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Age-Associated Role of Lingual and Pharyngeal Glands in Local Immunity in Oral Cavity

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The macroscopic and histological methods were employed to examine the autopsy specimens of salivary lingual glands obtained from 299 patients of both sexes and various age ranging from newborn to longevity. The age-associated alterations of minor lingual and pharyngeal glands were revealed, and the topographical relations between the glands and lymphoid cells were described. The characteristic sparsity of the glands in infancy is caused by nutritional uniformity at this period, when diminished production of secretory IgA results in frequent inflammatory processes in oral and pharyngeal cavities. With age, the glandular orifices widen, and their number increases thereby augmenting local immunity in the oral cavity and in oral aspect of the pharynx. Starting from elderly and senile age, the involutive alterations were observed, which were accompanied by diminished production of secretory immunoglobulin A and related degradation of local and humoral immunity.

Key Words: *oral cavity; local immunity; lymphoid tissue; lingual and pharyngeal glands; secretory IgA*

During the last decades, immunology of the oral cavity was actively studied. The rapid development of this scientific field can be explained, specifically, by the appearance of ample new data on antimicrobial activity of secretory IgA in the oral cavity [3]. The content of IgA in the saliva is known to surpass the levels of all other immunoglobulins indicating its especially important role in securing the local immunity. In the oral cavity, saliva is secreted from the greater and minor salivary glands; 30% of the saliva is produced by the minor ones [1]. The secret produced by these organs includes a variety of digestive enzymes, antibacterial components such as lysozyme, and immune cells. Availability of all these ingredients explains rapid recovery of oral mucosa integrity after lesions and/or infection. Of the factors determining the resistance of the oral mucosa, an important role is given to secretory IgA [9]. The glandular structures in the walls of hollow visceral organs play important role in vital processes in both norm and pathology [7].

Recently, the scientific views on morphophysiology of the glands in digestive tract, including the minor ones located in its walls (namely, in pharyngeal walls) were essentially modified [8]. Previously, the views on physiological role of pharyngeal glands were focused only on secretory defense of laryngeal mucosa against lesions provoked by the passage of air and food bolus, but now this proposition is being revised. Now, the pharyngeal glands are examined with due account for their endocrine activity [4] and implication (similar to the lingual glands) in local immunity. Thus, the secret of the minor glands not only promotes the most rapid regeneration of oral tunica mucosa, but it also participates in the formation of certain barrier against invasion of the antigens into the upper airways and initial part of the digestive tract [2,3].

At present, the glands of small and large intestines as well as their topographic relations with lymphoid tissue have been extensively examined in humans and

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animals in norm and under experimental conditions [8]. However, the morphology of human lingual and pharyngeal glands, and their topographic associations with lymphoid tissue were little studied. Evidently, the data on peculiarities of lymphoid-glandular relations in the depth of tongue and in the upper pharyngeal subdivisions are especially important for physiology and medicine. The importance of this problem is highlighted by the facts of implication of the lingual and pharyngeal glands (side by side with the immune structures) in maintaining homeostasis, which had been proved by numerous studies of the lymphoid-glandular microtopographic relations in tunica mucosa of various visceral organs.

This study was designed to reveal the morphological peculiarities and topographic relations between lingual and pharyngeal glands, on the one hand, and lymphoid tissue, on the other hand, in humans of both sexes and different ages under the conditions of conventional norm during the postnatal ontogeny.

MATERIALS AND METHODS

The autopsy specimens of lingual glands were obtained post mortem from 299 humans of both sexes aging from newborn to longevity. The specimens were taken from forensic mortuaries of the Bureau of Forensic Medical Expertise of the Moscow Health Department in compliance with Russian Federation Government Decree (Federal Law No. 323, Art. 47, 4180-1, 355n). The study excluded the cases with pathological alterations in the digestive system established by forensic medical expertise.

