Immunohistochemical Study on the Distribution of Lymphoid Tissues in the Upper Alimentary and Respiratory Tracts of Chickens

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ABSTRACT. The posthatching development of lymphoid tissues and the distributions of immunoglobulin-containing cells (cIg: cIgG, cIgA, cIgM) or thymus-derived lymphocytes (T cells) which appeared in the upper alimentary tract (AT) and the respiratory tract (RT) of chickens were estimated immunohistochemically and histoplanimetrically. In adults, a marked amount of well-developed lymph nodules was noted in the upper AT as esophageal tonsils, and also in the RT as mesobronchial lymph nodules. Histoplanimetry showed the highest relative frequency in each class of cIg in the area of esophageal tonsils. A predominant frequency of cIgG compared to cIgA and cIgM was observed throughout upper AT and RT, and during the course of posthatching development, a small number of cIgG were found first in the esophageal tonsils and in the mesobronchial lymph nodules at 5 days of age. In these regions an active increase of cIgG was shown until 2 weeks of age, when the formation of germinal centers (GC) was first noted in the lymphoid tissues. In the lamina propria of the upper AT and RT, a large number of T cells appeared on the 20th day of incubation. And then the frequencies of cIg and T cells gradually increased with aging. These results suggested that the esophageal tonsils and the mesobronchial lymph nodules might act as local immune system corresponding to the Peyer’s patches of cecal tonsils in the lower AT.—KEY WORDS: BALT, chicken, GALT, Ig-containing cell, immunohistochemistry.

It is known that a considerable amount of lymphoid tissues is contained in the walls of the AT and RT. In domestic birds, these lymphoid tissues are designated as peripheral lymphoid tissues [12, 16, 30]. The lymphoid tissues associated with the AT are formed as lymphocyte aggregations or lymph nodules in the lamina propria or submucosa from the pharynx to the rectum. In general, they are called “Gut-Associated Lymphoid Tissues” (GALT), which may include the mesenteric lymph nodes in mammals [32, 37]. In chickens, esophageal tonsils, Meckel’s diverticulum, Peyer’s patches and cecal tonsils are known as well-developed peripheral lymphoid tissues in the AT [11, 18]. However, there are few descriptions of the lymphoid tissues associated with the RT. Recently it was reported that lymphocyte aggregations existed in the bifurcations of the bronchi, and they were termed “Bronchus-Associated Lymphoid Tissues” (BALT) [7]. In mammals, BALT are found most extensively in rabbits, rats, guinea pigs [8] and humans [25]. In chickens, BALT were previously described as small finger-like lymphoid protrusions of the bronchial surface, and they were identified with the naked eye [6].

GALT and BALT are believed to serve a function in the secretary IgA-linked immune system of antigen-recognition and antibody-production [4, 21, 28, 31, 36]. In mammals, the distribution of cIg has been demonstrated in the mucosa of the AT of human [13], dog [19], pig [1, 10], mouse [14] and cat [39], and in the RT of human [33], pig [9], sheep [2] and cat [39]. The develop-
Table 1. Antisera used in this study

<table>
<thead>
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<th>Antiserum</th>
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<th>Source</th>
<th>Specificity</th>
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<tr>
<td>Chicken T cell</td>
<td>Rabbit</td>
<td>HOKKAIDO Ogata Univ. et al. (27)</td>
<td>1:400</td>
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tion for immunohistochemistry, then embedded in paraffin and cut into 4 μm semiserial sections. For histology, hematoxylin-eosin or methyl green-pyronin stainings were applied.

For immunohistochemistry, deparaffinized sections were exposed to absolute methanol and 0.1% H₂O₂ in 0.01M phosphate buffered saline (PBS) for 45 min for the elimination of endogeneous peroxidase activity. Thereafter, the peroxidase-labelled indirect method [26] was applied; sections were incubated according to the following schedule in: (1) 1% normal goat or rabbit serum for 1 hr at room temperature; (2) respective primary antiserum, as shown in Table 1, for 18 hr at 4°C; (3) peroxidase-labelled goat anti-rabbit IgG antiserum (Medical and Biological Lab., LTD: MBL, diluted 1:400 or 1:600) or peroxidase-labelled rabbit anti-goat IgG antiserum (Cappel Lab., 1:100) for 2 hr at room temperature; (4) 3,3'-diaminobenzidine and H₂O₂ in Tris-HCl buffer solution [17]. Sections for the control procedures were incubated in non-immunized goat or rabbit serum instead of the respective primary antiserum. To improve staining contrast, nuclei were counterstained with hematoxylin.

RESULTS

In the upper AT and RT of chickens, three types of lymphoid tissues were iden-
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Fig. 1. Tonsilar crypt of esophageal tonsil in an adult chicken. (a) Note the epithelium, which is particularly thin at the bottom of the crypt (arrow). GC: germinal center. Hematoxylin-eosin (H-E) stain. ×75. (b) Higher magnification of the epithelium, which is heavily infiltrated with small lymphocytes, macrophages and plasma cells (arrows). Methyl green-pyronin stain. ×300.

tified according to their morphology and size.

