Alpha-naphtyl acetate esterase (ANAE) activity and plasma cells in the oesophageal tonsils of chickens

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SUMMARY

The aim of this study was to determine the localization of T lymphocytes and of plasma cells in the oesophageal tonsils of avians using ANAE staining and methyl-green pyronin method respectively. Eighteen Ross 308 breed male broilers served in this study. Lymphoid tissue in the form of lymph follicles or lymphocytic infiltration was encountered in the lamina propria and between corpus glandulae of the mucous glands in parts of the oesophagus before and after the crop and at the oesophagus junction with the crop. However, tonsil-like lymphoid tissues could only be seen in the distal parts of the mucosal folding at the oesophagus junction with the proventriculus. ANAE positive cells were mainly observed in the germinal centres of the tonsils, and only rare positive cells were encountered in the IFA (inter-follicular areas) and in the lymphoepithelium. In oesophageal tonsils, plasma cells appeared to be stratified beneath the epithelium, but some B cells infiltrated the epithelium. The plasma cells were also encountered in the IFA and in the germinal centres. The oesophageal tonsils were presumed to form a barrier against foreign particles taken orally and also to play an important role in the cellular and humoral defence against various antigens.

Keywords : alpha-naphtyl acetate esterase - plasma cell - oesophageal tonsil-chicken.

RÉSUMÉ

Localisation de l’activité α naphtyl acetate estérase (ANAE) et des plasmocytes dans les tonsilles oesophagiennes chez le poulet. Par S. KUM, U. EREN and M. SANDIKCI.

Cette étude a pour objectif de déterminer la localisation des lymphocytes T et B dans les tonsilles oesophagiennes de poulets en détectant l’activité ANAE des lymphocytes T et en colorant spécifiquement les lymphocytes B par le vert méthyl et la pyronine. Au total, 18 poulets de chair de race Ross 308 ont été utilisés. Les tissus lymphoïdes sous forme de follicules lymphoïdes ou d’infiltrats lymphocytaires ont été localisés dans la lamina propria et entre les glandes muqueuses dans les portions oesophagiennes antérieures et postérieures au jabot et dans la zone de jonction avec le jabot. Cependant, des tissus lymphoïdes d’apparence tonsillaire n’ont pu être vus que dans les portions distales des plus muqueux de la zone de jonction de l’oesophage avec le proventricule. Les cellules ANAE positives ont été principalement localisées dans les centres germinatifs des tonsilles et seulement quelques rares cellules positives ont été observées dans les zones interfolliculaires et dans le lympho-épithélium. Dans les tonsilles oesophagiennes, les plasmocytes ont été principalement rencontrés en position sous-épithéliale. Quelques cellules B ont également infiltré l’épithélium et d’autres ont été détectées dans les zones inter-folliculaires et dans les centres germinatifs. Les tonsilles oesophagiennes constituerait donc une barrière à l’égard des exo-antigènes administrés oralement et joueraient un rôle important dans les défenses humorales et cellulaires contre des antigènes variés.

Mots-clés : α naphtyl acétate estérase - plasmocyte-tonsille oesophagienne- poulet.

Introduction

The mucosal immune system is especially important in food allergy, tolerance against undigested antigens and intestinal infections. Following the orally intake of foreign antigens, helper T lymphocytes as well as B lymphocytes are activated to give the mucosal immune response. This response is subdivided into two types as; NALT (Nasal-associated lymphoid tissue) and GALT (Gut-associated lymphoid tissue) [10]. In several species (for example sheep, rabbits and chickens) the GALT plays an important role in the development of B lymphocytes [21]. In avians, the GALT is a lymphoid structure containing lymphoid aggregates distributed within the epithelium of the Fabricius Bursa, caecal tonsils, Peyer’s patches, Meckel’s diverticulum and lamina propria of the gastrointestinal tract [10]. Investigators have studied the Bursa Fabricius [5-7, 11, 14], caecal tonsils [20], Peyer’s patches [1], and the Meckel’s diverticulum [19]. In recent years GALT has been reported to be an important component of the oesophageal tonsils [17]. The oesophagus in cross section is seen to be stellate-shaped with six to eight longitudinal folds. The lymphoid tissue is localized at the distal ends of the folds and infiltrated the stratified squamous epithelium. This epithelium has been named the lymphoepithelium. Oesophageal tonsils, because of their proximal location within the stomach, are exposed frequently to environmental as well as food antigens. The oesophageal tonsils are thought to offer a barrier to the continual stimulatory effects of these antigens on the immune system [17].

The histological and histochemical structures of the oesophageal tonsils considered as one of the components of GALT is of prime importance for understanding its role in cellular and humoral defence. This study was aimed to determine the location of T lymphocytes based on their activity with alpha-naphtyl acetate esterase enzyme and those of plasma cells by the methyl-green pyronin method.

