

Antigen-presenting cells in the hypertrophic pharyngeal tonsils: a histochemical, immunohistochemical and ultrastructural study

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Summary. The antigen presenting cells (APCs) with special interest to dendritic cells (DC), were investigated in 28 hypertrophic and 10 control pharyngeal tonsils of children by histochemistry, immunohistochemistry and electron microscopy. In this study, we are trying to clarify the function and classification of APC in pharyngeal tonsils using morphologic criteria, Human Leukocyte Antigen Monoclonal Antibody (HLA-DR MoAb), which is specific for APCs, and acid phosphatase (APh) reacting with both phagosomes and lysosomes.

The surface epithelium of the patient group examined by light microscopy, heavy infiltration of lymphocytes, degenerated columnar cells and a few HLA-DR MoAb (+) columnar cells was observed. Additionally, a significant number of APCs which were Langerhans cells (LCs), interdigitating dendritic cell (IDC), follicular dendritic cell (FDC) and macrophages were stained with both HLA-DR MoAb and APh in the epithelial, interfollicular-subepithelial and follicular areas. Ultrastructural examinations revealed that lymphocytes, macrophages, LC and M cells were found among the surface columnar epithelial cells of the patient group. The interactions between M cells and LC suggested that M cells probably passed antigens from surface to LC. In the interfollicular-subepithelial areas of the hypertrophic pharyngeal tonsil, IDCs were in close contact with lymphocytes, macrophages and plasma cells. Seven types of FDCs (FDC-1 - FDC-7) were recognised according to their ultrastructural appearances. Differentiated FDCs (FDC-4) were also in close contact with each active subtype of FDCs in follicular areas besides lymphocytes. These findings supported the idea that although the pharyngeal tonsils contained several types of active APCs, only DC were in close contact with immunocompetent cells and the other APC's. Therefore, these morphologic appearances of DC could be a sign of function to initiate the immune response of the pharyngeal tonsil.

Key Words: Dendritic cell, Antigen-presenting cell, Pharyngeal tonsil, M cell, Langerhans cell, Macrophage, Plasma cell.

Introduction

The stroma of the pharyngeal tonsil is composed by lymphoid and nonlymphoid cells (the reticular cells and antigen presenting cells (APCs) such as DCs, macrophages and epithelial cells) [1-3]. The function

of the APCs is to carry antigen and to present it to the lymphocytes resulting in the induction of an immune response [4-6]. The antigen presentation process depends on the interaction of lymphocyte receptor with antigenic peptide-major histocompatibility complex (MHC) on the surface of APCs [7].

DCs are potent APCs [3,8]. They consist of Langerhans cells (LCs) in the skin, interdigitating dendritic cells (IDCs) in the paracortex of the lymphoid follicles and follicular dendritic cells (FDCs) (in germinal center and mantle zone of lymphoid follicles) [8,9]. Although LCs were known as a classical cell of stratified squamous epithelium [10], these cells were also found in the pseudostratified columnar epithelium of the pharyngeal tonsil [11]. Additionally, M cells were located between the surface columnar epithelium of pharyngeal tonsils [12,13] and in close contact with underlying lymphocytes and macrophages that transport the antigen to the deeper part of the epithelium [3,12-16]. It is known that LCs and M cells were associated with immunologic reactions of pharyngeal tonsils [10-15], but the interactions between these cells were not shown.

Interfollicular-subepithelial areas consist of cellular immunity relating cells (such as IDCs, macrophages and lymphocytes) [16] and humoral immunity relating cells (plasma cells) [10]. The functional interaction between the IDCs with macrophages and lymphocytes was reported to provide the cells with an opportunity for immune regulation at the earliest stage of immune induction [17]. However, a morphologic interaction of IDCs and plasma cells has not been shown.

FDCs play a role in the presentation of the antigens to B cells in the germinal centre of lymphoid follicles [18-20]. Seven types of FDCs were reported on the basic cell localization and content of the cell organelles in the palatine tonsil by electron microscopy [20]. However, neither the classifications of the FDCs within the pharyngeal tonsils nor the interaction with other types of FDCs were reported until now.

In our previous study, we had clarified that the cellular differences of hypertrophic pharyngeal tonsil result in OME [21]. This study tried to understand the possible relationship between morphology and function of these cells by using histochemistry, immunohistochemistry and electron microscopy.

Materials and methods

Patients and Specimens

Specimens of pharyngeal tonsils were obtained during adenoidectomy operations of 28 children, with ages ranging from 3 to 12 years. Also, pharyngeal tonsil were taken from 10 age-matched control cases who were operated by bronchoscopy for foreign bodies in the airways. Adenoids were considered normal when children had had no upper airway and/or middle ear infections during the previous two months. In each of the control subjects, informed consent was obtained before surgery from the patient's parents to carry out biopsies for the purposes of the study. Each tissue

fragment was divided into three parts and each part was processed with different procedures.

One of the tissue fragments from each subject was fixed in neutral formalin fluid (10%), dehydrated in graded series with ethanol and water, and embedded in paraffin. Five micron thick serial sections were collected on slides. The first slides of this group were stained with Hematoxylin-Eosin. The second and third slides of the first group were immunostained with HLA-DR MoAb (1:100) (Dako no: 775, Glostrup-Denmark) by applying the streptavidin- Biotin technique.

