Pyloric tonsil as a novel gut-associated lymphoepithelial organ of the chicken

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Pyloric tonsil as a novel gut-associated lymphoepithelial organ of the chicken

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Summary

The pyloric tonsil is a novel peripheral lymphoepithelial organ of the gastrointestinal tract in the chicken. It forms a complete lymphoid ring at the beginning of the duodenum, where crypts of Lieberkühn are transformed to tonsillar crypts with lymphoepithelial lining. The pyloric and oesophageal tonsils (described previously) are characteristic of chicken, because they are absent in mammals. The lymphoid system develops from the middle germ layer, mesoderm and forms connections with the ect- and endoderm, namely the skin and gut, respectively. These connections are based on the lymphoepithelial lining of the crypts, and provide gates for environmental antigens. Recent findings taken together with the literature suggest that in birds the lymphoid system forms anatomically and histologically more extensive connections with the endoderm-derived organs than the ectoderm, which may be explained by the absence of regional lymph nodes, and the less developed lymphoid circulation of the skin.

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Introduction

Birds and mammals are warm-blooded animals that may also be reflected in the functional similarity of their immune system. The basic histological framework of the secondary lymphoid organs is comparable in these vertebrate species; namely, the separation of T and B dependent regions is completed.

The lymphoid system develops from the middle germ layer, the mesoderm. However, antigen information comes from the environment through the outer and inner germ layers, i.e. the ectoderm and endoderm, respectively. Therefore, the lymphoid system should create structural and functional

connections with both germ layers. The connection with the ectoderm is established by the skin–associated lymphoid tissue (SALT), while the gut- and bronchus-associated lymphoid tissues (GALT and BALT) contribute to the endodermal connections (Glick and Olah, 1981; Streilein, 1983; Fagerland and Arp, 1993; Finke and Kraehenbuhl 2001; Ratcliffe, 2002; Reese et al. 2005). In mammals both systems are well-developed, while in birds the SALT is poorly understood, which may be explained by the absence of regional lymph nodes (Olah and Glick, 1983).

Many parts of the chicken GALT have already described and named ceacal tonsil (Olah and Glick, 1979); Peyer's patches (Befus et al. 1980; Burns and Maxwell, 1986); diffusely infiltrated area of the cloaca (Dolfi et al. 1988); and Meckel's diverticulum (Olah et al. 1984; Jeurissen et al. 1989), esophageal tonsil (Olah et al. 2003; Nagy et al. 2005). These lymphoid tissue is stabil, permanent structures of the gastrointestinal tract, but scattered solitary nodules occur in the pharynx, on the top of the glandular units of the proventriculus, and in the apex of caeca (Bang and Bang, 1968; del Cacho et al. 1993; Kitagawa et al. 1996; Matsumoto and Hashimoto, 2000).

The systemic histological studies of the gastrointestinal tract of the chicken revealed an extensive lymphoid tissue at the beginning of the duodenum. This short report deals with this novel part of the GALT. Recent paper together with the esophageal tonsils provide evidence that the chicken stomach is immunologically highly protected unlike that of the mammals.

Materials and Methods

Animals: Fertilized White Leghorn eggs were obtained from CEVA-Phylaxia Hungary and incubated at 37,7°C in a humidified incubator. Tissue samples were taken from 6, 8, 12 and 16 week old chickens. Two animals per group of both sexes were used. Experimental design and condition of the animals were approved by the Animal Ethical Committee of Semmelweis University, Budapest, Hungary. In these chickens no appreciable histological differences occurred, therefore 8 week old

chicken is used to introduce the pyloric tonsil. Development of the lymphoid tissue at the entrance and exit of the stomach is under examination.

Histological procedures: For light and transmission microscopy, the tissue blocks were placed in 4% buffered glutaraldehyde for 3 h. Postfixation was carried out in 1% osmium tetroxide, followed by dehydration in ethanol, embedment in a Polybed/Araldite 6500 mixture (Polysciences), and sectioning. The 1-µm-thick semithin sections were stained with toluidine blue. For transmission microscopy, ultra-thin sections were contrasted with uranyl acetate and lead citrate. For hematoxylin-eosin staining specimens were fixed in buffered formalin and embedded in paraffin.

