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Light Microscopy of Bronchial Associated Lymphoid Tissue of Healthy Domestic Cat with Suggested New Nomenclature

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Abstract

Background: Bronchial associated lymphoid tissue (BALT) is present in humans and several other animal species including the cat which was reported that only present during infection. The role of BALT in antigen uptake and immune response is well established, and its location is varied in different species. The aim of this study was to investigate the presence and morphology of healthy cat lungs lymphoid tissue using traditional light microscopical techniques.

Methods: Tissues were collected from all lung lobes from (2 to 3 years old) healthy domestic cats and processed using standard histological techniques. The tissues were cuts into small pieces and embedded in an Epon-Araldite mixture. Tissue sections were stained with hematoxylin and eosin, and periodic acid Schiff stains.

Results: The majority of lymphatic tissue was located along the bronchial tree and associated neurovascular bundles. Both nodular and diffuse forms were present in healthy domestic cats. These lymphoid tissues extended distally into the wall of terminal bronchioles. The nodules had distinct marginal and central areas and were located in the tunica adventitia of the bronchi, bronchioles and diffusely infiltrated the adventitia of the wall of adjacent blood vessels. However, a diffuse form was found primarily in the submucosa of the bronchial tree intermingled with the submucosal glands. In addition, the diffuse form was also located around bronchioles, blood vessel and alveoli.

Conclusion: This study shows presence of diffuse and nodular forms of lymphoid tissues within the lungs of a healthy cat. The nodular form was not only associated with bronchial tree all the way down to terminal bronchioles, but also with veins, arteries and parenchyma of the lungs. Hence, PALT (Pulmonary Associated Lymphatic Tissue) seems to be a better nomenclature than BALT to describe the relationship and distribution of the distal lymphatic tissue in the lungs of healthy domestic cat.

Keywords: Cat; Pulmonary; Lymphoid tissue; BALT; Lung; Bronchial tree

Introduction

Intrapulmonary lymphoid tissue of lungs has been reported in different animal species and man [1-9].These lymphatic tissues have been given various names such as bronchial associated lymphoid tissue (BALT), intrapulmonary, sumps, pulmonary tonsils and lymphoid tissue. Bienenstock and co-workers [10,11] used the name BALT after observation of sub-epithelial follicular lymphoid aggregates in the parenchyma of most mammalian species including man. Classically, BALT as a term is used to indicate an intrapulmonary lymphoid tissue in association with the adventitia of the pulmonary vessels and bronchi [7,11-14].

Previously, Macklin [6] referred to this lymphoid tissue as "sumps" or "pulmonary tonsils" in which dust and organisms were trapped. BALT is occasionally present in mice [15,16] and in healthy humans [17]. However, there is great variability in the presence and/or distribution of BALT among animal species. Pabst and Tschernig [14] described BALT as a tertiary lymphoid tissue and are not present in all species or age groups. Reports indicate that BALT is normally absent in cats, dogs and Syrian hamsters [3,7]. However, it is frequently found in chickens [18].

Although lungs are generally not regarded as lymphoid organs, experimentally lungs have been shown to be an important area of antibody production [19]. In 2002, Ahmed and co-workers [20] found that lymphocytes originate from the marginal zone of BALT follicles and invade the epithelium to produce lymphoepithelial lesion-like structures. Furthermore, Guinee [21] described that both T and B cells are present in BALT with T cells being the major cellular elements. BALT, which is usually absent in naive mice and man, becomes well developed during certain disease conditions including pulmonary infections [8], and is induced by pulmonary infection, which is not restricted to the upper airways. Richmond [22] reported an increased incidence of BALT in smokers with chronic inflammation, which has been referred to as inducible BALT [16]. It has been observed that BALT participates in immune response through a cascade of adhesion processes by selectively recruiting naive T and B cells in high endothelial venules [23].

Contrary to the reports indicating the absence of BALT in the domestic cat [3,7], we present here the morphological characteristics and the extent of lymphatic tissue in healthy adult domestic cat by using traditional histological techniques.

