium and subepithelial region (Fig. 1) and variable number was found in extrafollicular areas. IgG-producing cells were numerous beneath the crypt epithelium and in crypt epithelium (Fig. 2), in germinal centres of the lymphoid follicles, as well as in extrafollicular areas. However, predominant localisation of IgM-producing cells was in lymphoid follicles and very rarely in other tonsillar morphological compartments (Fig. 3). The mean diameter of Ig-producing cells- \bar{D} was $7.82 \mu m$, while the real diameter- D was $9.97 \mu m$.

Numerical density of IgA and IgG -producing cells was significantly decreased in ITH ($p < 0.05$) and increased in RT ($p < 0.05$) compared to the control group (Table 1). There was no significant difference in numerical density between CHT and control.

The numerical density of IgM -producing cells in ITH and CHT was significantly increased ($p < 0.05$), while there was no difference between control and RT (Table 1).

Table 1. Numerical density of Ig-producing cells per mm^3 of tonsillar tissue.

| Group | Ig A | Ig G | Ig M |
|---------|---------------------------|---------------------------|-------------------------|
| Control | 23072 ± 2870 | 46462 ± 3930 | 2045 ± 273 |
| ITH | 5455 ± 365 ^a | 18550 ± 933 ^a | 2661 ± 215 ^a |
| CHT | 20670 ± 1257 | 44532 ± 2225 | 4234 ± 262 ^a |
| RT | 43248 ± 2027 ^a | 66056 ± 2559 ^a | 1970 ± 140 |

a - $p < 0.05$ vs. control. Data are given as means ± SEM

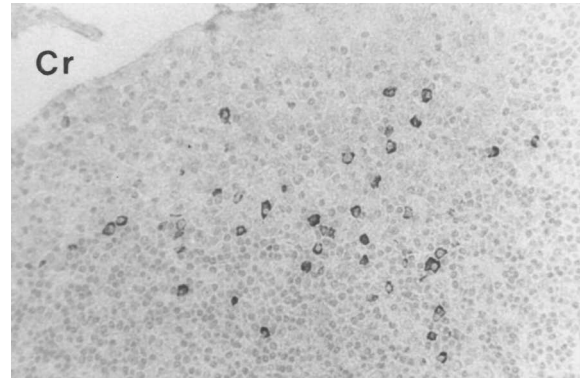


Fig.1. Tonsillar crypt (Cr) in control tonsil: IgA-producing cells are present in crypt epithelium and beneath that. PAP. x 200.

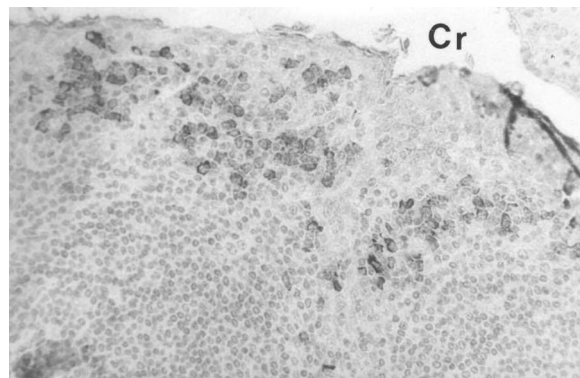


Fig. 2. A lymphoid tissue in tonsil with RT: Numerous IgG-producing cells are seen. PAP. x 200.

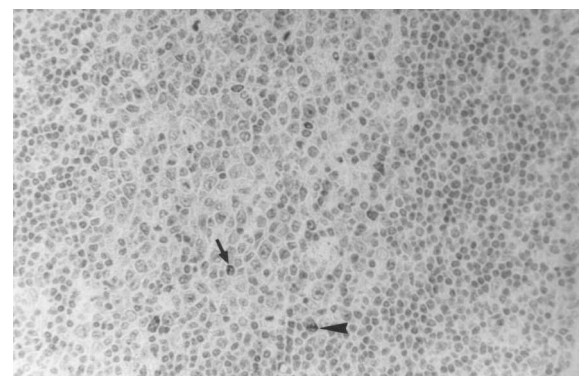


Fig. 3. Part of a lymphoid follicle in tonsil with CHT: IgM-producing cells are seen in germinal centre (arrow) and mantle zone (arrowhead). PAP. x 400.

