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Ultrastructure of the human palatine tonsil and its functional significance

MARKO JOVIĆ[1)], VERICA AVRAMOVIĆ[1)], PREDRAG VLAHOVIĆ[2)], VOJIN SAVIĆ[3)], ALEKSANDRA VELIČKOV[1)], VLADIMIR PETROVIĆ[1)]

[1)]*Institute of Histology and Embryology, Faculty of Medicine, University of Niš, Serbia*
[2)]*Department of Immunology, Center for Medical Biochemistry, Clinical Center, Niš, Serbia*
[3)]*Institute of Biomedical Research, Faculty of Medicine, University of Niš, Serbia*

Abstract

The human palatine tonsils represent a mucosa-associated lymphoid tissue with a significant function in mucosal protection against alimentary and airborne pathogens. The ultrastructure of different morphological compartments in the human palatine tonsil was studied in eighteen tonsils obtained from the patients who had undergone elective tonsillectomy due to chronic tonsillitis. The tonsillar specimens were analyzed by scanning and transmission electron microscopy. The results showed the presence of tight junctions between superficial epithelial cells of the oropharyngeal tonsillar surface. The crypt epithelium is a sponge-like structure infiltrated by non-epithelial cells, mostly lymphocytes, and is characterized by the presence of small pores – microcrypts occupied by large microvillus cells and/or lymphocytes. Antigen-presenting Langerhans cells with typical intracytoplasmic Birbeck granules were also found in the crypt epithelium. The lymphoid follicles are composed of lymphocytes and two types of non-lymphoid follicular cells: small fibroblast-like cells and large cells, morphologically consistent with antigen-bearing follicular dendritic cells or macrophages. The interfollicular areas consisted of a dense network of reticular cells and reticular fibers; many lymphocytes were interspersed between the reticular fibers. In addition to arterioles and high endothelial venules in the interfollicular lymphoid tissue, some fenestrated capillaries were seen intraepithelially and subepithelially. The complex ultrastructure of the human palatine tonsil provides a microenvironment necessary for antigen uptake, antigen processing and immune response.

Keywords: human palatine tonsil, crypt epithelium, reticular cells, blood vessels, electron microscopy.

Introduction

The human palatine tonsils represent a mucosa-associated lymphoid tissue located in the oropharynx, with a significant role in mucosal protection against alimentary and airborne pathogens. For that reason, there have been many studies dealing with the role of the palatine tonsil in the immune defense system [1–6]. Morphological compartments of the human palatine tonsil, *i.e.*, crypt epithelium, lymphoid follicles and interfollicular lymphoid tissue with high endothelial venules (HEVs) are specialized for the uptake of exogenous antigens, production of antibodies and lymphocyte passage, respectively.

Functional significance of the crypt epithelium structure was investigated primarily in the context of lymphoepithelial symbiosis, *i.e.*, cooperation between epithelial M-cells and T-cells localized in a very special intraepithelial microenvironment, as well as in subepithelial areas [7–10]. Maeda & Mogi [8] have described the microcrypts Type I, Type II and Type III in the crypt epithelium and have reported that these microcrypts serve as an entrance for exogenous agents and for the passage of "wandering" cells. The specificity of the crypt epithelium refers also to the reticular structure and keratinization followed by the expression of different cytokeratins during lymphocyte infiltration [11, 12].

Numerous immunohistochemical studies have reported that tonsillar lymphoid follicles possess a germinal centre with follicular dendritic cells, T-helper cells and proliferating B-cells [13–15]. Therefore, the role of apoptosis in clonal selection and differentiation of follicular germinal centre B-cells were demonstrated [16, 17].

The tonsillar microvasculature has been previously described histochemically and immunohistochemically [18–20], however, without any detailed use of scanning and transmission electron microscopy. It is known that blood vessels play an important role in the immunological function of the palatine tonsil, particularly those known as HEVs, through which lymphocytes migrate between endothelial cells and enrich lymphoid compartments.

The aim of the present study was to investigate the ultrastructure of the human palatine tonsil under a scanning and transmission electron microscope in the context of its ascribed immunological function.