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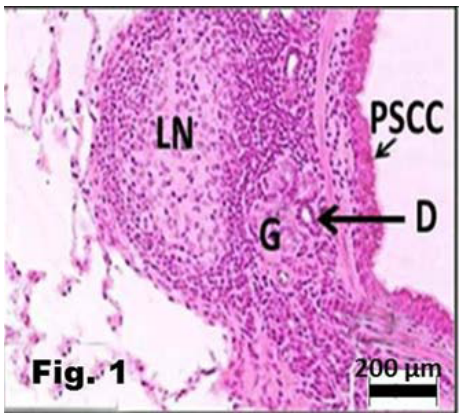

Figure 1: Mature nodular lymphoid tissue in the wall of bronchus. PSCC-Pseudostratified columnar ciliated epithelium; LN- lymph nodule; D- Duct; G-Gland, PAS-stain.

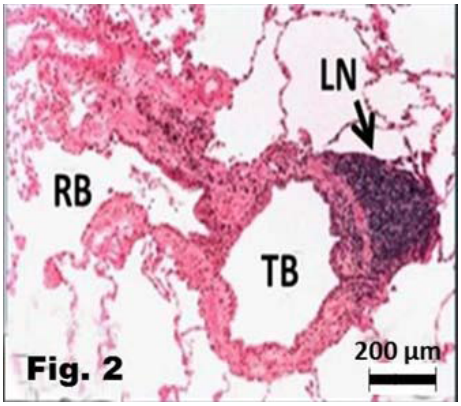

Figure 2: Lymphatic nodule in the adventitia of terminal bronchiole (TB), showing also lymphocytic infiltration of the lamina propria. RB- Respiratory Bronchiole; LN- Lymph Nodule, H&E stain.

Materials and Methods

Four healthy adult domestic cats (*Felis catus*) of known age (2 to 3 years old) were maintained on a regular diet in the animal facility (The University of Tennessee, Knoxville, TN 37901). The cats were euthanatized by intravenous injection of ketamine (5 mg/kg) and xylazine hydrochloride (4 mg/kg). Immediately after death, the trachea was exposed and cannulated for intraluminal infusion of 3% glutaraldehyde in phosphate buffer (pH 7.4, 1 M) at a pressure of 20 cm H_2O into the tracheobronchial tree. After fixation in situ for 30 minutes, the thorax was opened and the lungs with the trachea were carefully removed. Tissue samples were taken from the principle bronchus and from each lobe of the lung. The specimens were cut into small pieces and immersed in the same fixative for 48 hours before further processing.

Fixed tissues were washed three times in phosphate buffer (pH 7.4, 0.1 M). Subsequently, the samples were dehydrated in a graded series of ethanol and acetone, and embedded in an Epon-Araldite mixture. Three micrometer sections were cut using an ultratome. Sections were mounted and stained with hematoxylin and eosin and periodic acid Schiff stains. The slides were examined for the presence of lymphatic tissue in both lungs using Nikon Eclipse E400 light microscope.

Results

BALT nodules were identified along the bronchiolar tree down to the terminal bronchioles (Figures1,2). However, nodular aggregates were also noted in the interstitial tissue of lung parenchyma (Figure 3). Mature BALT nodules (Figure 1) had characteristic pale eosinophilic germinal centers and a dark stained peripheral mantle of lymphocytes, and was only found associated with bronchi (Figure 1). The lymphocytes in the mantle layer attained darkly stained nuclei with occasional mitotic figures and macrophages. Immature nodules represented by an aggregate of lymphoid tissue which lacks germinal centers were associated with segmental division of the bronchial tree (Figure 2), parenchyma of the lung (Figure 3) and blood vessels (Figure 4).

Diffuse lymphoid tissue was observed in the wall of the bronchi and bronchioles (Figure 5,6). The quantity of diffuse lymph tissue decreased gradually toward the terminal and respiratory bronchioles (Figures 2). Mononucleated cells infiltrated the wall of the pulmonary artery from the side abutting the nodular form of the bronchus (Figure 5). It was composed of variable number of mononuclear cells which includes lymphocytes and macrophages (Figure 6B). The cellular components were scattered within the connective tissue of the bronchus and intermingled with the glands in the lamina proprio-submucosa. A few high endothelial venules were present in the lamina propria (Figure 6A).