The additional microanatomical examination of the glands was performed in preparations obtained after evisceration (n=139). The tongue was stretched out and fixed on a piece of cardboard facing it with its bottom surface, thereupon it was placed in 10% neutral formalin. The tissue samples (1.5×0.5 cm) were cut strictly transversally in the regions of the apex of the tongue, circumvallate papillae at the tongue body, and tongue root. Three tongue specimens were taken from each region. The larger dimension of a specimen corresponded to transverse line of the tongue. The fixed specimens were alcohol dehydrated and embedded into paraffin blocks. Each specimen was cut transversally to prepare 5-7 μm sections (n=5-6), which were stained with hematoxylin-eosin and van Gieson picrofuchsin. Some specimens were stained according to Kreiberg to detect mucous type of secretion in a gland. A Biolam binocular microscope (objective 10×, ocular 64×) was employed to assess the topographic relations between the glands and lymphoid tissue on transversal sections of the tongue in each region (anterior, median, and posterior thirds) of the randomly selected glands (n=5). The following parameters were determined: *1*) thickness of initial part of the gland (its longitudinal dimension on the section); 2) area of initial part determined with ocular grid at 640×; *3*) the length and width of primary glandular unit (PGU) defined as the acinar sac of glandulocytes with the terminal duct; *4*) the number of PGUs in the section of initial part of the gland; *5*) the number of glandulocytes on PGU section; *6*) PGU area; *7*) PGU lumen area limited by the apical side of glandulocytes, which was determined with an ocular grid at 900× and field of vision 0.082 mm^2; *8*) the content of stroma (in %) at initial part of the gland determined with an ocular grid at 640×; and *9*) the major diameter of the lumen of the glandular excretory duct in its cross-section within the depth of tunica mucosa outside the region of ampullary dilation.

The data were processed statistically to calculate the mean value, *SEM*, as well as minimum and maximum of each parameter. Significance was assessed with confidence intervals.

RESULTS

PGUs of the salivary glands in posterior third of the tongue are situated in the bulk of muscular tissue and in loose fibrous connective tissue within the depth under the lingual tonsil (Fig. 1). In humans at elderly and senior age, as well as in the long-livers, the adipose tissue is typically observed near the posterior lingual glands and their stroma (Fig. 2, *a*).

The topographic relations between the glands and lymphoid tissue in the tongue were similar in all subdivisions of this organ, but they somewhat changed during the postnatal ontogeny. In the bulk of tongue of the

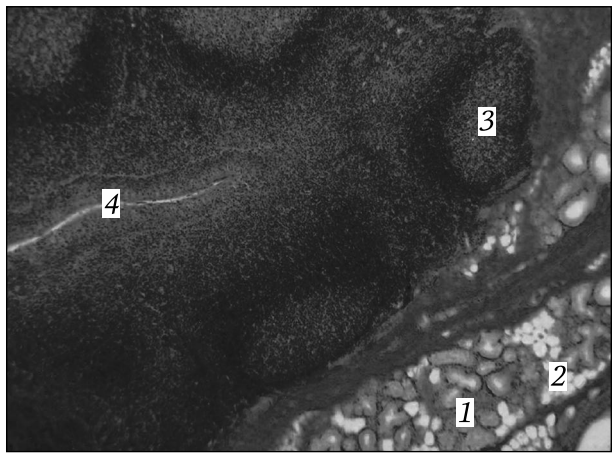

Fig. 1. Location of posterior lingual glands under the lingual tonsil in the posterior third of the tongue (at its root) of a 45-year-old woman. Hematoxylin and eosin staining, 60×. *1*) Initial part of a gland; *2*) adipose tissue in glandular stroma; *3*) lymphoid nodules with germinal centers; *4*) crypt of the lingual tonsil.