A second series of tissue fragments from each subject were placed in a cryoembedding medium and immediately frozen at -70°C . Five micron thick serial cryostat sections were fixed in cold acetone. The slides were incubated with alpha naphthyl acid phosphate (Aph) (Sigma No 7000, St Louis-USA) for 60 minutes at 37°C according to Barka and Anderson [22].

The third tissue from each subject was fixed in cold 4% glutaraldehyde in 0,1 mol cacodylate buffer (pH 7.4) for 4 hours at $+4^{\circ}\text{C}$ and postfixed in 1% osmium tetroxide in 0,1 mol phosphate buffer (pH 7.4) for 2 hours at $+4^{\circ}\text{C}$. The specimens were then dehydrated in a graded ethanol series, passed through propylene oxide and embedded in araldite. The compartments of the pharyngeal tonsils were determined by light microscopy on the semi-thin sections. Ultrathin sections of these areas were then carried out by a Reichert OMU ultramicrotome and stained with uranyl acetate and lead citrate. Observations were made with ZEISS EM 10B and ZEISS EM 900 electron microscopes (Carl Zeiss, Oberkochen, Germany).

Results

Light Microscopy

The surface epithelium of the pharyngeal tonsil in both the control group and the patient group was covered by pseudostratified ciliated columnar epithelium (Figures 1a, 2a). Many intraepithelial lymphocytes and degenerated columnar cells were found in the hypertrophic pharyngeal tonsils (Figure 2a). A few of the columnar epithelial cells of the control group were immunoreactive both HLA-DR MoAb (Figure 1b) and Aph, versus many in the patient group (Figure 2b.c). In addition, stratified squamous epithelium was observed like islets in the pseudostratified epithelium of the hypertrophic group and the epithelium was not stained with either HLA-DR MoAb and Aph activity.

Beneath the epithelium, the lymphoid tissue had similar histological properties in both control and hypertrophic pharyngeal tonsils, the only detectable difference being the presence of more and larger follicles in the hypertrophic group than in the control group. The APCs were recognised according to staining with HLA-

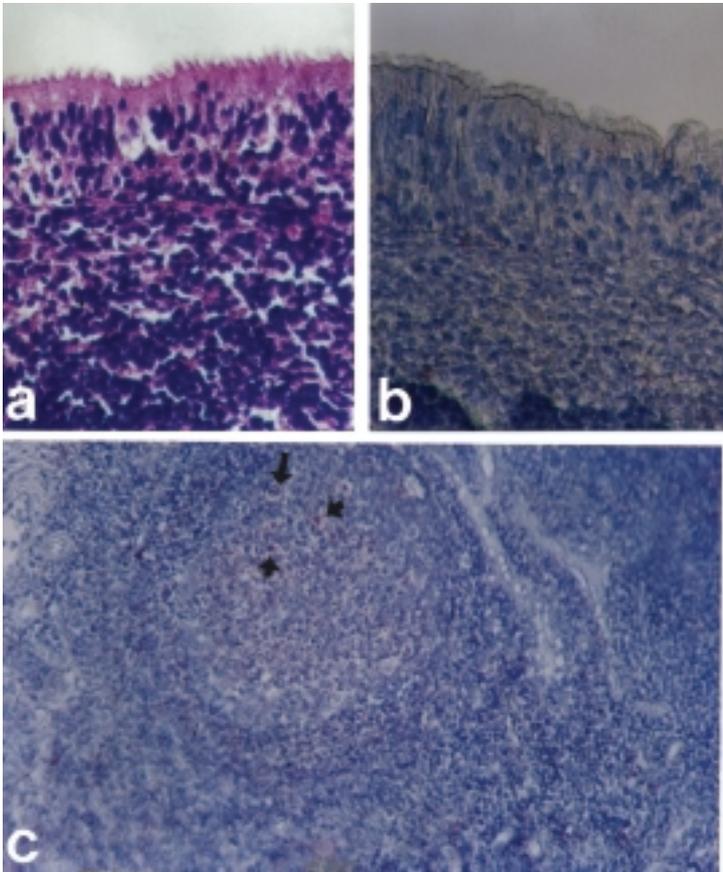


Figure 1. Light microscopy of normal pharyngeal tonsil:

a) Pseudostratified columnar epithelium is seen in the control group of pharyngeal tonsil. H.E; Original magnification X 200.

b) HLA-DR MoAb positive columnar epithelial cells were not seen in the control group of pharyngeal tonsil. Original magnification X 200.

c) Dendritic cell with thin long cytoplasmic extensions (arrowheads) and macrophages (arrow) staining with HLA-DR MoAb were seen in both follicular and interfollicular area of the control group of pharyngeal tonsil. Original magnification X 100.