The surface epithelium of the terminal bronchioles overlying

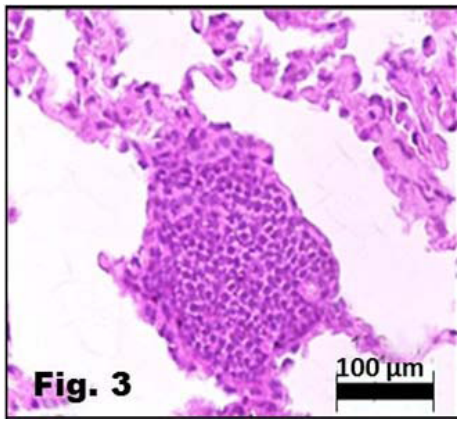

Figure 3: Lymphatic nodule in the interstitial space of alveoli. PAS-stain.

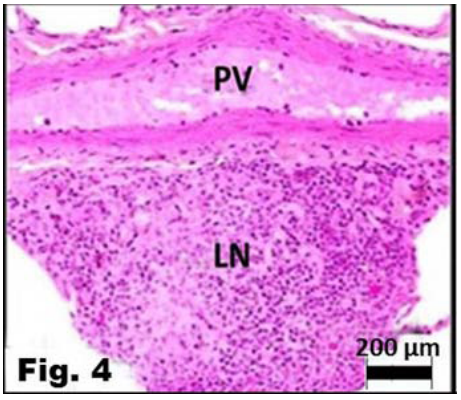

Figure 4: Well develop lymphatic nodule in the adventitia of pulmonary vein (PV), LN- Lymph Nodule.

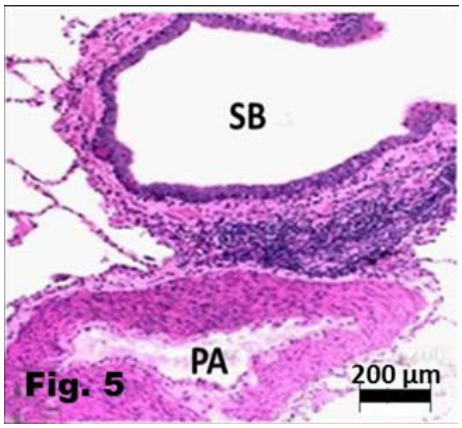

Figure 5: Tunica media of pulmonary artery (PA) was infiltrated with mononuclear cells; SB-Secondary bronchiole, PAS- stain.

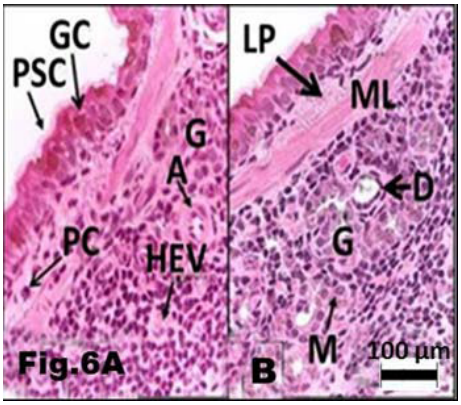

Figure 6: (**A**)- Lymphocytic infiltration of lamina propria of bronchus; PSC- Pseudostratified columnar epithelium; GC- Goblet Cell; G- Gland; A- Arteriole; HEV-High Endothelial Venule; PC-Plasma Cell. (**B**)- Diffuse lymphoid tissue scattered among the glands in the wall of a bronchus; LP- Lamina Propria; ML- Muscular Layer, G- Gland; D- Duct; M- Macrophage; H&E stain.

nodules was low columnar to high cuboidal and had no signs of lymphocytic infiltration. Few plasma cells were also observed at the periphery of nodules (Figure 6A). The bronchial muscular layers were interrupted in the area over a nodule. A number of arterioles and high endothelial venules were observed in the region between the smooth muscle cell layers of the bronchus and the nodule.

Discussion

Both nodular and diffuse lymphoid tissues were found in healthy domestic cats.The nodular form is found in interstitial tissue, adventitia of pulmonary vessels, bronchi and bronchiole walls reaching the terminal bronchioles of the lung. We suggest that pulmonary associated lymphoid tissue (PALT) should be used for the cat instead of BALT. PALT reached the base of the surface epithelium through narrow trails of loosely packed lymphocytes' thus interrupting the smooth muscle layers of the bronchi in the area above the nodule to allow free movement of lymphocytes and plasma cells to the subepithelial region.