Materials and Methods

Palatine tonsils were obtained from the ENT Clinic of the Clinical Center Niš, Serbia and were taken from 18 patients aged 19 to 26 years, who had undergone elective tonsillectomy due to chronic tonsillitis. Prior to tonsillectomy, a signed written consent was obtained from all the patients as well the approval of the Ethical Committee of Clinical Center Niš (No. 9060/14).

After tonsillectomy, a part of each tonsil was routinely

processed to paraffin blocks and 3–5 μm thick sections were stained by HE (Hematoxylin and Eosin) method for histopathological analysis. The rest of the tonsillar tissues were immediately rinsed in cold saline, and tissue samples about 3×3×3 mm (containing crypt epithelium and lymphoid tissue), were taken under a dissecting microscope. Tissue samples were fixed in the Zamboni's fixative [21] at 4^0C for 24 hours and transferred to 0.1 M cacodylate buffer (pH 7.2) at 4^0C for further 12 hours. The samples were post-fixed in 1% osmium tetroxide for 90 minutes, placed in phosphate buffer saline for 30 minutes, and dehydrated in increasing concentrations (30–100%) of ethanol. The samples were then placed in the ascending grades of acetone diluted in ethanol and in ascending grades of amyl acetate diluted in acetone (50–100%). Then, they were dried at a critical point, sputter coated with gold and examined under a JEOL JSM-5300 (Japan) scanning electron microscope (SEM). For the transmission electron microscopy (TEM), the tissue samples were embedded in EPON 812; ultrathin sections were stained with uranyl acetate and lead citrate, and were analyzed under a JEOL JEM-T7 transmission electron microscope (Japan).

Results

Tonsillar surface epithelium

The oropharyngeal surface of the palatine tonsil is covered with stratified squamous non-keratinized epithelium, whose superficial cells are polygonal in shape and possess microridges on its apical membrane. These cells are connected by tight junctions providing the continuity of the epithelial surface (Figure 1).

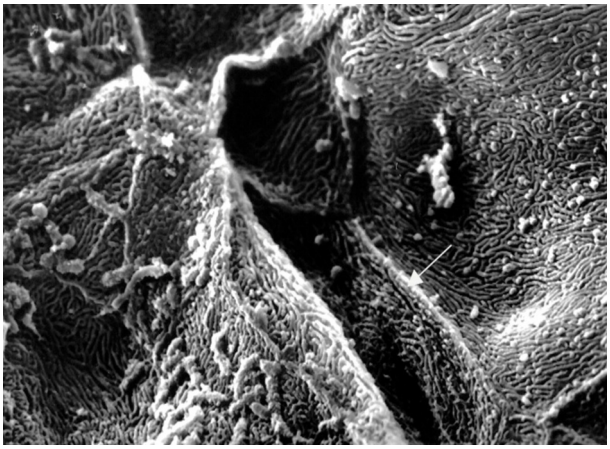

Figure 1 – ***Electron micrograph shows oropharyngeal surface of the human palatine tonsil. Superficial polygonal squamous cells of tonsillar surface epithelium display cell–cell tight junctions (arrow) and microridges on apical cell surface; cell detritus and bacteria are also present. SEM, ×3500.***

Tonsillar crypt epithelium

The surface cell layer of crypt epithelium consisted of polygonal squamous cells with numerous microridges on the apical surface; small pores – microcrypts were situated between these cells (Figure 2a). Microcrypts varied in size (between 5 μm and 25 μm), shape and content. The most common microcrypts Type III were 15 μm in diameter and were occupied by large (15 μm) oval cells with numerous microvilli on their free surfaces (Figure 2, a and b).