In adults, numerous small accumulations of lymphocytes were located randomly in the lamina propria of the whole upper AT and RT. These small lymph nodule-like structures (larger than 0.1 mm in diameter), which contained no GC, were detected in the upper esophagus rather than in the lower esophagus and the proventriculus. Middle-sized lymph nodules (smaller than 1 mm in diameter), which had one or two GC, were always present in the pharynx and pyloric regions and also in the larynx. Well-developed large lymph nodule aggregations (1–2 mm in diameter) containing more than 3 GC each were located exclusively in the border zone between the esophagus-end and the proventriculus. They became the so-called esophageal tonsils and the mesobronchium.

The esophageal tonsils were covered with a thick layer of stratified squamous epithelium and have tonsillar crypts. The epithelium was much thinner in the bottom region of the crypt (Fig. 1a). In the interfacing zones between the covering epithelium and the lymph nodules, there was a large number of small lymphocytes, plasma cells and macrophages (Fig. 1b).

In the mesobronchium, where the bronchial cartilages had disappeared, groups of lymph nodules in mushroom-like mucosal protrusions in the bronchial lumen were found (Fig. 2a). These lymph nodules, which contained one or two GC each, were constantly demonstrated in the protrusions. The epithelium overlying the lymph nodules was always infiltrated with lymphocytes. Clusters of macrophages containing foreign materials were observed around the GC (Fig. 2b).

In the adults, a number of cIgG, cIgA or cIgM were demonstrated immunohistochemically throughout the lamina propria of the upper AT and RT. They were recognized as typical plasma cells, or middle or large-sized lymphocytes. The relative frequencies of these three types of cIg per unit area of lamina propria were evaluated and shown in Fig. 3. In the pharynx, the cIg were found throughout the lamina propria,
Fig. 2. Mesobronchial lymph nodules showing mushroom-like mucosal protrusions. (a) lymphoid follicles, containing one or two germinal centers (GC). H-E stain. ×30. (b) Higher magnification of lymph nodule and germinal center (GC). Note the cluster of macrophages which ingested foreign materials. H-E stain. ×300.

Fig. 3. Relative frequencies of the three types of clg per unit area (0.156 mm²) in the lamina propria of the upper AT (1–6) and the RT (7–9) of adult chickens. 1. Pharynx, 2. Esophageal area (lower esophagus), 3. Esophageal tonsil, 4. Proventriculus, 5. Ventriculus, 6. Pylorus, 7. Larynx, 8. Trachea, 9. Mesobronchium.

and the relative frequency of the clgG predominated over that of the clgA and clgM. This tendency appeared commonly in each portion throughout the upper AT. Most of the clg were localized just underneath the epithelium and adjacent to the basal portions of the salivary gland, and especially the clgA or clgM showed a tendency to be distributed in the areas surrounding the acini (Fig. 4). Anti-thymus lymphocyte serum (ATS) reactive cells were also scattered in the lamina propria; and

Fig. 4. clgA in the pharynx of an adult chicken. Most of the cells are located in proximity to the glandular acini. ×300.

were predominantly located underneath the epithelium and in the extranodular areas.
The number of clg found in the mucosa of the upper esophagus, crop and lower esophagus was almost the same; however, the number was fewer in the pharynx. In the esophageal area, clgA or clgM were predominantly observed adjacent to the esophageal glands.

In the esophageal tonsils, the frequency of clgG was the highest in all the regions examined in this study as shown in Fig. 3. Most of the clg were found exclusively in the extranodular regions, and concentrated mainly near the GC and the epithelium (Fig. 5). In some GC, weak reticular staining specific for IgG and IgM and a few middle or large lymphocytes with cytoplasmic staining of IgG were observed (Fig. 6). ATS-positive cells were located preferentially in the areas surrounding the GC.

In the proventricular mucosa, the total numbers of clg were relatively fewer than those in the upper AT, but there was a predominant number of clgM or ATS-positive cells. In the pyloric region, although the relative frequencies of the three types of clg were almost the same as those in the esophageal region, the clg were concentrated in the extranodular areas, and a larger number of clgA was detected distally to the duodenum. In the follicle-

Fig. 5.  clgG in the esophageal tonsil area of an adult chicken. Most of the cells are concentrated in areas close to the germinal centers (GC) and the epithelium. ×150.

Fig. 6.  A GC in the esophageal tonsil area of an adult chicken, showing a few middle or large-lymphocytes with cytoplasmic staining and a weak reticular staining specific for IgG. ×300.
associated epithelial cells in this region, an intense positive reaction only for IgA, but not for IgG and IgM, was confirmed (Fig. 7a, b).

In the larynx, the highest frequency of cIgG was evaluated throughout the RT. And in the tracheal mucosa, the number of cIgG was predominant as compared with that of cIgA or cIgM; however, their relative frequencies were very variable. Throughout the tracheal mucosa, these cIg were scattered in the lamina propria in close association with the epithelium and glands. In the mucosa of the mesobronchium, in which many of well-developed lymph nodules were aggregated, the total number of cIg was relatively small in the mucosa of the RT, although many pyroninophilic plasma cells were localized just beneath the epithelium or around the GC. In this area the number of cIgA was fewer than that in any other mucosa of the RT. Some GC showed a weak reticular staining specific for IgG and IgM but not for IgA, and some middle or large lymphocytes with cytoplasmic reactions were also observed within the GC. Furthermore, ATS-positive cells were located in areas surrounding the GC (Fig. 8). Throughout the mucosa of the RT, intensive ATS-positive cells were widely distributed, and some of them were found extensively in the mucosal epithelium.

