

# Morphological study of development and functional activity of palatine tonsils in embryonic age

## *Studio morfologico dello sviluppo e dell'attività funzionale delle tonsille palatine durante l'età embrionale*

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### Key words

Palatine tonsils • Histology • Embryology • Lymphocytic tissue

### Parole chiave

*Tonsilla palatina • Istologia • Embriologia • Tessuto linfatico*

### Summary

Palatine tonsils play an important role in the development of the immune system, being the first organ in the lymph system which analyses and reacts to antigenic stimulation. In this study, the peritonsillar area of Waldeyer's ring was investigated in 88 normal human embryos which were examined histologically and immunohistochemically. The progressive development of palatine tonsils during embryonic life is discussed. The first appearance of tonsils is in about the 14<sup>th</sup>-15<sup>th</sup> week followed by a parallel development of B- and T-cell regions which accounts for the high incidence of non mucosa-associated lymphoid tissue lymphomas among all tonsillar lymphomas and the higher incidence of T-cell-lymphomas, in comparison to the mucosa-associated lymphoid tissue of the digestive system. The way in which the human body develops the palatine tonsils quickly and prepares them to react to the first antigenic stimulation, are discussed.

### Riassunto

*La tonsilla palatina come tutti gli organi a struttura linforeticolare esplica una funzione reattiva ed immunitaria nei confronti dei complessi antigenici batterici e non batterici. In questo studio sono state esaminate, attraverso preparati istologici ed immunoistochimici, le tonsille palatine di 88 embrioni umani sani (4 embrioni per ogni settimana di sviluppo embrionale, dalla 14<sup>a</sup> alla 35<sup>a</sup> settimana), al fine di valutare lo sviluppo progressivo della tonsilla palatina nel corso della vita embrionale. Si è osservato che la tonsilla palatina inizia il suo sviluppo tra la 14<sup>a</sup> e la 15<sup>a</sup> settimana di vita intrauterina, con uno sviluppo parallelo delle zone di T e B linfociti, il che giustifica l'elevata incidenza di "non-tessuto linfoide mucosa-associato" linfomi tra le forme linfoproliferative che interessano la tonsilla, e la più alta frequenza di linfomi a cellule T in confronto agli altri organi a struttura linforeticolare annessi alle mucose. In seguito vengono discusse le modalità di sviluppo della tonsilla e le modalità di preparazione a rispondere agli stimoli antigenici.*

## Introduction

Palatine tonsils play an important role in the development of the immune system. Complete development of the tonsils is achieved after birth under the influence of antigens. The embryologic development of palatine tonsils appears to be the same as that of the thymus gland. In the present investigation, 88 normal human embryos (4 embryos from each embryonic week were studied). Multiple histological sections were obtained and the study included also immunohistochemical analyses.

The progressive development of tonsils during embryonic life are discussed and conclusions are drawn. The purpose of this study was to show the way in which the human body develops the palatine tonsils and how this knowledge can be used in managing related pathological conditions.

## Patients and methods

This research was completed with the collection and systematic examination of 88 human embryos from the 14<sup>th</sup> to 35<sup>th</sup> embryonic week. More specifically, 4 embryos from every embryonic week.

Embryos were collected at the University Department of Gynaecology Hippokratio General Hospital Thessaloniki, Greece. This procedure was performed in accordance with the legal regulations for the collection of human biological material, and with the agreement of the parents and the Administration Council of the Hospital.

The embryos collected were normal and intact and referred to cases of abortion, premature birth, or detachment of the placenta.

Examination of the embryos was performed in the Histopathology Laboratory of the Medical Faculty in the Aristotelian University of Thessaloniki, Greece. At the initial examination, all embryos with congenital abnormalities or syndrome were excluded.

A total of 88 normal and intact embryos were, therefore, available. At this stage, the head of each embryo was preserved in a 4% solution of formol for 48 h. Removal of the tongue and the mandible was carried out in order to obtain histological sections from Waldeyer's area, and specifically from the palatine tonsils.

All this material was embedded in paraffin cubes from which thin section of 3-5  $\mu$  were cut and stained in preparation for microscopic examination.