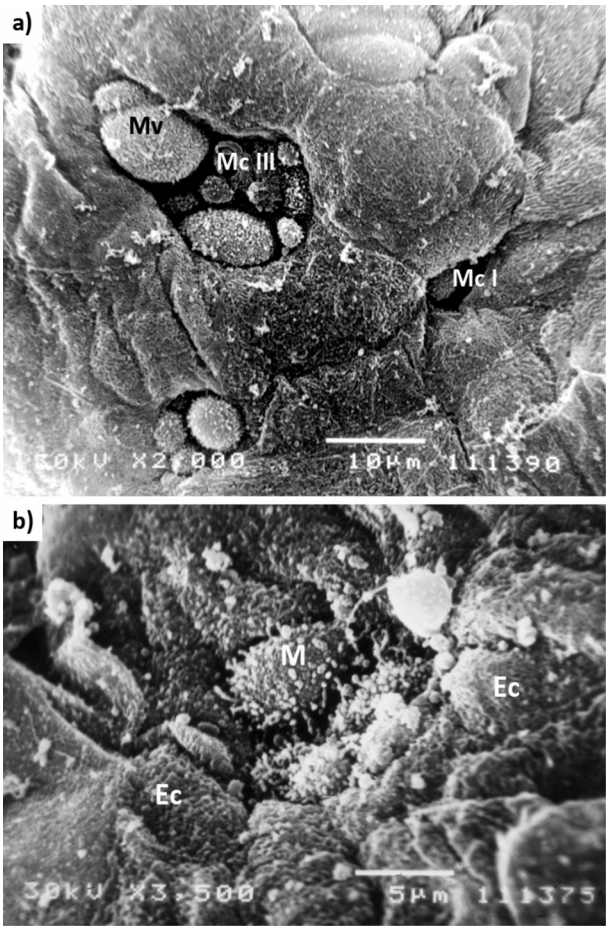

Figure 2 – ***Scanning electron micrographs of a tonsillar crypt epithelium surface: (a) Small pores-microcrypts (Mc I and Mc III) are between the superficial epithelial cells and contain large oval microvillous cells (Mv) and lymphocytes. (b) Superficial epithelial cells (Ec) border a microcrypt in which were noticed large oval microvillous cell (M) and several smaller microvillous cells (probably lymphocytes).***

The SEM analysis demonstrated a reticular structure of the crypt epithelium. Specifically, epithelial cells with their slender cytoplasmic processes formed a network with intercellular spaces filled with non-epithelial cells, mostly lymphocytes (Figure 3a). Transmission electron microscope analysis showed that the crypt epithelial cells were connected by numerous desmosomes (Figure 3b).

Fenestrated capillaries were frequently present in the subepithelial region (Figure 4, a and b). The crypt epithelium of some of the tonsils contained small blood vessels, 20 μm in diameter; some of these vessels passed the whole thickness of the crypt epithelium (Figure 4c).

The Langerhans cells, recognizable by the presence of intracytoplasmic Birbeck granules, were also found in the crypt epithelium (Figure 5).

Lymphoid follicles

Tonsillar lymphoid follicles consisted of the lymphoid and non-lymphoid cells. Two types of the non-lymphoid

cells were placed in the follicular germinal centre: small fibroblast-like cells (Figure 6a) and large cells (15–20 μm) with spherical protrusions and particles on their surface (Figure 6b). The first cell type belongs to reticular cells with thin reticular fibers forming the reticular framework. The second cell type had the appearance of antigen-presenting cells or macrophages. Lymphocytes were present inside the follicle reticulum and some of them appeared apoptotic (Figure 6a).

Interfollicular lymphoid tissue

Scanning electron microscopic analysis confirmed that the interfollicular lymphoid tissue consisted of the reticular cells, reticular fibers and lymphocytes (Figure 7a). Moreover, high endothelial venules (Figure 7b) and arterioles (Figure 7c) were found in the interfollicular areas. By TEM analysis of interfollicular regions, the large cells with morphological characteristics of interdigitating dendritic cells were also found (Figure 8).