Discussion

Palatine tonsil is a B- cell dominant lymphoid organ with important role in specific immunity (1). Ig-producing cells appear to be the result of B-cell activation, and differentiation, and they are also indicator of tonsillar tissue immunoreactivity. In this study the number of Ig-producing cells in diseased palatine tonsils was determined and compared with the healthy control tonsils. Considering that previous studies determined only the number of Ig-producing cells per area unit (1, 5, 6), we proposed that the number of cells per volume unit of tissue is more appropriate method for precise determination of Ig-producing cells number.

Surjan et al. (1978) found a reduced number of Ig-producing cells in all morphological compartments of diseased tonsils (5). Based on the quantification of Ig-producing cells, Korsrud and Brandtzaeg (1980) also reported alteration of tonsillar B-cell system in chronic tonsillitis and tonsillar hyperplasia (6). In contrast, Agren et al. (1996) confirmed that the number of cytoplasmic Ig-positive cells was not different between recurrent tonsillitis and tonsillar hyperplasia in childhood (10). In this study increased numerical density of IgA- and IgG-producing cells in RT was observed, while there was no difference concerning IgM-producing cells. This discrepancy could be explained by the variable morphological changes in analysed tonsils, especially with regard to the degree of

fibrosis as a consequence of chronic inflammation. Anyway, the results observed for RT group suggest that, although diseased, tonsils could be useful in humoral immunity.

Decreasing numerical density of IgA- and IgG-producing cells in ITH is consistent with previous findings (1, 5) and could be explained by the absence of signals important for differentiation and maturation of cells in germinal centre. At the same time, the reduced number of Ig-producing cells in ITH could be the result of apoptosis in germinal centre, because Rajewsky (1996) demonstrated that only cells expressing high-affinity antibodies can be selected and survive (11). Moreover, Bernstein (1993) documented the defective IL-2 production in response to mitogens and specific antigens in diseased tonsils, what may be considered as a cause of insufficient immunity in ITH (12). The increased numerical density of IgM-producing cells in ITH, is understandable. Namely, in tonsils with ITH, hyperplastic germinal centres are the sites of the earliest appearance of IgM-positive cells after B-cell activation. We consider that the age of patients may also influence the differences observed between ITH and RT groups.

In conclusion, the present results indicate the similarities in distribution and the difference in number of Ig-producing cells in tonsillar tissue from all studied groups, preferentially, in tonsils from ITH and RT. Moreover, they could be valuable in diagnostic procedure, as well as for indications for tonsillectomy.

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NUMERIČKA GUSTINA IMUNOGLOBULIN PRODUKUJUĆIH ĆELIJA U OBOLELIM PALATINALNIM TONZILAMA

Verica Avramović¹, Predrag Vlahović², Milan Stanković³

¹ Institut za histologiju i embriologiju, Medicinskog fakulteta, Univerzitet u Nišu

² Institut za nefrologiju i hemodijalizu, Kliničkog centra u Nišu

³ Klinika za uvo, grlo i nos, Kliničkog centra u Nišu, Jugoslavija

Kratak sadržaj: Analizirani su broj imunoglobulin (Ig)-produkujućih ćelija po mm³ tonzilarnog tkiva - numerička gustina i njihova distribucija u tonzilama pacijenata sa idiopatskom tonzilarnom hiperplazijom (ITH), hroničnim hiperplastičnim tonzilitisom (CHT) i rekurentnim tonzilitisom (RT). Numerička gustina je određivana na serijskim isečcima tkiva tonzile koje je ukalupljeno u parafin i bojeno peroksidaza-antiperoksidaza (PAP) metodom. Broj IgA- i IgG-produkujućih ćelija je bio značajno snižen u ITH, povećan u RT, i nepromenjen u CHT u poređenju sa kontrolnom grupom. U ITH i CHT numerička gustina IgM-produkujućih ćelija je bila povećana, dok nije uočena razlika u RT. Dobijeni rezultati mogu predstavljati informaciju koja je relevantna za imunološku kompetenciju obolelih tonzila.

Ključne reči: Tonzila palatina čoveka, stereologija, imunoglobulin produkujuće ćelije, tonzilitis

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