Material and Methods

In the study, the chickens were fed with corn-soy bean as predominant food ad libitum. Eighteen 42 day-old Ross 308 breed male broiler chicks of average fresh weight of 2000 g were used in this study. The chicken did not receive any treatment at the time of inclusion in the study. All animals were slaughtered and immediately after slaughter, tissues were taken. Tissue samples were obtained from oesophagus junction with the crop, from the oesophagus junction with the proventriculus and parts of pre and post crop of oesophagus. The parts of pre and post crop of oesophagus were first divided into four parts. Thereafter, each part was again divided into two parts; one for enzyme staining and the other for paraffin blocking. The tissue samples reserved for enzyme staining were first fixed in a formol-sucrose solution for 22 hours at + 4°C (pH 6.8) and then kept in Holt’s solution for another 22 hours. Serial sectioning of the tissue 6 µm thick was made at 50 µm intervals. Activity of ANAE was ascertained by incubation in a medium consisting of 40 ml of 0.067 M phosphate buffer, pH 5.0, 2.4 ml of hexazotized paraarsaline (paraarsaline was purchased from Sigma P-3750), and hexazotization was done with 4 % sodium nitrite) and 10 mg of α naphthyl acetate (Sigma N-8505) in 0.4 ml acetone. The mixture was adjusted to pH 5.8 using 2 N NaOH. The sections were incubated at room temperature for 5 minutes [13] and counterstained with methyl green.

The tissue samples reserved for paraffin blocking were first kept in 10% Neutral Buffer Formalin (NBF) solution and then embedded in paraffin after the necessary tissue treatment. Serial sections of 6 µm thickness at 50 µm intervals apart were cut from the paraffin blocks. For the demonstration of plasma cells the sectioned tissues were stained by the methyl-green pyronin staining method [4]. ANAE positive cells from various parts of the tissues and the localization of plasma cells were done under examination with a light microscope (Leica DMLB microscope). Photographs of the tissues were taken when seemed necessary with a Leica DC-200 camera.

The number of ANAE positive T cells and plasma cells were determined by counting 10 microscopic fields (400X magnification) in each tissue section per animal. To determine whether or not statistical significance among the groups Kruskal-Wallis test was employed. The Duncan’s test was used for the determination of an eventual difference of cell distribution according to the tissue origin. For this purpose SPPS 10.0 for Windows® statistic package program was used. The difference was considered as significant when p level was less than 0.05.

Results

The numbers of ANAE positive T cells and plasma cells were given in Table I according to their localization in the upper gastrointestinal tract. The T cells were mainly found in lamina propria, then on tonsils and between the corpus glandulae. The B cells were predominantly evidenced into tonsils, and underneath the surface epithelium at a lesser extend. In lamina propria and in areas between the corpus glandulae, their numbers declined. Whereas the distribution of B cells was quite similar for the 18 chickens examined, some birds exhibited a higher proportion of ANAE positive T cells into lamina propria (birds n°5, 8, 10, 14 and 18), or into tonsils (birds n°2) or in both (birds n°9 and 19).

Examination of the serial sections revealed lymphoid tissue evidencing by ANAE positive cells (Figure 1) in the form of lymph follicles and lymphocytic infiltrations within the lamina propria and between the corpus glandulae of the mucous glands (Figure 2). However, these tonsil-like lymphoid tissues were also observed in the distal parts of the mucosal folds in the transitional zone between the oesopha-
gus and proventriculus, spreading across the lamina propria. Within these areas, as well as in the other parts of the oesophagus, the lymphoid tissues were found to be covered by a stratified squamous epithelium with reduction in thickness and constituted the lymphoepithelium (corresponding to epithelium with lymphocytic infiltration). In these transitional zones, proventricular glands (PG) were also observed. The lymphoid tissues surrounding the crypts were of tonsillar form, with germinal centre (GC) and interfollicular areas (IFA) (Figure 3). The lymphoid tissue were observed surrounding the mucous glands or in areas where corpus glandulae opened into the excretory ducts or between two corpus glandulae. The epithelium here was transformed into lymphoepithelium (Figure 4). Whereas ANAE positive cells were mainly densely packed in the germinal centres within the tonsils, scattered ANAE positive cells were also observed in IFA (Figure 5). Some ANAE positive cells were found scattered across the lymphoepithelium that lined the lymphoid tissue. In the oesophageal tonsils plasma cells were mainly found underneath the epithelium, but some plasma cells infiltrating the epithelium could also be noted (Figure 6). Plasma cells were also encountered in the IFA and in germinal centres (Figure 7). The presence of plasma cells (Figure 8) and of few ANAE positive cells were also found in the lymphoid tissue surrounding mucous glands.