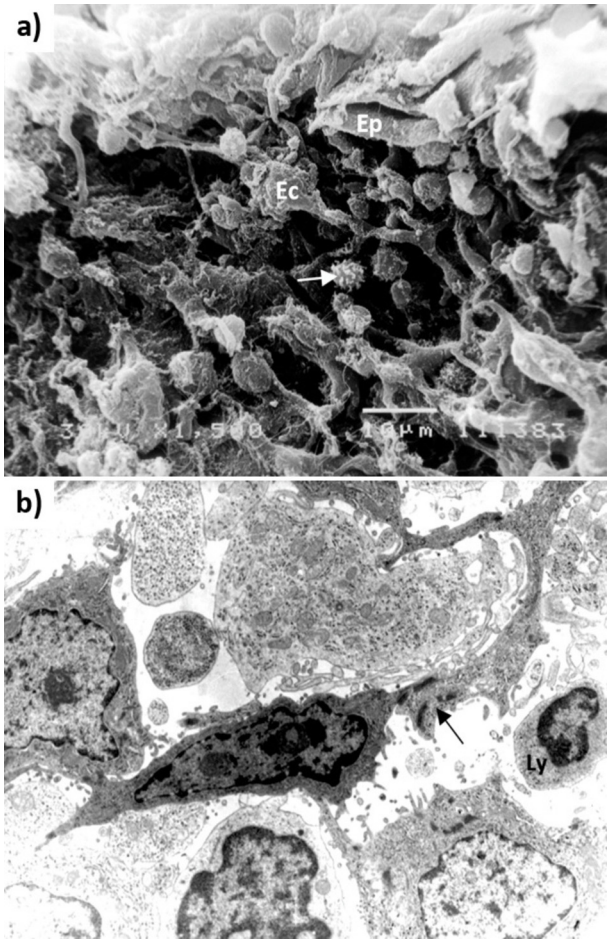

Figure 3 – ***Electron micrographs of a tonsillar crypt epithelium: (a) Scanning electron micrograph shows a reticular structure of the crypt epithelium; the surface of epithelium contains two superficial layers of horizontally oriented squamous cells (Ep); underlying epithelial cells (Ec) are in contact with each other via cytoplasmatic processes forming intercellular spaces filled by lymphocytes (arrow). (b) Epithelial cells with different morphology are connected by desmosomal junctions (arrow); note lymphocyte (Ly) between epithelial cells. TEM, ×3300.***