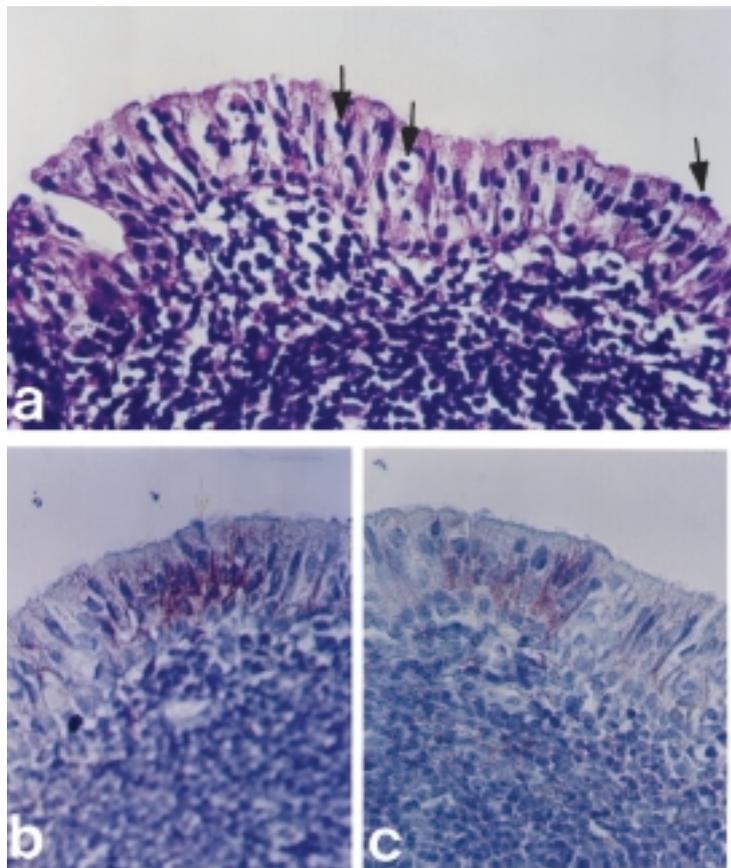


Figure 2: Light microscopy of hypertrophic pharyngeal tonsil:

a) Pseudostratified columnar epithelium and lymphocyte infiltration (arrows) are seen in hypertrophic pharyngeal tonsil. H.E; Original magnification X 200.

b, c) HLA-DR MoAb positive columnar epithelial cells in serial sections. Original magnification X 200.

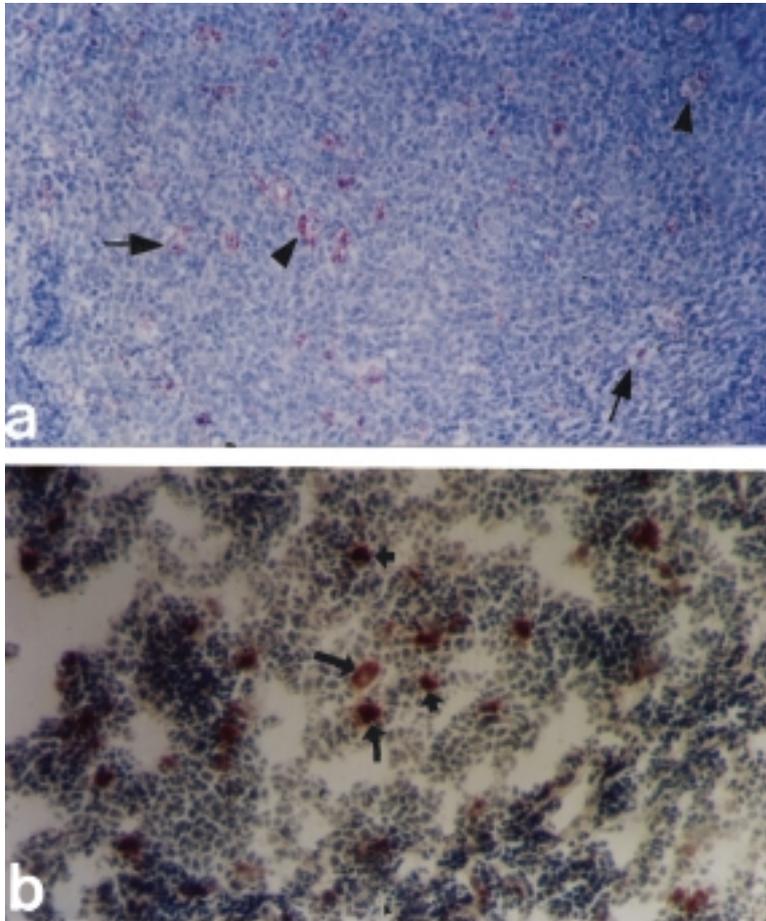


Figure 3. Light microscopy of epithelium of hypertrophic pharyngeal tonsils.

a) Dendritic cells with thin long cytoplasmic extensions (arrows) and macrophages (arrowheads) staining with HLA-DR MoAb were seen in both follicular and interfollicular area of the hypertrophic pharyngeal tonsil. Original magnification X 100.

b) Dendritic cell showing acid phosphatase activity in central spot (arrows) but macrophages showing acid phosphatase activity throughout their cytoplasm (arrowheads) in both follicular and interfollicular area of the hypertrophic pharyngeal tonsil. Original magnification X 100.

DR MoAb and APh activity in the interfollicular-subepithelial, and follicular areas of both groups (Figure 1c, 3a, b). DCs stained in a central spot near the nucleus but macrophages stained throughout the cytoplasm (Figure 1c.3a, b). Many DCs cell were seen in the hypertrophic pharyngeal tonsils but few in number in the control group (Figure 1c.3a, b).

Electron Microscopy

Surface epithelial cells of the pharyngeal tonsils of all groups were classified as columnar cells having cilia and microvilli, having only microvilli, intermediate cells and goblet cells (Figure 4). In the patient group, cytological signs of degeneration that were nuclear pyknosis, vacuolization and rupture of plasma membrane and also complete cell degeneration were shown in some of the columnar epithelial cells. Additionally, M cells having very thin cytoplasm and few organelles were observed among the columnar cells. Immediately under the M cells there were lymphocytes, macrophages and Langerhans cells (Figure 5).