During the course of posthatching life, the relative increase in the number of cIg in the mucosa of each region was shown in Fig. 9 a, b.

At 5 days of age, a few cIg were detected in the esophageal tonsils and in the mesobronchium areas, and cIgG were extensively predominant. In all the areas, except for those of the esophageal tonsils and mesobronchium, less active increases of respective cIg in number were shown until 2 weeks of age, when a marked increase of cIgG and an
active formation of GC occurred in the lymphoid tissues. Numerous ATS-positive cells in clusters had appeared previously at the 20th prehatching day in these areas (Fig. 10). In other lamina propria both in the upper AT and RT, the relative numbers of the three types of cIg and the ATS-positive cells showed a tendency to increase constantly up to around 4 weeks after hatching as aging proceeded. A predominant number of cIgG was observed in all the regions and at every stage examined.

DISCUSSION

It was reported previously that chicken esophageal tonsils developed at the distal end of the esophagus [18]. In the present study, it was confirmed that the structures of chicken tonsils were similar to those of palatine tonsils of mammals. In parallel with these structures, BALT were also described in chickens [6, 29], and these lymphoid tissues in the mucosa of the adult mesobronchium corresponded to the tonsils. In mammals, cIg distributed in the lamina propria of the intestinal and respiratory mucosa are considered to have close functional correlations with local immunity, and in the mucosa, the predominance in frequency of
cIgA has been shown [2, 9]. However, in the chicken respiratory and general intestinal mucosa, two conflicting findings were presented. One was that the cIgA were predominant in these mucosa as described in mammals [5, 24]. The other was that the cIgG were more frequent than the cIgA in the mucosa of the intestine and lung [3, 23], and present histoplanimetric results suggested that the cIgG were predominant throughout the mucosa of both the upper AT and RT.

These findings may support the idea that the esophageal and mesobronchial lymphocyte aggregations are not only characteristic of lymphocyte proliferation for IgA supply, but also a part of the systemic immune system by IgG.

Hoshi and Mori [20] detected GC in the cecal tonsils of chickens on the 8th day posthatching, although in the present study the GC were observed first in the lymph nodules of esophageal tonsils and mesobronchium at 2 weeks of age, which corresponded to the active period of increase of the cIg in these areas. Furthermore, two types of GC were reported in chick spleen, suggesting that type I GC might have produced the precursors of antibody-producing cells, and the quantitative changes of the cells agreed well with those of type I GC [27]. The present results showed that the GC appeared at any stage beyond 2 weeks were furnished with immunoreactive reticular cells specific for IgG or IgM, and that a few juvenile large lymphocytes had cytoplasmic reactivities for IgG or IgM.

The GC of chickens were reported to be bursa-dependent in ontogeny [12] and more recently, it was reported that T cells were important for the formation of new GC in nude mice [22], and that in newborn rats, Peyer's patches were mainly populated first by T cells, then by B cells as time passed [34]. In the present study, some ATS-reactive lymphocytes were observed in the esophageal tonsils and in the mesobronchial areas on the 20th prehatching day. In these areas, at any stages beyond 2 weeks of age, numerous lymphocytes inclusive of ATS-reactive cells were located mainly in the areas surrounding the GC.

The past evidence and the present
findings suggest that the GC in lymphoid tissues may have some relation with the differentiation and proliferation of peripheral cIg, although no cellular transformations from lymphocytes to plasma cell series in GC have been suggested from pure morphology [15], and that such ATS-reactive cells might present the optimum conditions for a new formation of GC.

The results of this study provided evidence for the following conclusions: 1. the esophageal tonsils in the upper AT and the lymph nodules in the mesobronchium of the RT have some function in chicken local mucosal immunity as well as Peyer’s patches or cecal tonsils in the lower AT, and 2. the lymphocytes that actively populated these lymphoid tissues up to the 2nd week of age may be differentiated and proliferated into competent cIg with the possible cooperation of ATS-reactive cells.

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REFERENCES


要　約

ニホントクリの上部消化管および気道付属リンパ組織に関する免疫組織化学的研究：荒井延明・橋本善春・北川浩・佐賀郡美（北海道大学教職員図書館図書館）——ニホントクリの上部消化管および気道付属リンパ組織の分布について組織学的に観察し、粘膜固有層中に出現するIg含有細胞（IgG、IgA、IgM）およびT細胞の分布を計測した。結果、特にT細胞の分布が顕著であった。上部消化管および気道付属リンパ組織においては、特に胃の粘膜固有層に多く、気道付属リンパ組織においては気管支を除く。胃の粘膜固有層に多く、気道付属リンパ組織においては気管支を除く。