Immunocytochemistry: Immunostaining was performed on ten-micron thick cryostat sections. Briefly, the junction of the gizzard and small intestine was excised and fixed in 4% paraformaldehyde in Dulbecco's phosphate buffered saline (PBS) for 1 hr. After rinsing in PBS the tissue samples were infiltrated with 15% sucrose/PBS at 4^{o}C overnight. The medium was changed for 7,5% gelatin containing 15% sucrose at 37^{o}C for 1 hr. Tissue samples were rapidly frozen at -60^{o}C in isopentane (Sigma, Hungary). The cryostat sections were collected on poly-L-lysine-coated slides (Sigma), air-dried, and rehydrated in PBS before immunostaining. Rehydration was followed by incubation with primary antibodies (Table 1), biotinylated horse anti-mouse IgG (Vector Laboratories, Inc., Burlingame, CA), and avidin-biotinylated peroxidase complex (Vectastain Elite ABC kit, Vector) for 45 min. each. Before ABC incubation, the endogenous peroxidase activity was quenched by 3% hydrogen peroxide (Sigma) in PBS. The binding sites of the primary antibodies were visualized by 4-chloro-1-naphtol (Sigma). For hematoxylin-eosin staining the gizzard-duodenal junction was collected and fixed in 4% buffered paraformaldehide for 24 hours and embedded in paraffin. Sections were covered with aqueous Poly/Mount (Polyscience Inc., Warrington, PA) and examined with Zeiss Axiophot photomicroscope. Images were captured with an Olympus DP50-CU digital camera.

Results

Lymphatic tissue of the pyloric region occupies the entire wall of the gastrointestinal tract, forming a complete ring (Fig. 1A), unlike the Peyer's patches, which are found only in the anti-mesenteric side of the gut. The pyloric lymphatic tissue is sharply limited in both proximal and distal directions (Fig. 1B) and consists of at least 15-20 tonsillar units in the circumference of the gut.

The tonsillar units are well observed on both paraffin embedded, hematoxylin-eosin stained (Fig. 1C) and immunocytochemical stained sections (Figs. 1D,E,F,G). CD45-positive hemopoietic cells occupy both the interfollicular regions and the germinal centers (Fig. 1D), while the majority of the B cells are restricted to the germinal centers (Fig. 1E). The dense interfollicular regions are loaded with T cells, but many of them (possibly helper cells) immigrate into the germinal centers (Fig. 1F). Collagen III, the main collagen type occurring in the reticular fibers, is present in the „capsule" of the tonsillar units, but surprisingly, completely absent in the germinal centers (Fig. 1G).

Crypt of Lieberkühn of the duodenum are transformed to tonsillar crypt and lined by lymphoepithelium (LE) (Fig. 1H). The number of Bu1b-positive B cells (Fig. 1I) is low in the LE compared to CD45-positive cells, suggesting that the majority of the lymphoid cells in the LE are T cells. Among the epithelial cells lining the crypt M cells also occur (Fig. 1J). Many lymphocytes, probably T cells, are present at the lateral surface of the M cells (Fig. 1J).

Remarkable numbers of germinal centers are present in the tonsillar unit (Figs. 1E,F,G). The majority of the germinal centers are close to the muscular layer of the intestine and their collagen „capsule" fuses with the „capsule" of the unit. The ovoid- or round-shaped germinal centers, unlike the mammalian ones, do not show polarization or topographical connection with the crypt epithelium (Fig. 1E,F,G). Inside the germinal center, besides the supportive reticular cells, follicular dendritic cells (FDC), few macrophages, and lymphoid cells can be histologically and immunocytochemically distinguished. The supportive reticular cells produce desmin, a muscle-specific intermediate filament

(Fig. 1K), but unexpectedly do not produce collagen III positive reticular fibers (Fig. 1G), like mammalian GC. The FDC can be identified by vimentin intermediate filament (Fig. 1L) and anti-IgG because their surface membrane is „sticky" for IgG. The IgG staining is restricted to the „perikaryon", the elongated cell processes far from the nuclear region do not bind anti-IgG. This staining indicates that the FDCs are localized in the center of the GC, leaving free a peripheral rim of the follicles (Fig. 1M). Toluidin blue stained semithin section reveals two morphologically different forms of FDCs. One of them is highly elongated and contains many cytoplasmic granules in one of the cytoplasmic processes, while the other shows ovoid shaped nucleus and few cytoplasmic granules surrounding the Golgi region (Figs. 1N,O). They represent mature and immature forms of FDC, respectively. The 74.3 monoclonal antibody identifies cytoplasmic antigen in the FDC (Fig. 1P). The cytoplasm produces spike-like processes and the intercellular space is filled with electron dense substance (Fig. 2).

