

ORIGINAL PAPER

Chronic tonsillitis: histological and immunohistochemical aspects

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Abstract

Objectives: Chronic tonsillitis represents the most common inflammatory lesions of the pharynx determining numerous local or distant evolutive complications. We decided to study the histological and especially immunohistochemical expression of this pathology. **Material and methods:** We have studied 112 surgical samples representing tonsils resected from 56 patients with chronic tonsillitis. The tonsillectomies were performed in the ENT Clinic of the Emergency County Hospital of Craiova, between 01.01.2007–31.12.2007. The processed histological samples were stained using Hematoxylin–Eosin, light green trichromic and argental impregnation. For the immunohistochemical study, we used LSAB method with CD20, CD45 RO, CD68 antibodies in order to reveal and differentiate T- and B-lymphocytes and also the macrophages. **Results:** In all samples, we found hyperplasia and hypertrophy of the lymphoid follicle with excessive developing of the clear germinal center as a normal reaction to antigens presence. In some cases, we remarked micro hemorrhages and hematic extravasations inside the follicles, probably due to the excessive virulence of the pathogens causing endothelial lesions. The conjunctive stroma was enriched in collagen fibers, in some cases organized in strong fascicles with an obvious tendency to divide the tonsils in lobules. The young fibroblastic type cells were numerous. The specific reticulin fibers had a low representation being disorganized. The immunohistochemical study proved that the clear center of the lymphoid follicles was occupied by B-lymphocytes, but the T-lymphocytes were present in the cortical region of the follicles, perifollicles and in the surface epithelium. **Conclusions:** In some pathological cases, the predominant cellular population of the clear center was formed by T-lymphocytes.

Keywords: chronic tonsillitis, immunohistochemistry, tonsil's hypertrophy.

✉ Introduction

Palatine tonsil belongs to the Waldeyer lymphatic ring, lymphoid structure placed at the antigens main protruding “gate” into the body, having an extremely remarkable role in the antimicrobial defense of the body [1]. The palatine tonsils may be the center of some acute or chronic inflammations. Under these circumstances, they became hypertrophied and red or white-grey false membranes may cover them [2]. Tonsillitis is frequently recurrent and rebel to antibiotherapy [3]. According to the oropharynx obstructive syndrome, five amygdalian hypertrophy degrees were described, such as:

- 0 degree – intraventric tonsils;
- Ist degree – slightly hypertrophied tonsils, 25% reducing the air flow;
- IInd degree – medial amygdalian hypertrophy, 25–50% reducing the airflow;
- IIIrd degree – increased amygdalian hypertrophy, 50–75% reducing the airflow;
- IVth degree – huge amygdalian hypertrophy, achieving more than 75% airflow reduction [4].

Chronic tonsillitis represent the most frequent lesions within pharynx inflammatory pathology with multiple complications both local-regional

(acute median otitis, catarrhal otitis, fibro-adhesive otitis, suppurative otitis with hypoacusis, chronic mucopurulent rhinitis, sinusitis, ocular and lachrymal pathways infections, descending respiratory infections) and at the distance (glomerulonephritis, joint rheumatism, endocarditis, enteritis, appendicitis, persistent albuminuria, etc.) [2, 5–7]. Chronic tonsillitis can be also the location of some specific infections such as tuberculosis and syphilitic but also malignant lesions [6–7].

Anatomopathologically, chronic tonsillitis can be described like this: focal tonsillitis, hypertrophic or scleroatrophic caseous cryptic tonsillitis as recurrent forms, and simple hypertrophic tonsillitis soft form in children and hard form in adults [6].

Starting from this dates in the present study we suggested to investigate some histological and immunohistochemical changes of the lymphoid tissue and amygdalian stroma in patients diagnosed with chronic amygdalitis who needed amygdalectomy.