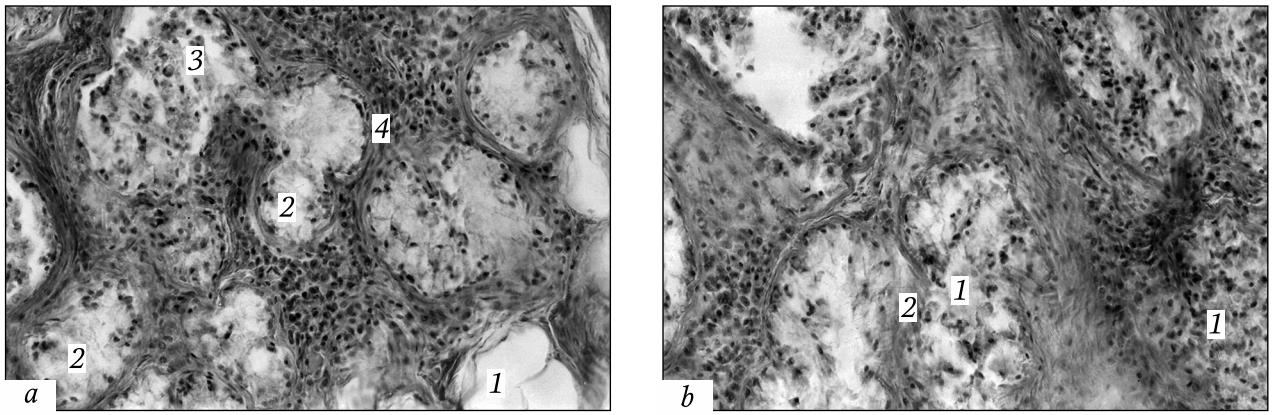

Fig. 2. Adipose tissue near PGU and excretory ducts of posterior lingual gland of a 67-year-old woman (*a*) and almost complete absence of lymphoid cells at the posterior lingual glands of a 70-year-old (*b*). The posterior third of the tongue. Van Gieson staining with picrofuchsin, ×250. Fragment *a*: *1*) adipose tissue; *2*) PGU; *3*) excretory duct with the excreted substance; *4*) glandular stroma; fragment *b*: *1*) initial part of the gland; *2*) amorphous loose fibrous connective tissue.

newborns, the glandular-lymphoid relations were minimal. In postnatal and infant babies, the small numbers of lymphoid cells (lymphocytes, macrophages, plasma cells, *etc*.) were observed near the glandular excretory ducts and their initial parts. Similarly, such cells were rarely found in the glandular stroma; in contrast, no clusters of lymphoid cells were observed in glandular stroma of postnatal and infant babies.

The lymphoid nodules and diffuse lymphoid tissue are located in loose fibrous connective tissue between the muscle fibers and near the initial parts of all groups of lingual glands (anterior, median, and posterior). The lymphoid cells and their clusters (usually of irregular shape) were also observed in the depth of loose fibrous connective tissue, which separates PGU and their lobular groups (Fig. 3, *a*). These cells were detected in glandular stroma near the striated excretory ducts (Fig. 3, *b*) and at the common excretory duct of all glands (Fig. 3, *c*). Predominantly, the lymphoid cells were lymphocytes, which formed a 3-5-layer rim around the excretory ducts along their entire length. The lymphoid nodules with and without the germinal center were observed at the orifice of common excretory duct near its posterior portion.

In senile persons and long-livers, the lymphoid nodules were observed in no more than 5-7% sections; a few of them were located at the common excretory duct of the gland and near the first-order excretory ducts. In all histological sections, these nodules had no germinal centers. Similarly, at these ages the diffuse lymphoid tissue was not pronounced near all lingual glands. In the stroma of initial part of the lingual glands, the lymphoid cells were found in small numbers (Fig. 2, *b*).

The study of pharyngeal glands focused on examination of their topographic relations with lymphoid tissue in the pharynx revealed the following age-related peculiarities of their localization [10]. During entire postnatal period and in infancy, the lymphoid tissue was always detected in lamina propria of mucous tunic and in its submucous layer. However, despite typical development of the glands at this age, the lymphoid cells were little observed near their excretory ducts and initial parts of the glands. The stroma of pharyngeal glands of newborns and infants incorporated rare and solitary lymphoid cells. In contrast, such cells were always detected near the glands in the juvenile and older persons. After juvenile age, pronounced lymphoid-glandular associations were typical. In young, adult and senile persons, the cells of lymphoid family were always observed near initial parts of the glands; in the stroma of these parts, they formed the clusters of irregular shape. The lymphocytes were also found between the glandulocytes of PGU. There were no lymphoid nodules in the pharyngeal wall of senile persons and long-livers. At these ages, the lymphoid cells were rarely observed near the excretory ducts and in the glandular stroma.