Discussion

The pyloric tonsil is a novel member of the GALT of the chicken, but recently to explain its regional role immediately after the stomach but at the beginning of the small intestine could be speculative.

The classical tonsilar structures at the entrance and the exit of the stomach occur in birds which are absent in mammals, but in mammals may occasionally be found solitary nodules. The absence of these well-organized lymphoid tissues in mammals, could be the reason, that was out of attention of avian immunologists. The presence and absence of esophageal and pyloric tonsils in birds and mammals, respectively suggest that these lymphoid tonsils are avian peculiarities. Thus the esophageal and pyloric tonsils at the entrance of the stomach and the beginning of the small intestine immunologically sentinel the stomach and the small intestine, respectively.

The gut-associated lymphoid tissues occur in two forms: i.) solitary and aggregated nodules under the surface epithelium; and ii.) tonsils, where the surface epithelium produces simple or branching pits, crypts or fossulae to increase the surface contact of the lymphoid tissue. In the aggregated nodules and tonsils the lymphoid tissue is organized to B and T dependent regions, which are represented by germinal centers and interfollicular dense lymphoid tissue, respectively. In the tonsil the lymphoid tissue is arranged around a crypt forming tonsillar unit, which is separated from the others by a fine connective tissue capsule, and establishes functional, immunological, pathological unit of the tonsil. No „capsule" occurs around the aggregated nodules. The epithelium lining the tonsillar crypt or covers the aggregated nodules may be infiltrated with mobile lymphoid cells, transforming the surface epithelium to LE tissue, which provides a „gate" for antigen access into the lymphoid tissue. In this aspect the LE may serve functionally as an „afferent lymphatic" for the GALT. In mammals, antigens from the GALT can get access into the mesenteric lymph nodes via afferent lymphatics, which may form a second barrier for antigen spreading. However, in the chicken, due to the absence of the mesenteric lymph nodes, the antigen can enter the blood via mesenteric lymphatics, thus the second barrier of antigen spreading lacks, which may be balanced by the highly developed tonsillar system at the entrance of the stomach, and intestine.

Earlier we described a tonsillar structure in the birds at the cardia, named esophageal tonsil (Olah et al. 2003; Nagy et al. 2005), and recently we identified another tonsil at the pyloric region. Thus, the beginnings of the stomach and intestine are immunologically sentinelled by the oesophageal and pyloric tonsils, respectively.

The plumage of birds may provide a better protection for the chicken against minor trauma, but parasites can reside in it. Parasites may initiate an immune response by means of Langerhans cells, but the route of Langerhans cells to the lymphoid tissue is still unknown, because of the lack of regional lymph nodes. But, recently small dermal lymphoid nodules were identified, which received

Langerhans-like cells from the epidermis (Igyarto et al. 2006). In spite of this new findings the SALT is still not well-established and less developed in birds than in mammals. Thus, in contrast with the GALT, the SALT is not well established and poorly developed in birds.

In conclusion, the pyloric tonsil is a novel member of the avian GALT, and its location suggests some functional role in the immunological sentinel of the small intestine.

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Tables.

Table 1. Primary antibodies used in this experiment.