✉ Material and methods

Hundred-twelve palatine tonsils resected from 56 patients aged between three and 40-year-old after surgical interventions performed in the ENT Clinic of the Emergency County Hospital of Craiova, during

01.01.2007–31.12.2007 was available for our study. Tonsillectomy and tonsilotomy was imposed in those patients as a therapeutic necessity. The biologic material was then fixed in neutral formalin solution (pH 7.4) and wax embedded; seriate sections were then performed for microscopic study. We used Hematoxylin–Eosin stains, Gömöri–silver impregnation and light green trichromic (Goldner–Szeckely technique) to reveal histological changes. For the immunohistochemical study, in the present study we used LSAB technique with CD20 (Monoclonal Mouse Anti-Human CD20cy clone L26, DAKO) and CD45 RO (Monoclonal Mouse Anti-Human CD45, clones 2B11 + PD7/26 DAKO) in order to reveal B- and T-lymphocytes and CD68 (Monoclonal Mouse Anti-Human CD68, clone PG-M1 DAKO) for macrophages revealing.

✚ Results

Immunohistochemical, histological and clinical studies revealed multiple changes of the tonsil structures, both of the lymphoid and stoma tissue levels. Therefore, in children aged between 3 and 10, it was clinically revealed the predominance of a simple soft amygdalian hypertrophy type tonsillitis where the tonsil was increased, pale, soft, friable, depressible when touched; microscopically it was revealed the rich lymphoid tissue with intense follicular hypertrophy, with excessive increase of the germinative clear center and the decrease of the follicular cortical, but the covering epithelium, capsule and interlobular septae had about normal structure (Figure 1).

In most of the hard chronic tonsillitis, the hypertrophic type appeared in adult and elder child, the tonsils were hypertrophied, congested, with diminished mobility in the amygdalian space with obvious crypts eliminating spontaneous caseum, but also when they were pressed by spatula on the anterior pillar. Microscopically, in those cases the lymphoid follicles appeared as hyperplastic and hypertrophic with excessive development of the germinative clear center as a response reaction to the presence of some antigens.

The histological study using high magnification allowed us to highlight the presence of numerous typical or atypical mitosis of the cells in the clear germinative center of the follicles (Figure 2).

In other cases we observed the presence of some microhemorrhages or subepithelial or in the structure of the lymphoid follicles and interfollicular hematic extravasates; this aspect can be given by the excessive virulence of some pathogenic agents determining capillary wall lesions; intima thickening and vascular thrombosis lesions. Conjunctive stroma appeared enriched of collagen fibers, here and there organised in thick fascicles having the tendency of dividing the tonsil in lobules with many fibroblastic type young cells with reactional aspect (Figure 3). Reticulin fibers appeared more reduced quantitatively and disorganised as a consequence of the fact that the follicular hypertrophic processes developed faster than the changes of the specific stroma (Figure 4). Amygdalian crypts appeared much elongated even sinuous with many cell

reminiscences, many lymphocytes and even pyocytes in lumen (Figure 5).

In focal scleroatrophic tonsillitis; palatine tonsils were of reduced sizes, intravelic, almost firmly into the amygdalian space; they were a little unequally congested, having a pale mucosa here and there with hardly visible crypts, expressing a small quantity of a latescent or caseous liquid when pressed on the anterior pillar. Microscopically the following were remarked: surface epithelium erosions especially at the crypts level, unequal hyperplasia of the lymphatic tissue; capsular and pericapsular cell tissue lesions with scleroses not regulated thickening of the interlobular septae and subepithelial abundant collagenous fibrosis. Most often induced by the repeated inflammatory accesses and different microbial associations between aerobic and anaerobic germs, but especially by the A group hemolytic beta streptococcus, scleroatrophic focal amygdalitis by pathogen agents retention and by accumulation of local cell degrading products, by crypts orifices blockages constitute a chronic infectious hotbed responsible for the most of the local and distant complications. Immunohistochemical reactions using CD20 antibodies revealed that the clear center of the lymphoid follicles was preponderantly occupied by the B-lymphocytes (Figure 6). The B-lymphocytes increased density showed the intensification of the cell differentiation and proliferation processes taking part into the tonsil lymphoid follicles as a consequence of the antigens presence at the Waldeyer lymphatic circle level. A minute analyze of the microscopic images revealed the presence of some gaps of reaction as a proof that other cells also existed into the germinative clear centre which did not give positive reaction to CD20, there for they did not belong to the B-lymphocytes lymphoblasts categories.