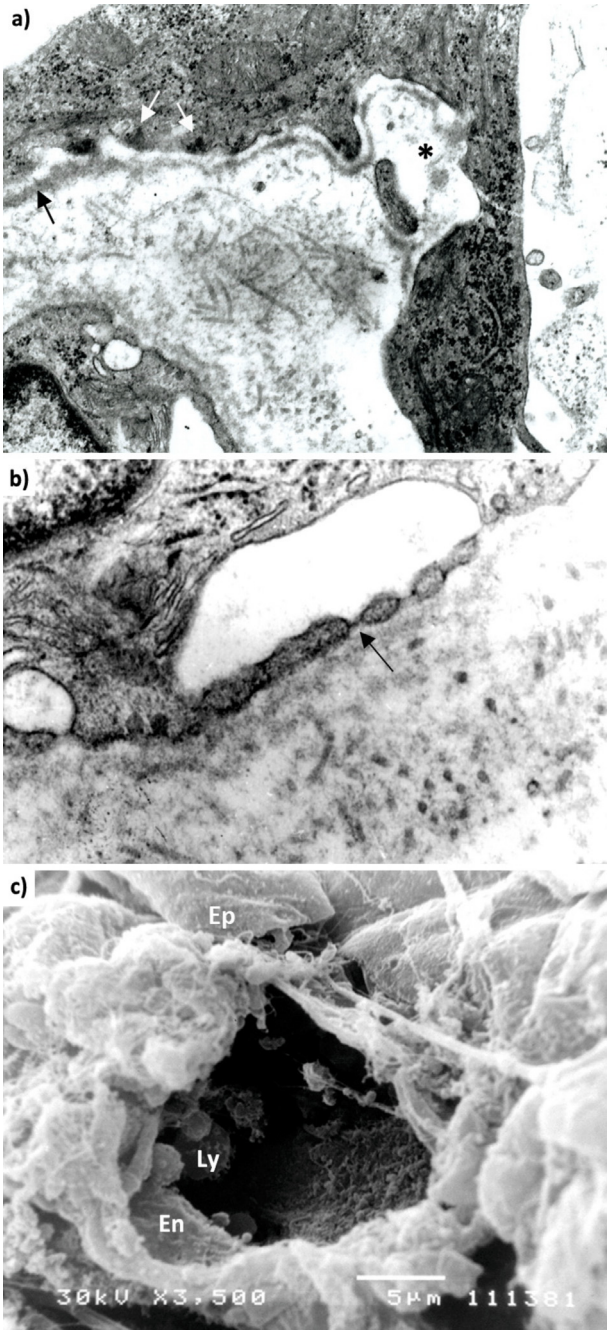

Figure 4 – ***Blood vessels and tonsillar crypt epithelium: (a) Crypt epithelial basal cell with electron dense cytoplasm is attached to the underlying basal lamina by hemidesmosomes (white arrows); note different thickness of lamina densa (black arrow) of the basal lamina and its interruptions (asterisk). A portion of capillary is seen at the bottom of micrograph. TEM, ×22 000. (b) The same capillary from the Figure 4a displays pores in the capillary wall bridged by an ultrathin diaphragm (arrow). TEM, ×26 000. (c) Scanning electron micrograph of the intraepithelial blood vessel; note endothelial cell nucleus bulging into the lumen (En) and lymphocyte (Ly) inside the vessel. One layer of horizontally oriented squamous cells (Ep) is above that blood vessel.***

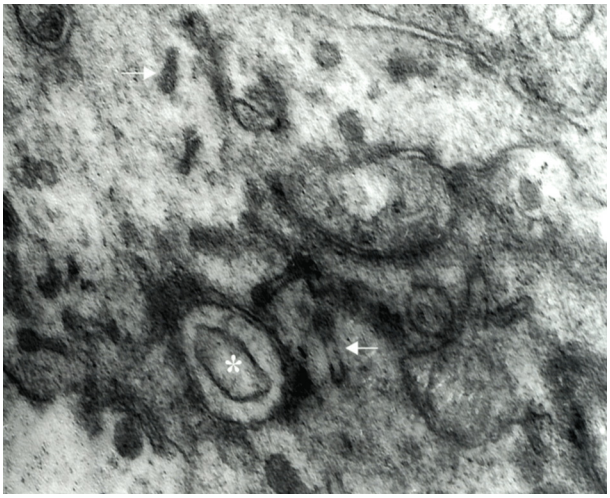

Figure 5 – ***Langerhans cell in tonsillar crypt epithelium. The figure shows a part of the cell cytoplasm with Birbeck granules (arrows) that resemble ping-pong paddles; some phagosomes (asterisk) are also present. TEM, ×33 000.***