The study was completed under the microscope using multiple histological sections stained by haematoxylin-eosin (H&E) which in the stain routinely employed for specimens in the Histopathology Laboratory.

This examination revealed the progressive development of the epithelial and lymphoid tissue of the embryonic palatine tonsils.

Some sections studied immunohistochemically were stained using the peroxidase-antiperoxidase (PAP) method. The purpose was to identify B-lymphocytes with the L26 marker, T-lymphocytes with the UCHL-1 marker and epithelial cells with the epithelial membrane antigens (EMA) and keratin.

## Results

### 14<sup>th</sup>-15<sup>th</sup> WEEK

In the area of palatine tonsils, the epithelium of the embryonic oropharynx is becoming thicker. This epithelium forms invaginations inside the underlying mesenchymal cells with multiple branches at each end. Some of these are becoming independent epithelial "islands".

In some epithelial invaginations or islands, a central necrosis and the start of a canal formation can be recognised. Parallel to the former procedure, marked infiltration of the epithelial and mesenchymal cells, by lymphoid cells, is observed. Another significant point is the appearance of post-capillary venules, at this stage.

### 16<sup>th</sup>-17<sup>th</sup> WEEK

The development of lymphocytic tissue results in the formation of primary lymph follicles. At the same time, the appearance of primary T-cells is the first stage in the formation of the parafollicular areas. A progressive development of complex epithelial invaginations can be found with formation of tubules and the appearance, in the above-mentioned structures, of primary Langerhan's cells.

### 18<sup>th</sup> WEEK

The formation of tubules in the epithelial invaginations is completed and the above-mentioned struc-

tures are the primary crypts of the embryonic palatine tonsils. The primary lymph follicles often display a central necrosis and are morphologically, similar in appearance to Hassall's corpuscles in the thymus gland.

### 19<sup>th</sup>-22<sup>nd</sup> WEEK

The crypts are becoming more complex and numerous and the follicular- parafollicular areas are more visible. There is a progressive development of fibrotic tissue and this tissue, under the pressure of lymphocytic growth, is becoming the primary tonsillar capsule.

### 23<sup>rd</sup>-28<sup>th</sup> WEEK

The above areas are now clear. The well-organised lymph follicles have a relation with the bottom of this crypt branches and the addition of fibrotic septula has, as a result, the primary functional tonsillar units (crypt – primary lymph follicle – parafollicular area).

### 29<sup>th</sup>-35<sup>th</sup> WEEK

There is a progressive development in the lymphocytic tissue which is characterised by an increased population of lymphocytes in the primary follicles (B-zone) and parafollicular area (T-zone), with a synchronous development of reticulocytes in the above areas and post-capillary venules in the T-zone. Another interesting observation is that, in embryonic life, germinal centres, in tonsillar lymph follicles, are never observed.

## Results of immunohistochemical study

From the 14<sup>th</sup> week, lymphoid cells are positive to lymphoid markers. The majority of positive cells to



Fig. 1. Early epithelial invaginations (solid or with tubules) in the mesenchymal cells. The beginning of tonsil formation (14th embryonic week) (H&E x100).



**Fig. 2.** Embryonic palatine tonsil with multiple epithelial invaginations. Canal formation with central necrosis is noted in some. Peripheral lymphocytic infiltration creates the primary follicles (16<sup>th</sup> embryonic week) (H&E x40).

L-26 marker (marker of B-lymphocytes) were subepithelial cells and cells positive to UCHL-1 marker (marker of T-lymphocytes) were mainly from post-capillary venules.

During embryonic development, specifically, in the 16-17<sup>th</sup> week, the follicular and parafollicular areas are more obvious with B-lymphocytes present mainly in the follicles and T-lymphocytes in the parafollicular areas.

The above areas are becoming more obvious and well-organised, week by week, especially after the 20<sup>th</sup> embryonic week.

Moreover, the study with the EMA and keratin showed similar results as far as concerns the epithelium and how this is organised.