Unlike the B-lymphocytes, the T-lymphocytes, specially revealed by the immunohistochemical reaction to the CD45 antibody, appeared preponderantly disposed at the lymphoid follicles periphery, into the follicular cortical, but also into inter- and perifollicular lymphocitary infiltrate (Figure 7).

Immunostaining with CD68 revealed the presence of macrophages. In the cases studied by us we could established the existence of an increased number of CD68 positive cells of great sizes, which were present relatively homogenous at the germinative clear center level (Figures 8 and 9). The existence of macrophages at that level pointed that following the lymphoblastic proliferation intense processes, abnormal B-lymphocytes could appear being recognized and taken away by macrophages. An intense positive reaction to CD68 was also observed in the superficial tonsil chorion; immediately under the basal membrane of the covering epithelium and even in the structure of the epithelium (Figure 10). The macrophages presence at that level is ordered by the existence of the pathogenic germs from the tonsil surface and even from the superficial chorion.

CD68 positive cells were more numerous into the amygdalian crypts epithelium, probably due to the microorganisms and cell detritions accumulation and remainings (Figure 11).

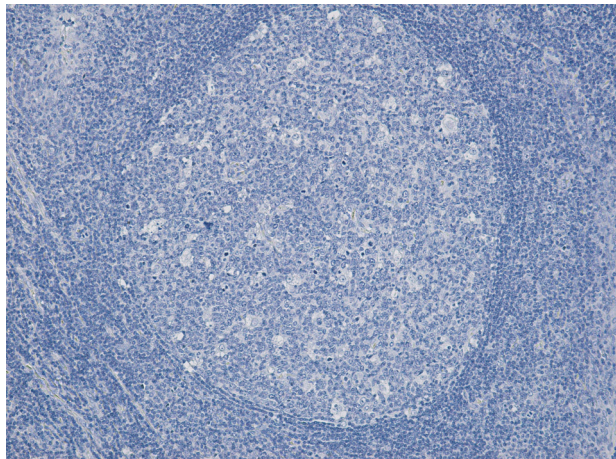


Figure 1 – Tonsillar follicle showing hypertrophied germinal center
(HE stain, $\times 100$)

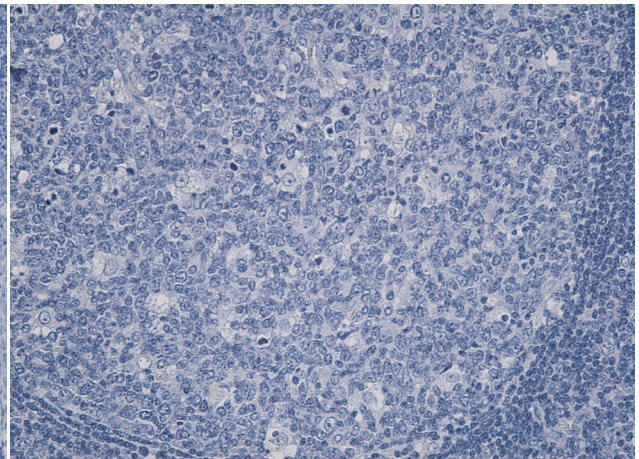


Figure 2 – Detail of the previous image. We remarked the presence of numerous mitosis and cells with foamy cytoplasm, suggesting macrophages in the clear germinal center of the follicle
(HE stain, $\times 100$)

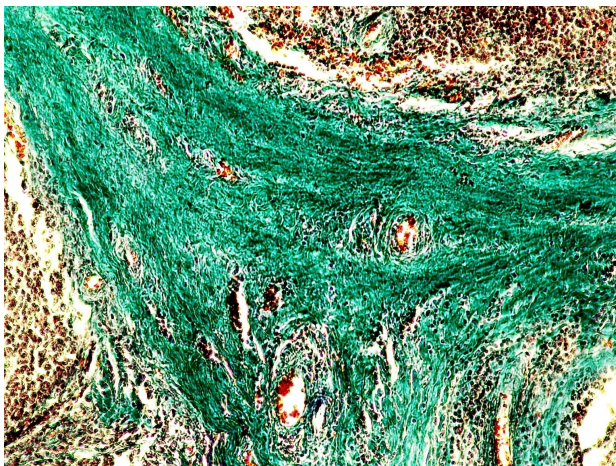


Figure 3 – Collagen fibbers organized as thick fascicles with a subcapsular disposition
(GS stain, $\times 100$)