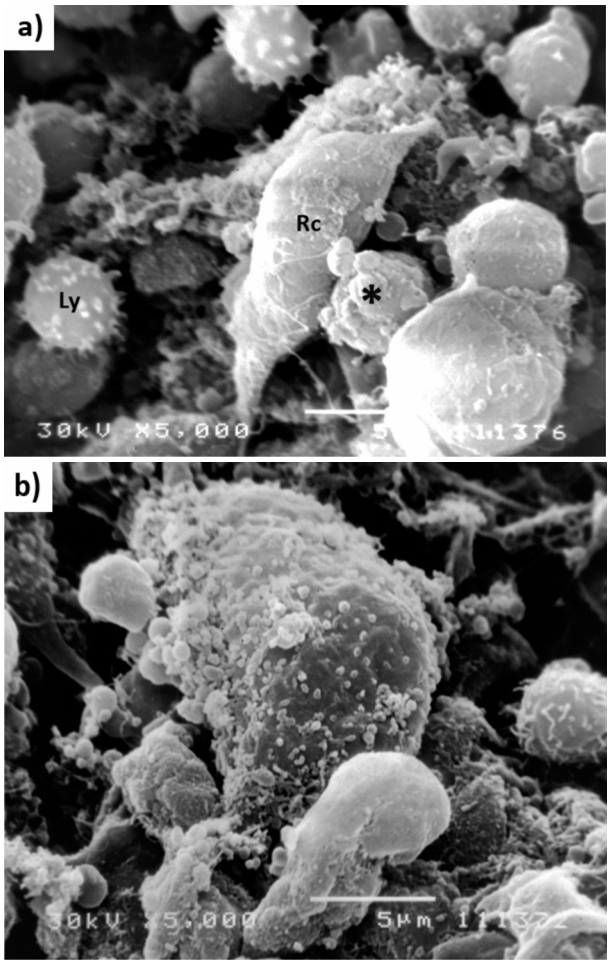

Figure 6 – ***Scanning electron micrograph of a palatine tonsil lymphoid follicle: (a) Reticular fibroblast-like cell (Rc) forms the thin reticular fibers evident as a fine spiral on the cell surface; smaller round microvillous cells – lymphocytes (Ly) are situated around and some of them are apoptotic, with many blebs on smooth cell surface (asterisk). (b) Large cell with small spherical protrusions and particles on its surface shows the morphological characteristics of follicular dendritic antigen-presenting cell or macrophage.***

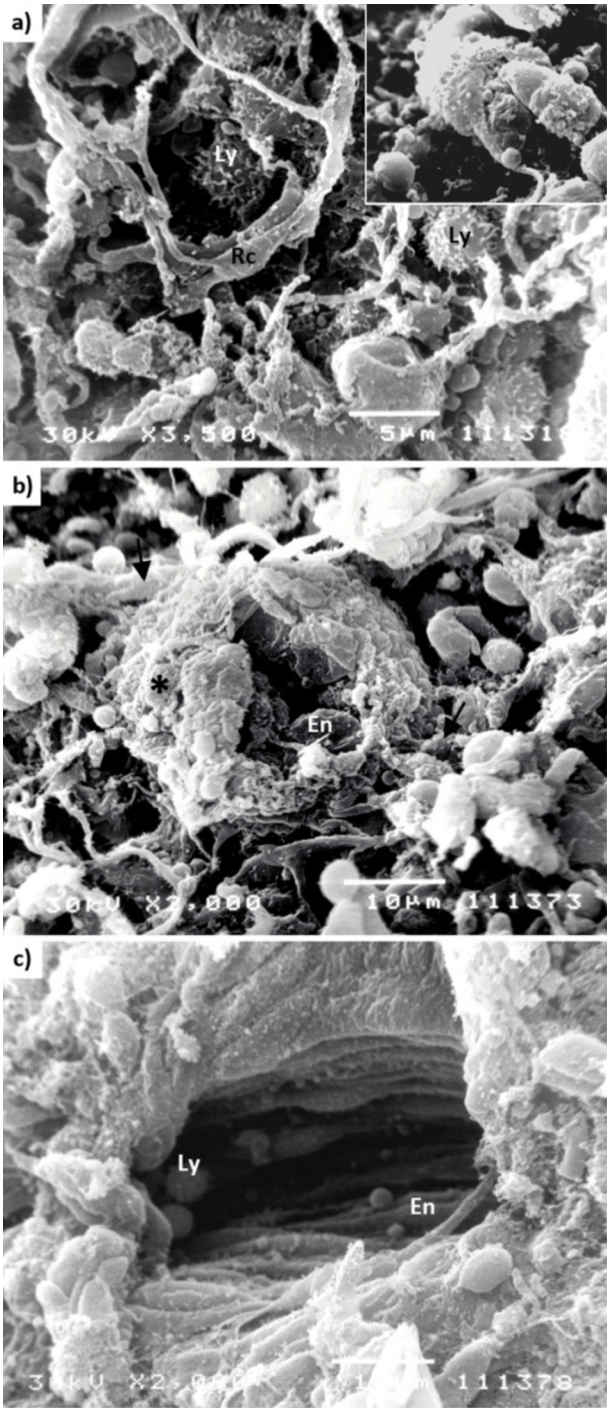

Figure 7 – ***Electron micrographs of a palatine tonsil interfollicular areas: (a) Reticular cells (Rc) form the framework; note lymphocytes (Ly) in intercellular spaces. The insert in the upper part shows the unusual reticular cell with cytoplasmic processes and small particles on the surface – possibly an antigen-presenting cell. (b) Cross section of a HEV; note cuboidal endothelial cells (En) which nuclei bulge into the lumen; the external tunica (asterisk) is made up of reticular fibers integrated with the extensions of the surrounding reticular cells (black arrows). (c) The section of an arteriole shows circular oriented closely grouped endothelial cells whose nuclear portion bulges into the lumen as a spindle-shaped thickening (En). Lymphocytes (Ly) are present inside the lumen of the arteriole. SEM, ×2000.***