IDCs, located in interfollicular-subepithelial areas, had an irregular nucleus, moderately developed organelles and long thin cytoplasmic extensions (Figure 6).

Junctional complexes were not seen between the cytoplasmic extensions of these cells but close membranous contacts were observed between IDCs and lymphocytes, between plasma cells (Figure 7) and macrophages (Figure 8).

According to the morphological properties of the FDC in the pharyngeal tonsil, they were classified into seven types in the lymphoid follicles. Primitive (FDC-1) (Figure 9) and undifferentiated (FDC-2) (Figure 10) FDC had very sparse organelles. Intermediate FDCs (FDC-3) were characterised by moderately developed granular endoplasmic reticulum and several Golgi complexes (Figure 11). These three cells were localized in the cortex of the lymphoid follicles and were in close contact with the cytoplasmic elongation of differentiated FDCs (Figure 9, 10, 11).

Differentiated FDCs (FDC-4) had well-developed organelles and their cytoplasmic elongations enveloped lymphocytes in the follicles. Desmosomal junctions were observed between the cytoplasmic extensions of FDC-4 (Figure 12). Secretory FDCs (FDC 5) had an euchromatic nucleus and large cytoplasm that contains many mitochondria, several Golgi complexes and a well-developed granular endoplasmic reticulum (Figure 13). The cells were in close contact and proximity with the

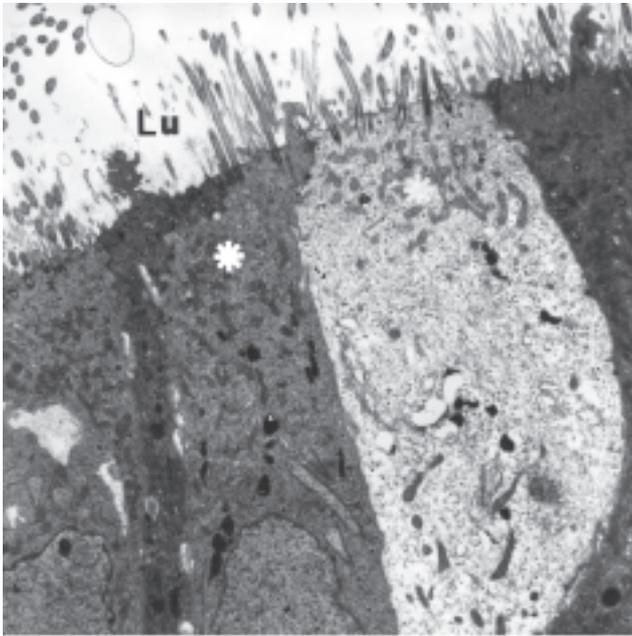


Figure 4: Electron microscopy of epithelium of control group of pharyngeal tonsils. Pseudostratified ciliated columnar cells (□) cover the surface of the control group of the pharyngeal tonsil. Lumen (Lu). Original magnification X 6000.

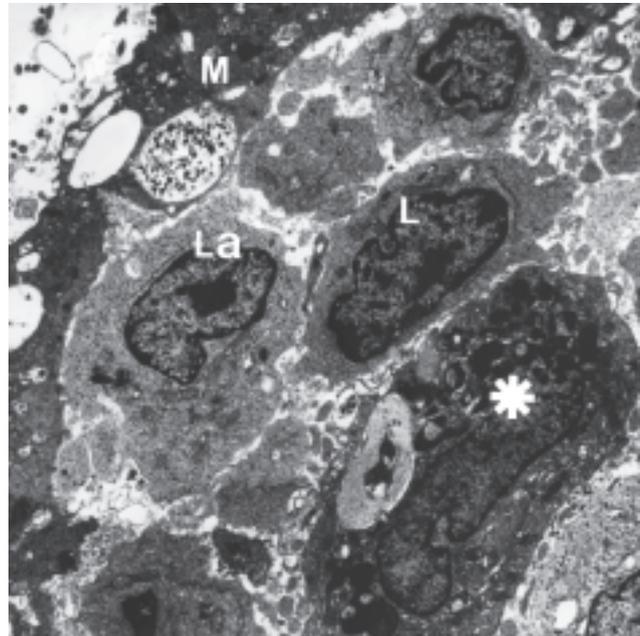


Figure 5. Electron microscopy of epithelium of control group of pharyngeal tonsils. The thin cytoplasm of the M cell (M) is in contact with Langerhans cells (La), lymphocytes (L) and macrophages (□). Original magnification X 6750.