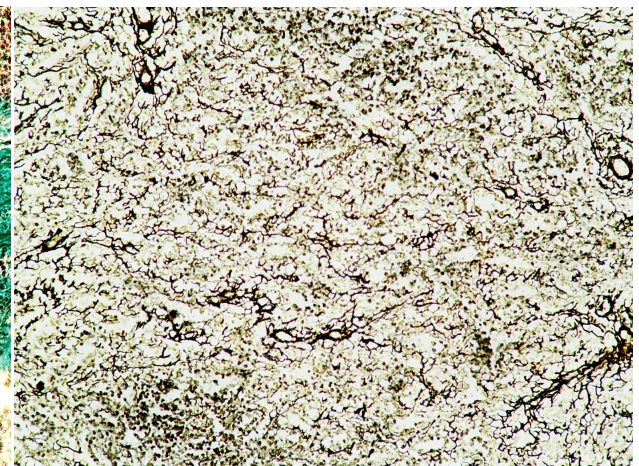


Figure 4 – Disorganized reticulin fibbers in the germinal center (Gömöri-silver impregnation, $\times 100$)

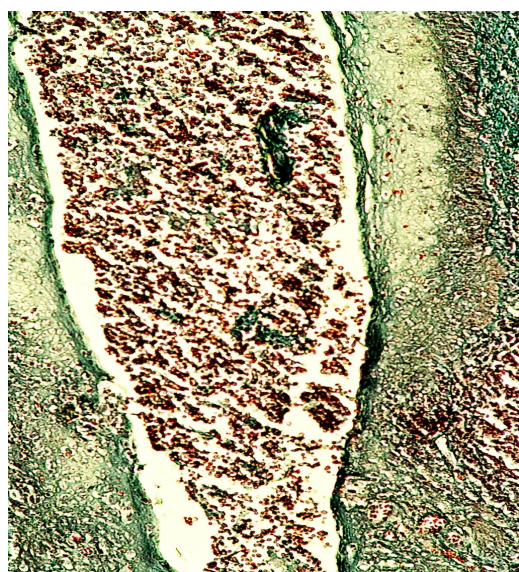


Figure 5 – Tonsillar crypt with dead cells remainings and lymphocytes in the lumen. Trichromic Green Sunlight (Goldner-Szeckely technique) stain, $\times 100$

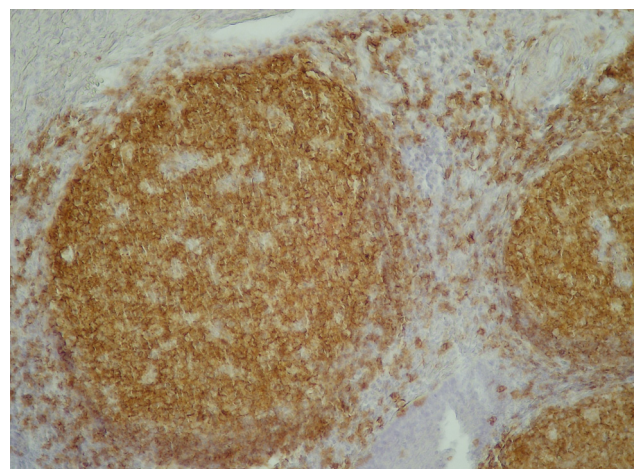


Figure 6 – B-lymphocytes preponderantly disposed in the follicular center. Immunohistochemical stain for CD20, $\times 100$