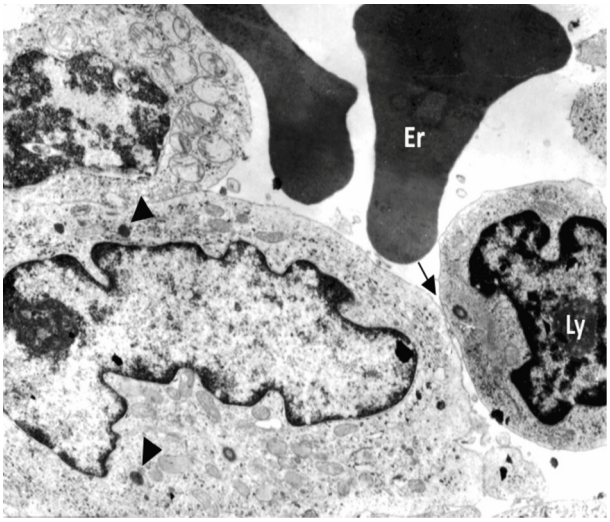

Figure 8 – ***Electron micrograph of a tonsillar interfollicular region: In this figure, a large cell, morphologically consistent with an antigen-presenting interdigitating dendritic cell dominates the microphotograph. This cell possesses an elongated irregularly shaped euchromatic nucleus and electron-lucent abundant cytoplasm with moderately developed organelles and some phagosomes (arrowheads). Note the adjacent lymphoid cell (Ly) attaching to the cell membrane of interdigitating dendritic cell (arrow). The two erythrocytes (Er) can be seen, which were probably extravasated during surgical intervention. TEM, ×15 000.***

Discussion

The ultrastructure of the human palatine tonsil was analyzed using the electron microscopy, with the reference to its significance for the tonsillar function. The oropharyngeal surface of the palatine tonsil was found to be covered by stratified squamous non-keratinized epithelium – tonsillar surface epithelium, whose superficial cells were closely connected by tight junctions. Epithelial cells of the surface and crypt epithelium of the palatine tonsil expressed some of the cell adhesive proteins – occludins and claudins [22]. It is well known that these molecules participate in the formation of tight junctions and are responsible for the obliteration of the intercellular spaces. In that way, the tonsillar surface epithelium is the first line of defense against invading pathogens.

By using SEM analysis, the structural differences between tonsillar surface epithelium and crypt epithelium were demonstrated in this study. It was also observed that the crypt epithelium possessed pores – microcrypts that were filled with large oval microvillous cells and/or lymphocytes. Maeda & Mogi [8] classified microcrypts as Type I, Type II and Type III, and reported that these microcrypts serve as an entrance for exogenous agents and for the passage of "wandering" cells. The localization and morphological properties of large oval microvillous cells, as well as their close contact with lymphocytes inside microcrypts, indicate a possible role of these cells in the uptake and transport of antigens to lymphocytes. Moreover, Maeda & Mogi [8] reported that large microvillous cells in microcrypts were tonsillar M-cells. Furthermore, Gebert [23] proposed that M-cells in the monocryptic palatine tonsil of rabbit represented a special cell type, different from the rest of epithelial cells in their morphology, function and cytoskeleton composition. Additionally, we demonstrated the Langerhans cells in the crypt epithelium, which confirmed the participation of crypt epithelium in the uptake of antigens and initiation of immune response.

Our study revealed the reticulation of the crypt epithelium as well as the lymphocyte infiltration from the subepithelial lymphoid tissue. Reticulation represents morphogenetically determined processes that start during the fourteenth week of gestation and continue after birth [24]. Clark *et al.* [12] reported that infiltrating lymphocytes secrete lymphokines that induce alteration in cytokeratin expression and, consequently, the changes of shape of epithelial cells and epithelial architecture. This characteristic of the crypt epithelium is very important regarding the creation of a microenvironment necessary for the initial phase of immune response in the palatine tonsils.