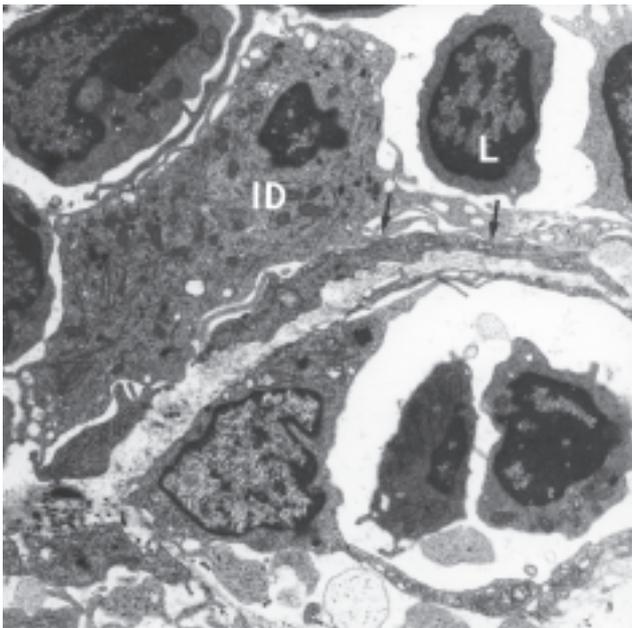


Figure 6. Electron microscopy of interfollicular areas of hypertrophic pharyngeal tonsils. An interdigitating dendritic cell (ID) with fine extension surrounding the capillary (arrows). Lymphocytes (L). Original magnification X 9900.

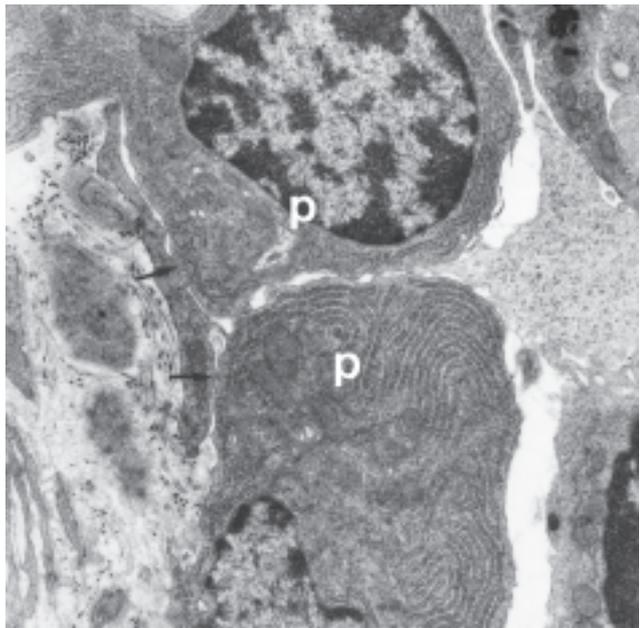


Figure 7. Electron microscopy of subepithelial areas of hypertrophic pharyngeal tonsils. Plasma cells (P) are in close contact with the cytoplasmic extension of dendritic cells (arrows). Original magnification X 12400.

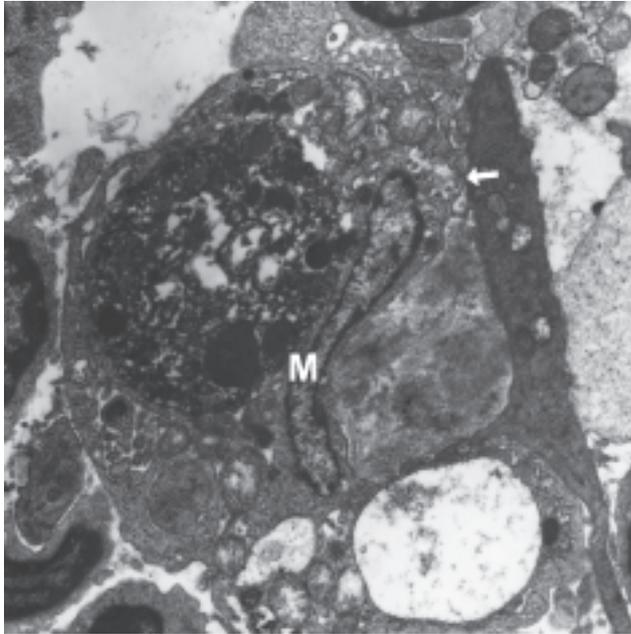


Figure 8. Electron microscopy of subepithelial areas of hypertrophic pharyngeal tonsils. Macrophage (M) is in close contact with the cytoplasmic extension of dendritic cells (arrow). Original magnification X 6000.

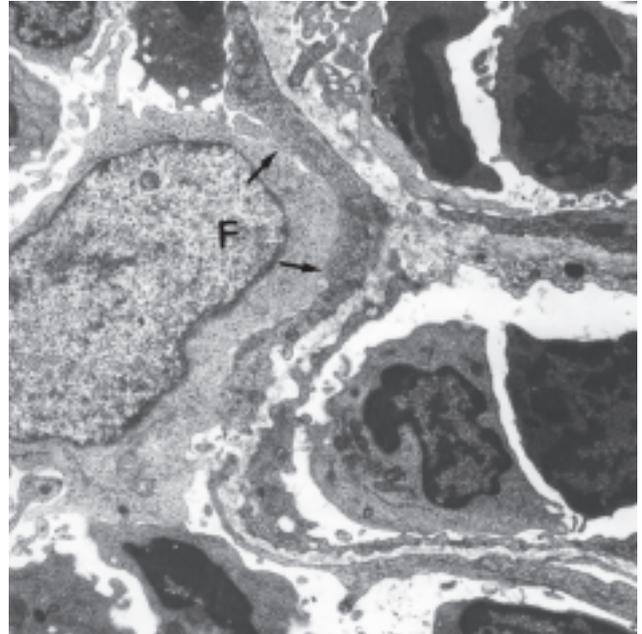


Figure 9. Electron microscopy of germinal center of hypertrophic pharyngeal tonsils. Primitive follicular dendritic cell (F) with few organelles is in close contact with cytoplasmic extensions of follicular dendritic cell (arrows). Original magnification X 9900.