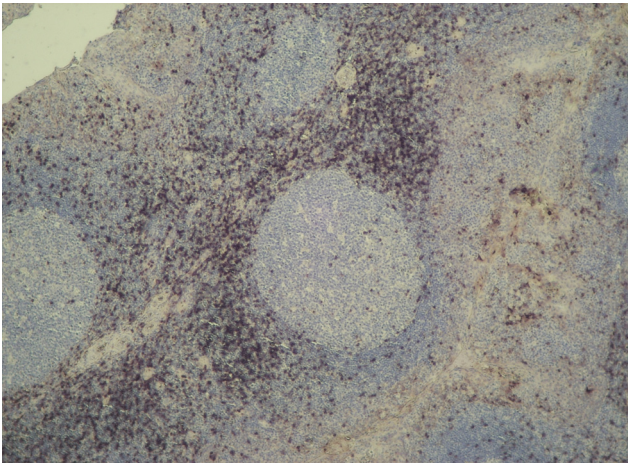


Figure 7 – *T-lymphocytes predominating in the cortical area of the follicle and interfollicular. Immunohistochemical stain for CD45 RO, $\times 40$*

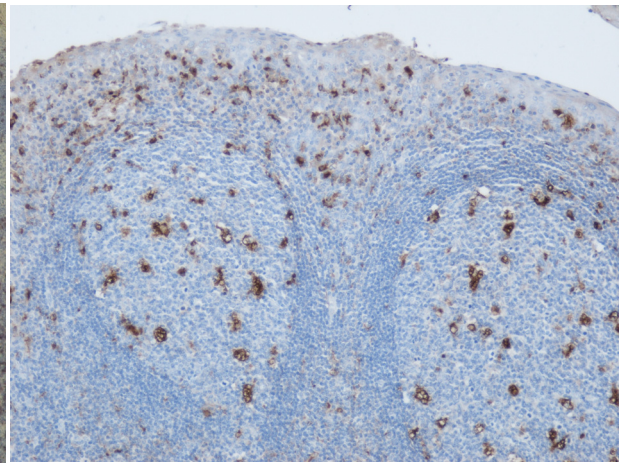


Figure 8 – *Numerous CD68-positive cells (macrophages) present in the clear germinative center and at the subepithelial level. Immunohistochemical stain for CD68, $\times 100$*

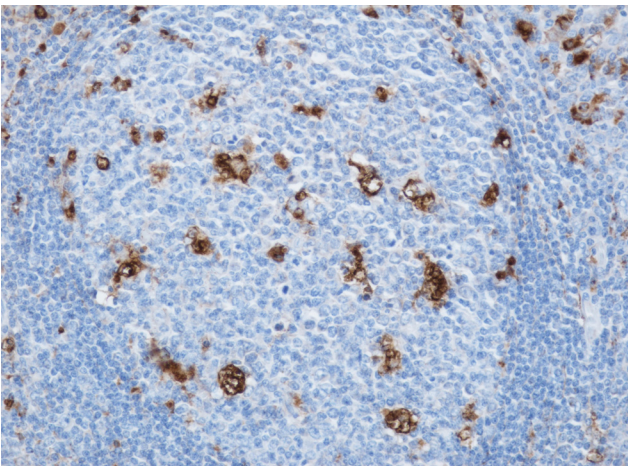


Figure 9 – *Detail of the previous image. Numerous macrophage cells in the clear germinative center. Immunohistochemical stain for CD68, $\times 200$*

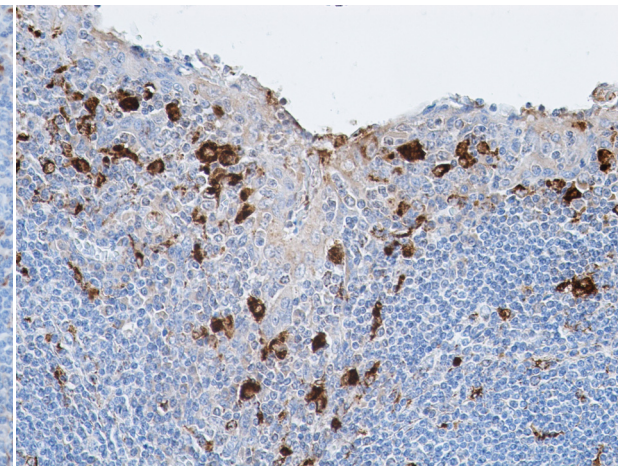


Figure 9 – *Subepithelial and intraepithelial disposed macrophages. Immunohistochemical stain for CD68, $\times 200$*