## Discussion

As can be seen from our findings, the first appearance of the palatine tonsils occurs at about the 14<sup>th</sup>-15<sup>th</sup> week and is characterised by the development of invaginations of the epithelium in the underlying mesenchymal cells and the infiltration of the stroma by lymphoid cells<sup>1</sup>. Palatine tonsils are often referred to in the literature as branchiogenic structures derived from the second branchial cleft<sup>2</sup>. Since at this time, the branchial system, which is a primary structure, has completely disappeared, we must consider the tonsils as deriving indirectly from the branchial cleft tissue. Therefore, the palatine tonsils are not branchiogenic structures like the thymus gland, even if they have a similar pattern of development (epithelial invaginations, etc.)<sup>3</sup>.

A characteristic point is that the whole development of the palatine tonsils follows the creation of the ep-

ithelial invaginations and crypts. In the majority of the above structures, there is a central canal formation (tubules) with a parallel creation of independent epithelial islands, some with central necrosis, and others without which are similar to Hassall's corpuscles of the thymus gland<sup>4</sup>.

The mechanism of the canal formation, in the crypts, probably plays a role in the development of lymphocytic tissue. It is possible that substances from the necrotic epithelial cells act as a local antigenic stimulation for the local development of immature lymphoid cells.

We agree with many scientists especially von Gan-decker<sup>5</sup> and Slipka<sup>6</sup> concerning the time of appearance of the palatine tonsils (14<sup>th</sup>-15<sup>th</sup> week), and the appearance within them of primary lymph follicles and parafollicular areas at about the 16<sup>th</sup>-17<sup>th</sup> week.

We also agree with the more recent literature<sup>7</sup> that germinal centres were never observed in the tonsillar lymph follicles.

After birth, tonsils are one of the first organs to accept external antigenic stimuli with respiration and deglutition. As a result, development of the lymphocytic tissue takes place with germinal centres in the lymph follicles and, finally, an increase in size of the tonsils in the pharynx. Therefore, palatine tonsils are, correctly, characterised as the first immunological analyzers of the organism.

The particular synchronous development of the B- and T-cell regions may explain the high incidence of "non MALT-lymphomas" in the palatine tonsils and the high incidence of "T-cell-lymphomas" in comparison to the MALT of the digestive system. Palatine tonsils differ from all other MALT but similarities do exist.

As far as concerns the immunohistochemical study, our material showed a significant decrease in the appearance of the EMA in comparison to sections from tonsils with chronic inflammation<sup>8</sup> (material from tonsillectomies). This would appear to support the hypothesis that the increased appearance of the epithelial antigen in the crypts is inflammation related. Very recent studies have shown a rupture of the basilar membrane of the epithelium in the crypts during inflamed conditions<sup>9</sup> confirming that the reticular epithelium of the crypts is a specialised part of tonsillar function.

## Conclusions

The following conclusions have emerged from this investigation:

1. Palatine tonsils must be considered as being derived indirectly from branchial cleft tissue, not unlike the development of the thymus gland.
2. Tonsils start to appear at about the 14<sup>th</sup>-15<sup>th</sup> week of embryonic life.

3. Development of lymphocytic tissue follows the creation of epithelial invaginations and crypts.
4. Appearance of primary lymph follicles takes place at about the 16<sup>th</sup>-17<sup>th</sup> week with parallel appearance of T-cells (parafollicular).
5. A progressive development of complex epithelial invaginations (tubules and crypts) takes place with parallel development of lymphocytic tissue.
6. In embryonic life, no germinal centres are observed in tonsillar lymph follicles.
7. The progressive and synchronous development of B- and T-cell regions make palatine tonsils different from the rest of the MALT, however, similarities with both MALT and lymph nodes do exist. This fact accounts for the high incidence of "non MALT-

- lymphomas" among all tonsillar lymphomas, and the higher incidence of "T-cell-lymphomas", in comparison to the MALT of the digestive system.
8. The immunohistochemical study of our material demonstrated that changes in epithelial cell differentiation result after antigenic stimulation. Apparently, the reticular epithelium of the crypts represents a specialised component in the immunological function of the tonsils.

It is obvious that the human body, during embryonic life, is scheduled in such a way that the palatine tonsils can develop quickly and make them ready to answer the first antigenic stimulation. These antigenic stimuli would go across this area, after birth, to the Respiratory and Digestive System.

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