During postnatal ontogeny, the topographic relations between lingual glands and lymphoid tissue in the bulk of the tongue revealed the age-specific peculiarities. In newborns, the number of lymphoid cells near the gland is relatively low. In juvenile, mature, and senile persons, the study persistently revealed the clusters of lymphoid cells near initial part of the glands as well as in their stroma and excretory ducts. In senile persons and long-livers, the cells of lymphoid family were rarely observed near the lingual glands, and their number was low. Similar age-related peculiarities of lymphoid-glandular relations were previously established in esophagus wall [5]. Probably, a rather low number of lymphoid cells located near the lingual glands and implicated in the immune surveillance, which is typical for the first months of life, can

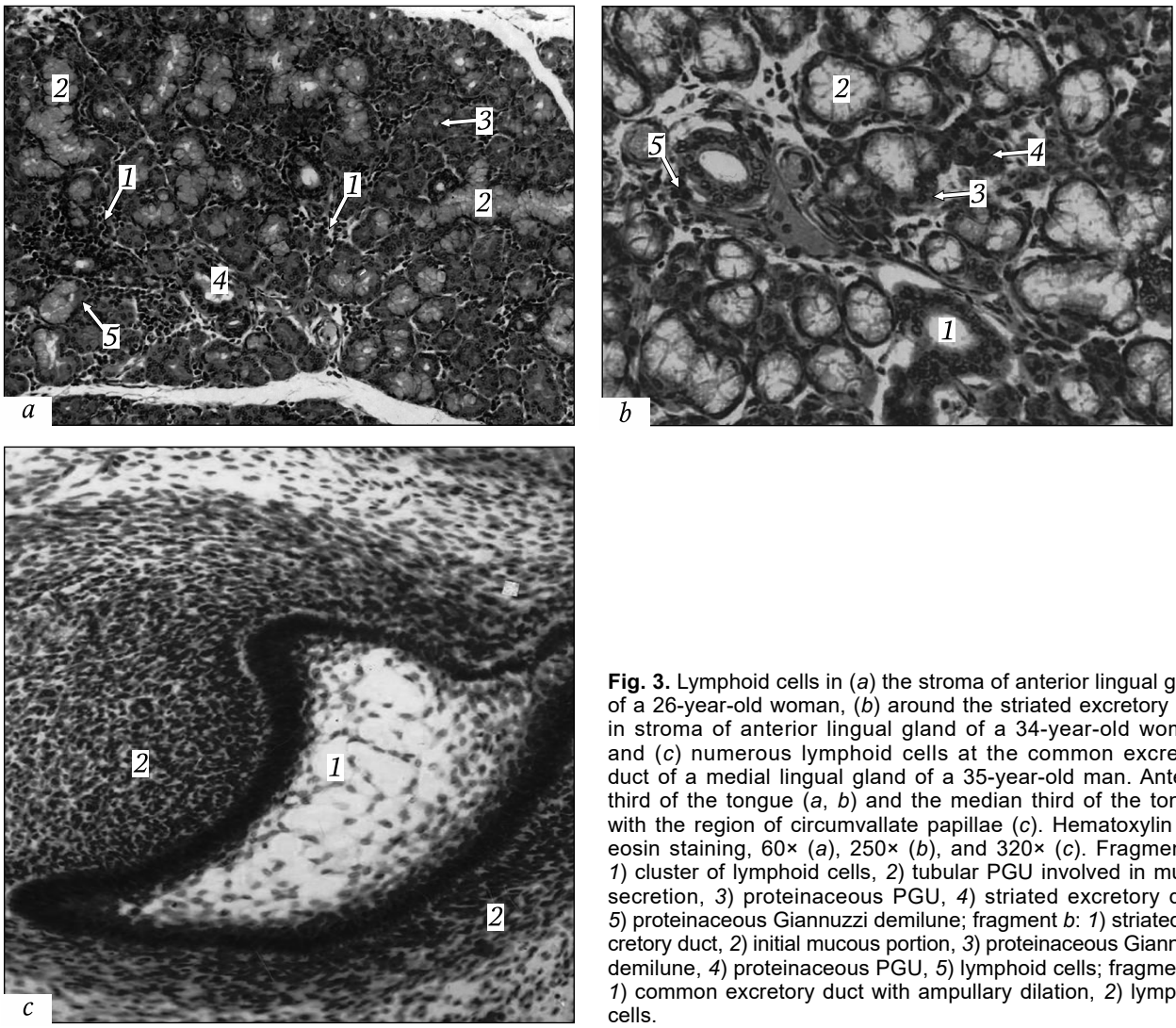

Fig. 3. Lymphoid cells in (*a*) the stroma of anterior lingual gland of a 26-year-old woman, (*b*) around the striated excretory duct in stroma of anterior lingual gland of a 34-year-old woman, and (*c*) numerous lymphoid cells at the common excretory duct of a medial lingual gland of a 35-year-old man. Anterior third of the tongue (*a*, *b*) and the median third of the tongue with the region of circumvallate papillae (*c*). Hematoxylin and eosin staining, 60× (*a*), 250× (*b*), and 320× (*c*). Fragment *a:* *1*) cluster of lymphoid cells, *2*) tubular PGU involved in mucus secretion, *3*) proteinaceous PGU, *4*) striated excretory duct, *5*) proteinaceous Giannuzzi demilune; fragment *b*: *1*) striated excretory duct, *2*) initial mucous portion, *3*) proteinaceous Giannuzzi demilune, *4*) proteinaceous PGU, *5*) lymphoid cells; fragment *c*: *1*) common excretory duct with ampullary dilation, *2*) lymphoid cells.

be consequential to nutritional uniformity (the regimen, a set of food products, *etc*.) at this age.