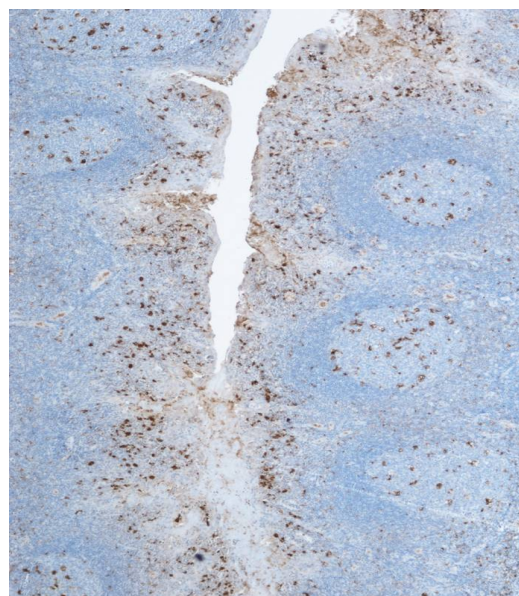


Figure 8 – *The disposition of the macrophages at the tonsillar crypt level. Immunohistochemical stain for CD68, $\times 40$*

Discussion

Palatine tonsil, just like the entire Waldeyer lymphatic circle, contains an increased immunologic reactivity tissue, thus building a barrier against the pathogenic agents' penetration into the respiratory and digestive tracts [9]. They take part intensely both in the humoral immunity by synthesis and secretion of a great quantity of immunoglobulins, which neutralize a part of the oropharynx cavity flora and in the cell immunity by T-lymphocytes penetrating the epithelial barrier [1, 10].

Chronic inflammatory pathology of tonsils most often affects children at the first decade of age, but also adults, probably due to a local dysfunction of the epithelial structures [11]. The histologically complex structure of the amygdalian parenchyma is essential for taking over and presenting the immunocompetent cell antigen from the subepithelial level [12]. That allows the entire organ to function unitarily and to play an important part concerning the antimicrobial defense. That is why the persistence of the local inflammatory reactions in the chronic adenotonsillitis, in the end, leads to histo-morphological changes and immunologic deficiencies at the level of that antimicrobial defense barrier [11, 13].

The microscopic study we had performed allowed us to observe that amygdalian chronic inflammation in children determined the amygdalian follicles hyperplasia and hypertrophy, while in adults, chronic inflammation led to the appearance of a fibrous type process originating fascicles of collagen fibers which seemed to septate the tonsilary parenchyma.

Some authors [14] established an increased number of amygdalian lymphoid follicles, in direct relation to antigenic stimulation, during the amygdalian hypertrophy. In Nave's opinion [15] the amygdalian hypertrophy was direct proportional to the numbers of lymphocytes and the immunitary activity developed after constant antigenic stimulation, in amygdalian recurrent diseases. Just like many other authors [11] we also observed that follicular hyperplasia is generated by the proliferation of B-lymphocytes which occupied the most part of the germinative clear center, with significant reduction of the intrafollicular T-cells compartment. T-lymphocytes were identified in a greater quantity both in the follicular cortical and interfollicular, too [16]. The presence of numerous mitosis at the level of the clear center, associated to follicular hypertrophy and hyperplasia pointed the immunitary process intensification stimulated by antigens. Some authors showed that cell proliferation and cell death by apoptosis were preponderantly produced of the level of the reactive lymphoid follicles and in a small part, at the level of the interfollicular zone and at the surface epithelium level.

Kučera T *et al.* [17] showed that in the case of the amygdalian chronic inflammation, cells bearing proliferation and cell death were mostly B-lymphocytes during their maturation, before clonal selection.