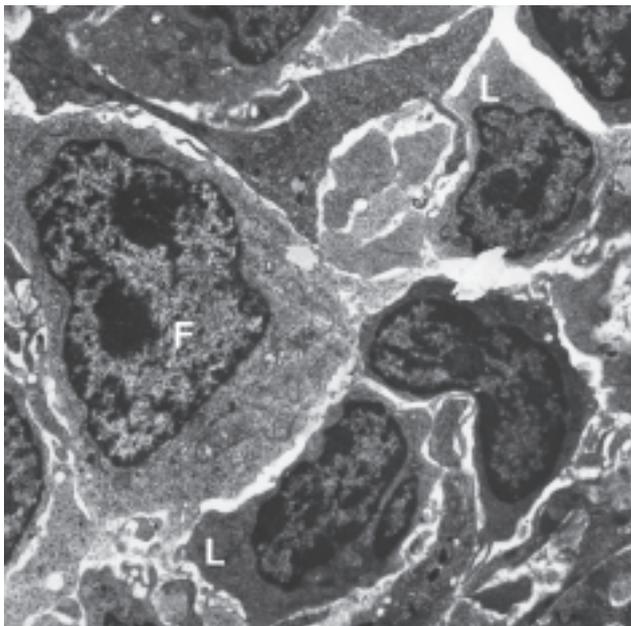


Figure 10: Electron microscopy of germinal center of hypertrophic pharyngeal tonsils. Undifferentiated follicular dendritic cell (F) containing few organelles is in close contact with cytoplasmic extension of follicular dendritic cell (arrow). Lymphocyte (L). Original magnification X 6750.

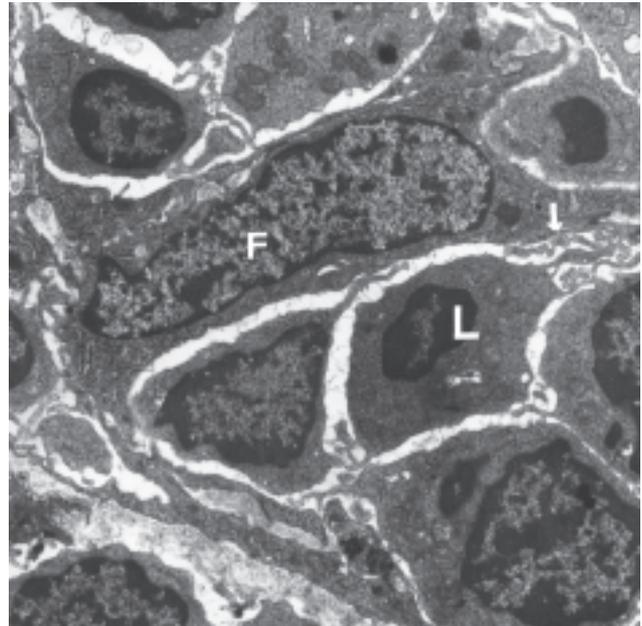


Figure 11: Electron microscopy of germinal center of hypertrophic pharyngeal tonsils. Intermediate follicular dendritic cell (F) containing few organelles is in close contact with cytoplasmic extensions of follicular dendritic cell (arrow). Lymphocyte (L). Original magnification X 9900.

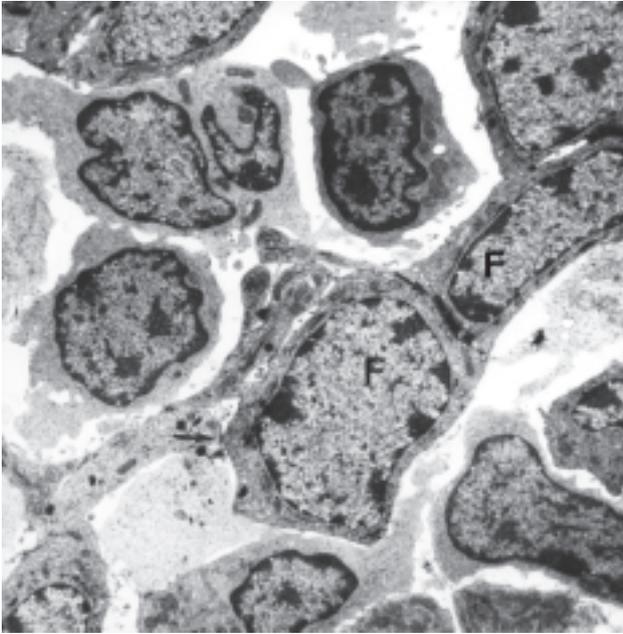


Figure 12. Electron microscopy of germinal center of hypertrophic pharyngeal tonsils.

Differentiated follicular dendritic cell (F) containing well developed organelles is connected with the other follicular dendritic cells by desmosomes (arrows). Original magnification X 6750.