**Discussion**

Lymphoid tissue in the avian oesophagus has been named the oesophageal tonsil [17]. In the study presented here, the distribution of T lymphocytes within the oesophageal tonsils and the localization of plasma cells were determined using the activities of the ANAE stain and the methyl-green pyronin methods respectively.

The ANAE enzyme has been reported to serve as a marker of T lymphocytes in tissue sections and peripheral blood [2]. Monocytes, macrophages and reticular cells also show ANAE activity. In T lymphocytes the enzyme staining pattern is granular, whereas it is diffuse in monocytes, macrophages and reticular cells [16]. The T lymphocytes express IgM or IgG receptors and according to the type of Ig receptors, they exhibit different functions: those with IgM receptors facilitate B cell proliferation and differentiation while the IgG receptor T lymphocytes probably have suppressive effects. GROSSI et al., [9] reported that the IgG receptor car-

rying by T lymphocyte was associated to ANAE positivity.

OLAH et al., [17] reported that the lymphoid tissues in the oesophageal tonsils were embedded in the lamina propria in egg-laying hens. Mostly B lymphocytes are found in the germinal centre but some B cells were scattered in IFA. On the other hand, T lymphocytes occurred in large numbers in the germinal centres and in the T cell dependent areas of IFA. By contrast, in this study, whereas enzyme positive T lymphocytes were predominantly found in the germinal centres of the oesophageal tonsils, only few positively ANAE stained T cells were distributed sparsely in the IFA. Plasma cells, however, were found to densely populate the sub-epithelial areas and were encountered in the germinal centres and also in IFA.

In their study on avians, MATSUMOTO and HASHIMOTO [12] reported lymphocytic aggregations localized in three different regions of the lamina propria in the proventricular mucosa: i) in the area immediately lying beneath the surface epithelium ii) in the area adjacent to the excretory ducts of the proventricular glands and iii) in the glandular tissue itself. Whereas T lymphocytes occupied the centres of the lymphoid structures underneath the surface epithelium and those surrounding the excretory ducts, B lymphocytes were found to be located in periphery. In this study, the localization of T lymphocytes was in agreement with the findings of MATSUMOTO and HASHIMOTO [12]. Besides, MATSUMOTO and HASHIMOTO [12] demonstrated that the germinal centres of the lymph follicles in the proventricular glands were rich in B lymphocytes while T lymphocytes occupied the peripheral areas.

B cells and a few T cells and macrophages have been encountered within the lymph follicles in the medulla of the Bursa Fabricius [18]. Areas of diffuse infiltration by lymphoid cells could also be seen in the Bursa of Fabricius. These areas demonstrated characteristics and histological properties of the thymus dependent areas seen in mammals, with lot of endothelial venules, reticular filamentous components and lymphocytes that infiltrated the epithelium. In this area the ANAE enzyme staining demonstrated numerous T lymphocytes [15]. CORTES et al., [3] who investigated the presence of a T cell dependent area in the Bursa of Fabricius suggested that the diffuse infiltrative areas play an important role in the immune response against antigens that reach the bursal lumen through the cloaca.
The Peyer’s patches which are lymphoid aggregates in the small intestines contain lymphocytes located under the epithelium and T lymphocytes occupying the centre [1]. In this study, it was also observed that ANAE positive T lymphocytes were mainly found in the germinal centre whereas plasma cells were located in the sub epithelium.

In avians, the caecal tonsils which have germinal centres similar to the Peyer’s patches, are structures with diffuse lymphoid tissues. Both B and T lymphocytes have been encountered in their germinal centres [10]. In another study conducted on the caecum of birds [8], the lymphoid tissue was predominantly constituted by CD4+ and CD8+ cells while the scarce B lymphocytes found expressed either IgM or IgA. Results obtained from the present study presented demonstrated similar lymphoid structures to the caecal tonsils.

In birds, the Meckel’s diverticulum is a remnant of the vitelline pouch in the small intestines and is regarded as a lymphoid tissue [19]. LILLEHOJ and TROUT [10] have found B lymphocytes and macrophages within the germinal centres in the Meckel’s diverticulum. In addition to the ANAE positive T lymphocytes found within the germinal lymphoid tissue [19]. LIILEHOJ and TROUT [10] have demonstrated similar lymphoid structures to the caecal tonsils.

In conclusion, lymphoid foci in the form of lymph follicles or lymphocytic infiltrates were observed in the lamina propria and between the corpus glandulae of the mucous glands in the areas laying the crop and in the oesophagus junction with the crop. However, tonsil-like lymphoid tissues could also be found within the distal ends of the mucosal folds in the oesophagus junction with the proventriculus. The location of ANAE positive T lymphocytes and plasma cells in the oesophageal tonsils was found to be similar to the pattern in other GALT sites. The oesophageal tonsils were thought to offer a transit gate for foreign materials taken orally, and to play an important role in the fight against antigens both cellularly and humorally.

References