Legend of figures

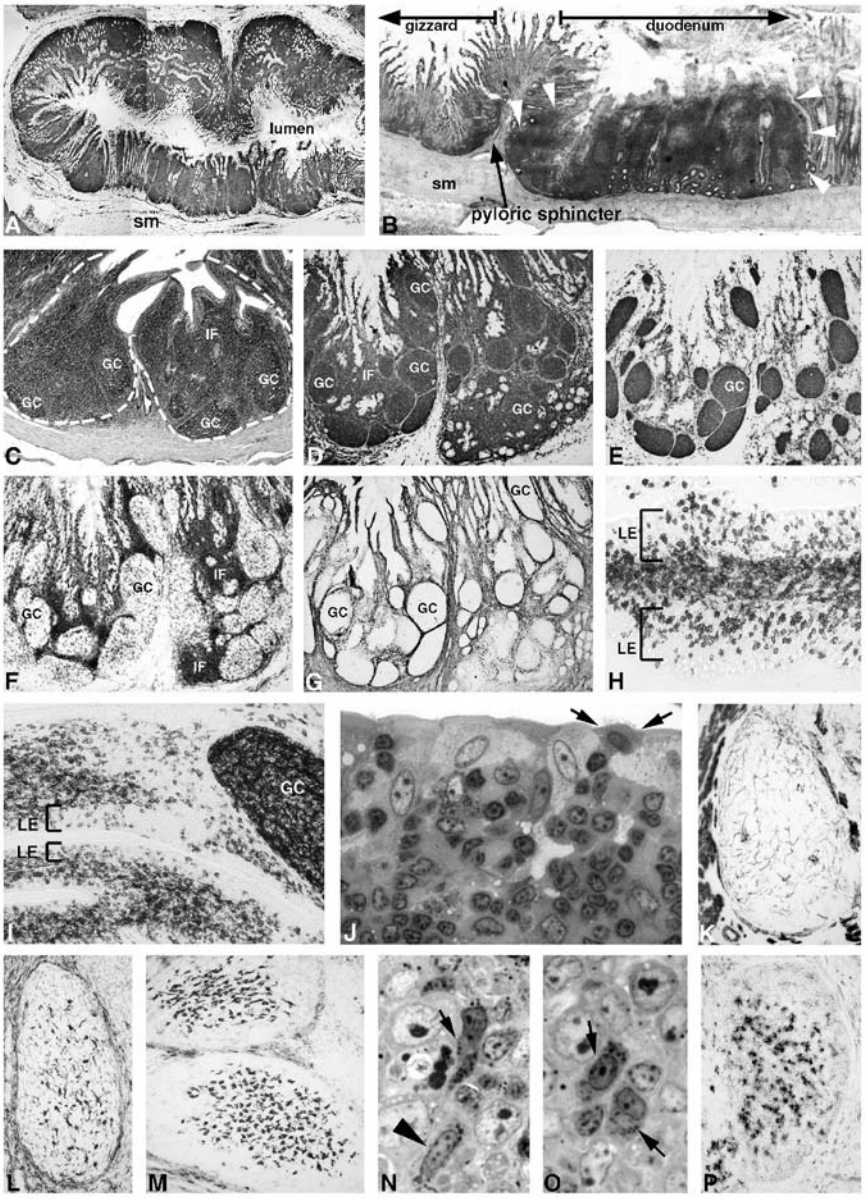
Figure 1.

Pyloric tonsil of an 8 week old chicken. A.) CD45 stained cross-section of the pyloric region. Lymphoid tissue occupies the entire mucosal layer of the gut. Magnification: 8x. B.) Hematoxylin-eosin (HE) stained longitudinal section of the pyloric region. Lymphoid tissue shows sharp borders to both proximally and distally. Mag.:12x. C.) HE stained cross-section of the pyloric region. The tonsillar units are outlined by dashed lines. Mag.: 24x. D.) CD45+ hemopoietic cells from the germinal centers (GC) and fill up the interfollicular region (IF) in the tonsillar units. Mag.: 24x. E.) Bu-1b+ B cells are densely packed in the GCs, majority of which are close to the capsule of the tonsillar unit. Mag.: 24x. F.) CD3+ T cells are accumulated in the interfollicular regions, but significant number of CD3 positive cells also occurs in the GCs. Mag.: 24x. G.) Anti-collagen III monoclonal antibody staining outlines the GCs, but the GC itself is free of collagen III. Mag.: 24x. H.) CD45+ cells infiltrate the crypt epithelium creating lymphoepithelial (LE) tissue. Mag.: 140x. I.) The number of Bu1b+ B cells in the LE are low compared to CD45+ cells. Mag.: 140x. J.) Semithin section stained with toluidin blue. Close to the M cell (arrows) lymphoid cells heavily infiltrate the surface epithelium. Mag.: 1200x. K.) The supportive reticular cells through the GC form a fine desmin intermediate filament web. Mag.: 130x. L.) Anti-vimentin staining recognizes intermediate filaments in the