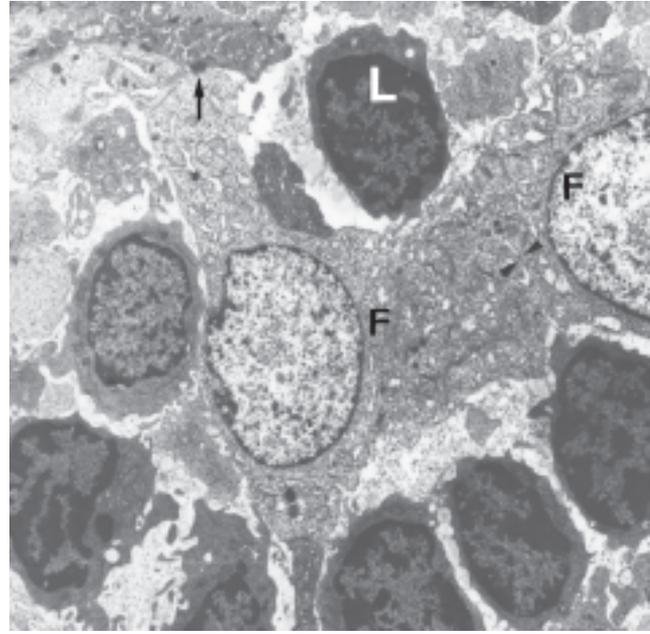


Figure 13. Electron microscopy of germinal center of hypertrophic pharyngeal tonsils.

Two secretory type follicular dendritic cells (F) contact the cytoplasmic extensions of follicular dendritic cell with desmosomes (arrow) and membranous contact (arrowheads). Lymphocyte (L). Original magnification X 6750.

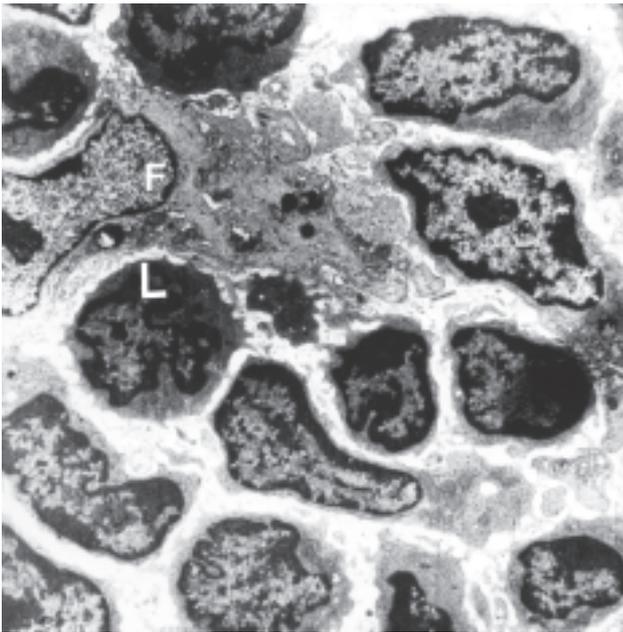


Figure 14. Electron microscopy of germinal center of hypertrophic pharyngeal tonsils.

Pale type follicular dendritic cell (F) has moderate organelles. Lymphocyte (L). Original magnification X 6750.

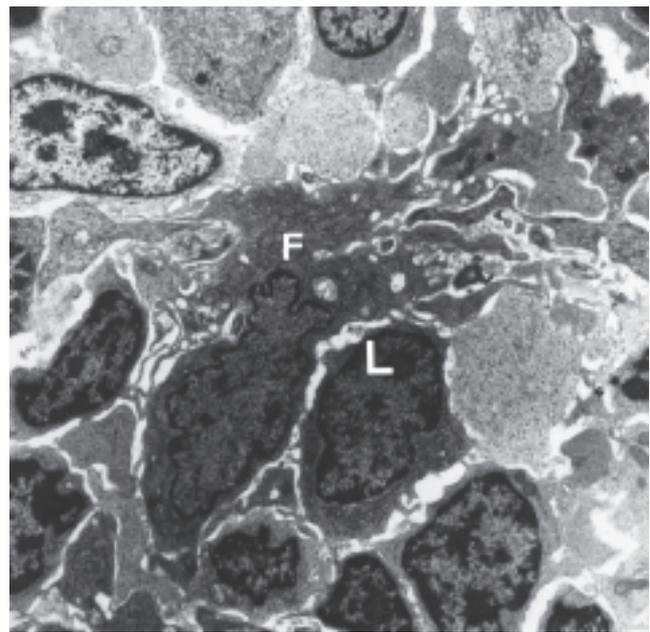


Figure 15. Electron microscopy of germinal center of hypertrophic pharyngeal tonsils.

Dark type follicular dendritic cells (F) with scarce organelles. Lymphocyte (L). Original magnification X 6750.

cytoplasmic extensions of FDC-4 in terms of both desmosomal interaction and membranous contact (Figure 13). Regressive pale (FDC-6) and dark type (FDC-7) FDC with abundant cytoplasm and moderate organelles were rarely detected (Figure 14, 15). These two types of FDC were not in close contact with the cytoplasmic extension of FDC-4 (Figure 14, 15).

Discussion

Different kinds of APCs were determined in the pharyngeal tonsil in the study. The cells have close interaction with each other according to their morphology. Different kind of APCs, the epithelium of the pharyngeal tonsils, LCs, IDCs, FDCs and macrophages were investigated in the pharyngeal tonsil in this study. We detected morphologically that the cells have close interactions with each other.