Epithelia, as is well known, do not have vascular supply, with the exception of a few sites in the human body. We demonstrated in this study that the tonsillar crypt epithelium was an exceptional structure, with fenestrated capillaries. This capillary type enables an intensive transport between blood and immunoglobulin (Ig)-producing cells located in the tonsillar epithelium and subepithelial region [2, 5].

The present study also showed that the tonsillar lymphoid follicles were specific morphological units with a characteristic relationship between the lymphoid and non-lymphoid cells inside a follicle. The largest part of lymphoid follicles consisted of two types of non-lymphoid cells: small fibroblast-like cells – reticular cells, and large cells – follicular dendritic cells or, perhaps, macrophages. The role of the reticular fibroblast-like cells is to provide mechanical support to the lymphoid cells and to produce reticular fibers. Scanning electronomicroscopic features of the lymphoid follicle reticular cells in our study (elongated shape, cell processes and close contact with lymphoid cells) is in accordance with recently published data of TEM observation of the same cells in the tonsils of sheep [25]. The other cell type inside a lymphoid follicle belongs to large cells whose morphological properties as well as close contact with the lymphoid cells suggest that these cells are, possibly, follicular dendritic cells. Described non-lymphoid cells in the lymphoid follicles are necessary to establish a microenvironment in which stimulated B-cells proliferate, undergoing somatic hypermutations, selection and differentiation into memory B-cells and plasma cells [5, 15, 17]. By SEM analysis, the presence of apoptotic lymphocytes in tonsillar lymphoid follicles was proven. This finding is in accordance with previous immunohistochemical studies that have shown that follicular B-cells, which do not synthesize the proper Igs undergo apoptosis and are destroyed by follicular macrophages [16, 17].

The interfollicular lymphoid tissue appeared as a reticular network occupied by lymphocytes. Reticular cells are the structural supporting elements for lymphocytes

situated between them, similarly to the reticular cells seen in the lymphoid follicles. Interdigitating dendritic cells observed in the interfollicular lymphoid tissue are the antigen-presenting cells that phagocytose, catabolise, process and present antigen on their plasmalemma to T-cells [4].

High endothelial venules are the most important structures in the tonsillar interfollicular regions participating in the continuous migration of lymphocytes from the blood to the tonsillar lymphoid tissue [9, 19, 26]. Our observation of lymphocytes that are in close contact with the luminal surface of the HEV – endothelial cells possibly indicates the first step of migration (adhesion) through the blood vessel wall. Hitherto, research on tonsillar tissue has confirmed the expression of a large number of the vascular adhesion molecules (*e.g.*, VCAM-1, VAP-1, P-selectin, L-selectin), which provide the adhesion of lymphocytes to endothelial cells of HEVs and their migration from the blood into the tonsil [2]. In this way, HEVs are involved in the lymphocyte traffic, which is essential for the tonsil immunocompetence. Furthermore, HEVs could be considered responsible for the extravasation of neutrophils during acute inflammatory episodes in the tonsil, probably assisted by disruption of the endothelial cell junctions [2, 3].

Conclusions

The knowledge of the palatine tonsil ultrastructure, primarily the crypt epithelium microarchitecture and microvasculature, provides better understanding of the mechanisms responsible for the decline of immunocompetence during recurrent bouts of tonsillitis. Damage to the crypt epithelium (including the loss of intraepithelial capillaries) as well as replacement of parenchymal structures by fibrous tissue in intrafolicular regions (which reduces the network of HEVs) gives rise to an inadequate initial phase of an immune response and decreased migration of lymphocytes into the tonsil, respectively. The consequence of these processes is attenuation of immune response in the tonsil as well as weaker systemic immunity. In clinical practice, this could be the guidance for the treatment of chronic tonsillitis – either conservatively or by surgical intervention.

Conflict of interests

The authors declare that they have no conflict of interests.

Acknowledgments

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Corresponding author
Marko Jović, Institute of Histology and Embryology, Faculty of Medicine, University of Niš, 86 Dr. Zorana Djindjića Avenue, 18000 Niš, Serbia; Phone +381 603510360, e-mail: marko.jovic@medfak.ni.ac.rs

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