Reduced content of lymphoid tissue in the lingual mucosa in senile age exemplifies the total reduction of lymphoid tissue at this period. In the pharyngeal wall, the lymphoid-glandular relations greatly vary during human postnatal ontogeny [10]. Actually, despite essential development of lymphoid structures in newborns and infants, the lymphoid structures were extremely rarely observed immediately near their pharyngeal glands. However, the lymphoid cells were almost persistently found near the pharyngeal and tubular tonsils during this period. Probably, a small number of immune cells at the pharyngeal and lingual salivary glands results from nutritional uniformity of newborns and infants. In juvenile, mature, and senile ages, the lymphoid-glandular relations were most pronounced both in lingual and pharyngeal glands. In addition, distinct immunocytes were detected in PGU epithelium, where they were distributed between the secretory cells. At this, the lymphoid cells always encompassed the proximal portions of the ductal apparatus of the glands. Seemingly, such microtopography can be explained by the direct role of these cells in production of IgA to be carried through the glandular excretory duct to the surface of mucous tunica [6]. Less pronounced lymphoid-glandular relations in the pharynx of senile and long-living persons can be associated with general involution of the lymphoid tissue at this age and with common features in their lifestyle and diet [10]. Thus, the digestive system plays a peculiar role in formation of general and local immunity [11].

The revealed topographic relations between the minor glands, on the one side, and the family of lymphoid cells and their clusters, on the other side, explain the presence of lymphocytes in the secretes of these glands, which are implicated in local immunity reactions in the oral cavity in parallel with IgA. Evidently, the immunological practice in clinics needs

fundamental and comprehensive knowledge on the normal morphology of minor glands in order to better understand the mechanisms underlying the processes associated with maintenance of local immunity in the oral cavity and oral portion of the pharynx.

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