The antigen presenting role of the pseudostratified columnar epithelium of pharyngeal tonsils is controversial in previous studies. Nieuwkerk et al [1] reported that the antigen-presenting columnar epithelial cells which were HLA-DR MoAb positive were found in the hypertrophic pharyngeal tonsils. However, Bani et al [23], suggested that the epithelial cells of the pharyngeal tonsils did not express detectable amounts of HLA-DR MoAb and the epithelium may not play an efficient role in the immune activation. Our results clearly showed that some columnar epithelial cells of the hypertrophic pharyngeal tonsils were stained with HLA-DR MoAb, and they were less in number in the control group. This reaction further supported our previous studies in that the columnar epithelial cells of the hypertrophic pharyngeal tonsil have the capacity of antigen presentation in infectious conditions [21].

We observed that the areas of stratified squamous epithelium were not immunoreactive to HLA-DR MoAb in the surface epithelium of the hypertrophic pharyngeal tonsils. Therefore, we speculated that stratified squamous epithelium may not have antigen presentation functions and the aim of metaplasia may be to prevent the excessive access of antigens passing from epithelia to lymphoid tissue. However, Fusioshi et al [24] revealed that the epithelium in the columnar epithelium of pharyngeal tonsil was prompting squamous metaplasia under infection conditions and these changes might be a defence against antigen-trapping and antibody-producing systems.

LCs and M cells were observed with electron microscopy between the columnar epithelial cells of the hypertrophic pharyngeal tonsil. It is known that M cells transport antigens to lymphocytes and macrophages, which circulate into the epithelium and back to the lymphoid follicle [3,12,13,15]. We noticed that M cells were in close contact with LCs cells in addition to lymphocytes and macrophages. These findings suggested that LCs may take antigens from M cells and then process

and present them to lymphocytes in the epithelium. It is a possibility that an immune response may occur in a short time against foreign antigen within the columnar epithelium in this way.

The APCs of pharyngeal tonsils in the interfollicular-subepithelial and follicular areas with IDCs and macrophages were identified using morphology with MHC class II and APh positivity (1). We reported in our previous study that the number of APCs staining with HLA-DR MoAb in total cells was low (1%) and that of the patients was significant compared with the count [21] and the number of APC increases under inflection condition like epithelial cells.

We observed the interaction between macrophages and IDCs in the interfollicular-subepithelial areas by electron microscopy. However, Okata et al [16] showed the presence of morphological interaction among these cells and suggested that DCs seem to mediate antigen presentation from macrophages to helper T-lymphocytes. Also, it is known that DCs carry out endocytosis and processing independently [14]. Therefore, the aim of these interactions among these cells can be explained as a sign of the regulation of immune responses.

Our electron microscopic studies clearly showed that many IDCs in the interfollicular-subepithelial areas were in close contact with lymphocytes, macrophages and plasma cells. The IDCs are related to cellular immunity and they present antigen to T-lymphocytes [2,3,7,8,10,16]. However, the precursors of plasma cells were found in the follicular areas where FDCs present antigen to these cells [4,5,10,18]. It is known that the plasma cells precursors found in the lymphoid follicles differentiate to plasma cells for secretion of one type of immunoglobulins [10]. Therefore, the interaction between IDCs and plasma cells may not be responsible for antigen presentation in the subepithelial areas but may be a co-ordinating sign of regulation between humoral and cellular immunity.

The cytoplasmic elongations of different types of FDCs are connected by desmosomes and become a reticulum in the follicles [3-5,8,15,16,18,20]. We suggested that the reticulum might supply and control passing antigens and protect virgin B-lymphocytes. However, similar structures were known related to the thymus in which the thymic epithelial reticular cells surround within the capillaries in order to control the passage of antigen from vessels to parenchyma of thymus [10].

In this study, seven types of FDCs were identified in the lymphoid follicles of pharyngeal tonsils of the related patient group as reported in human palatine tonsils [20]. Our ultrastructural examinations showed that primitive and undifferentiated types of FDCs have fewer organelles compared with differentiated types, and were located in the cortex of the lymphoid follicles which is the antigen passing pathway. A few numbers of HLA-DR MoAb positive stained cells were observed in the cortex compared to that in the germinal centers of follicles. This result suggested that primitive and undifferentiated

type FDCs might play a role in transporting the antigens from the cortex to the germinal center of the lymphoid follicles where the differentiated FDCs are present. Moreover, Henien et al. showed that already 30 minutes after gold particle injection, FDCs in contact with the cortex of follicles were faintly positive but negative in the center. Colloidal particle twenty-four hours after injection were found in cytoplasmic extensions of FDCs in all parts of the germinal centers [5].

Our electron microscopic examinations showed that there was close contact between secretory and differentiated types of FDCs. These connections were considered in functional co operation among the cells. Secretory FDCs might provide some chemical agents for maturity and viability of the lymphocytes, while the differentiated FDCs present antigen to lymphocytes. However FDCs in the germinal centre of lymphoid follicles were responsible for the differentiation and maturation of B cells [26]. Moreover FDC-containing suspension consisted of PGF₂, IL-1 and TNF molecules [4], lymphocyte survivals were long in the FDC containing suspensions [27].

In conclusion, numerous APCs were found in the pharyngeal tonsil. The cells were in close contact with immunocompetent cells and with each other. Therefore the APCs were constricted in a complex system to initiate immune response.

Acknowledgments

This study was supported by the Research Foundation of Çukurova University, Grant N. T.F.E.50.

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