follicular dendritic cells of the GC. Mag.: 130x. M.) Anti-IgG staining. FDCs locate in the center of the GC. Mag.: 130x. N.) Toluidin blue-stained semithin section shows elongated-shaped mature FDC, an FDC process with cytoplasmic granules (arrow), and an immature one FDC (arrowhead). Mag.: 1600x. O.) Toluidin blue-stained semithin section shows immature FDC with bulky cytoplasm containing few granules (arrows). Mag.: 1600x. P.) 74.3 mAb recognizes cytoplasmic antigen in the FDC's. Mag.: 130x.

Figure 2.

Transmission electron micrograph of a follicular dendritic cell. In the FDC the cytoplasmic granules are localized on one side of the nucleus and the cell surface is covered by electron dense extracellular substance (arrows). L: lymphoblast. Mag.: 4500x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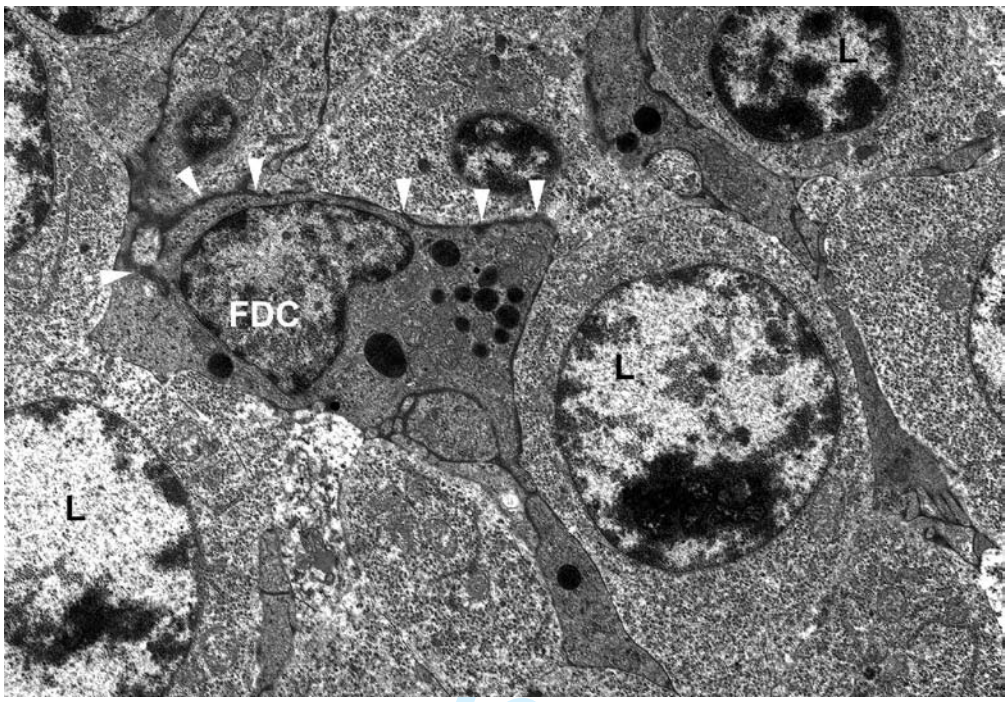

Table 1.

Cell	Antibody specificity	Antibodies	Source of antibody
Hemopoietic cell	CD45 antigen	clone HIS-C7	CEDI-Diagnostics
T cell	CD3 antigen	clone CT3	Gift from Chen-Lo Chen, Birmingham, AL, USA
B cell	Bu-1b antigen	clone 11G2	Gift from Olli Vainio, Turku, Finland
Dendritic cell	vimentin intermediate filament	clone AMF-17b	DSHB, Iowa, USA
Dendritic cell	surface IgG	clone CG-106	Sigma-Aldrich Kft, Hungary
Dendritic cell	unknown	clone CVI-ChNŁ 74.3	CEDI-Diagnostics
Connective tissue	collagen type III	clone 3B2	DSHB, Iowa, USA
Mesenchymal reticular cells	desmin	clone D3	DSHB, Iowa, USA