Macrophages in the amygdalian parenchyma appeared as distributed in two different areas: along the clear germinative center and at the level of the covering

epithelium or immediately subepithelial. We think that, into the clear follicular center, macrophages played the role of fagocytating the cell remainings obtained by B-lymphoblasts or B-lymphocytes division recognized as non-self particles, while those localized at the level of the surface epithelium or immediately subepithelial levels function as antigen presenting cells [15].

On our preparations, at the level of tonsils covering epithelium, we observed relatively frequent erosions of it, leading to a direct contact of the amygdalian parenchyma to the saprophyte or pathogenic flora present in the pharynx. We considered that the erosions of the surface epithelium were determined by the aggressiveness of the pathogenic agents or by the decrease of the local defense capacity of the covering epithelium.

In Nave's opinion [15], amygdalian cryptic epithelium also named lympho-epithelium due to the increased number of lymphocytes contained into it, played a significant part into the immune response initiation at the amygdalian level as, the luminal antigens from the crypts were taken over and presented to some specialized cells localized at the amygdalian epithelium level. Other authors [18] considered that bacteria settled at the level of the tonsilary crypts level were resistant to the antibiotics and specific humoral immunity, which would explain the frequent recurrence. The presence of the bacterial biofilms in crypts, in tonsilary inflammations, explains why some tonsillitis forms get chronic and recurrent [18].

Passali D *et al.* [11], by using electronic microscopy techniques, observed discontinuities just like fissure, at the level of the amygdalian epithelium, especially at the level of the tonsilary crypts. Surface epithelium hurting enables gates for the pathogenic agents to enter which explains the recurrence of the amygdalian inflammatory processes, turning the tonsils into hotbed diseases, thus forming a vicious chain that can be solved only by the tonsillectomy [10, 19].

Hofman P (2003) [20] showed that the close junctions of the surface epithelium cells were destroyed both by microbial toxins and by the direct action of pathogenic agents joining to the epithelial cells surface. Rescigno M *et al.* (2001) [20] discovered a trajectory through which certain pathogenic microorganisms could invade the epithelial barrier, passing through the epithelial cells. There for, they demonstrated that antigens penetrate through the pathway of the dendritic cells sending prolongations through the covering epithelium cells up to the tonsils surface. We have to mention that the greatest amygdalian immunologic activity was established to belong to the first decade of age (3–10 years) to the age of 60: B- and T-lymphocytes numbers decreased in all the tonsilary compartments. The number of the follicular and interfollicular amygdalian dendritic cells was reduced with age and it explained the tonsilary involution [15].

Amygdalian inflammatory processes led not only to the proliferation and to activation of the immune system cells, but also the fibroblastic type conjunctive cells, which by producing collagen succeeded to replace the immunologic active tissue with fibrous tissue and thus the appearance of clinical form of sclero-atrophic

tonsillitis. We observed that fibroblastic proliferation was more intense in the deep part of the tonsils, into the subcapsular area, on our preparations. The presence of the greater number of monocyto-macrophagic type cells at the intra- and subepithelial cryptic level revealed the presence of an increased quantity of antigens at that level.

✉ Conclusions

As concerning chronic tonsillitis, after repeated antigenic stimulation, a lymphoid follicles hypertrophy and a hyperplasia took place, proportionally to the amygdalian hypertrophic degree. Those processes are joint by the excessive increase of the germinative follicular clear center, process that was explained by the B-lymphocytes excessive proliferation and follicular cortical reduction. T-lymphocytes appeared as preponderantly disposed into the follicular cortical but also interfollicular. Macrophages were identified both into the germinative clear center and in the intra- and subepithelial zone. As regarding the sclero-atrophic chronic tonsillitis, besides the immunitary system cells proliferation, we also observed an intense proliferation of the fibroblastic type conjunctive cells, with fibrillary collagen increased quantity especially into the peripheral subcapsular area. Our study results explain the mechanisms responsible for the chronic inflammation in some cases, of its persistence and recurrence in other cases and of the way, the immune response unfurls to the persistent antigenic stimulation.

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