Schummer and coworkers [24] have reported the presence of lymph nodes along the primary bronchi in the cat. However, other reports indicated that BALT is normally absent in cats and Syrian hamsters [3,7]. Nodular lymphoid tissue has also been shown in subpleural, peribronchial, perivascular, and as a diffuse pulmonary lymphoid tissue in pigs [4]. Breel and co-workers [2] described the location of BALT between the bronchus and its associated artery in mouse lungs.

Histological features have shown that BALT is part of mucosal-associated lymphoid tissue (MALT) with active follicle, marginal B-cell zone and infiltration of the submucosa by plasma cells [21]. Gregson and his colleagues [25] stated that the amount of BALT in rodent lungs varies with the age of the animal and the conditions under which it has been maintained. However, they did not observe any plasma cells within BALT. Our results showed several plasma cells at the periphery of the lymphatic nodules. BALT has been described and named by Bienenstock and coworkers [10,26] as consisting of lymphoid tissue closely associated with and infiltrating bronchial epithelium to form lymphoepithelial lesion. However, these lesions should not be considered as part of the normal physiological BALT once they infiltrate the epithelium. They usually indicated some kind of infection which led to lymphocytic infiltration. Other studies have shown that BALT is not present in naive mice and humans but become obvious when pulmonary infections occur [8] in smokers [22] and in low-grade B-cell lymphoma [20].

Nodular forms of BALT may differ in the presence or absence of germinal centers depending on whether that specific animal has been exposed to antigen or not. Therefore, a single sampling is not sufficient to establish the basic histological features of these structures, and additional studies are needed to elucidate the function of BALT in cats and its role in the process of antibodies formation.

In the cat, the epithelium of the terminal bronchioles over BALT was simple columnar and bulged into the lumen. The absence of infiltrating lymphocytes was an indication that there is no infection in that region. On the contrary, Bienenstock et al. [10] found that the epithelium overlying BALT and gut- associated lymphoid tissue (GALT) was flattened, contained no goblet cells, and was heavily infiltrated with lymphocytes. We believe their findings were due to a pathological insult to this specific region and not in healthy animals.

In agreement with Bienenstock and co-workers [10], the diffuse form of BALT was found in several places that include the wall of pulmonary artery, mucosal surface epithelium of the bronchial trees and the alveoli. The infiltration of pulmonary artery suggests that brochial nodules might serve as a secondary center for lymphocytic proliferation. In contrast to earlier reports in rabbit and man, the nodular form of the cat has distinct marginal and central zones [10]. Although BALT bears morphological resemblance to Peyer's patches and other GALT, its role in immunoglobulin production is still obscure [10,11].

Nohr and Weihe [27] proposed that the neuroimmune connections in BALT play a significant role in the regulation and/or modulation of physiological/pathophysiological mechanisms of the lung. BALT may also be an important part of the psycho-neuro-immune axis. On the other hand, Bienenstock et al. [10] suggested that one of the main functions of the BALT is to act as a depot of immature, potentially IgA producing lymphocytes which, under antigenic stimulation, will populate the lamina propria. It has also been hypothesized that BALT is involved in sampling the contents of the bronchial lumen [25]. In our study, the extension of nodular forms to the terminal bronchioles and the presence of plasma cells in the epithelium observed support the concept of Xu et al., in the mice [23] that BALT is involved in sampling the bronchial lumen air to produce antigenic induced IgA in cats.

We believe that the development and morphology of BALT during

diseased conditions [8] and pulmonary infection [16] are completely different from normal BALT. Therefore, aggregation of lymphoid follicles or diffuse lymphoid tissue should be carefully analyzed for the presence of inflammatory cells, congestions of blood vessels and other signs of inflammation to distinguish between normal and diseased conditions.

The presence of high endothelial venule in cat BALT is in accordance with Gerda et al. [1], who proposed that memory cells may not be able to migrate as freely as recirculating small lymphocytes due to the type of high endothelial venule present in Wistar rats. These endothelial venules in the cat warrant further elucidation of their specific binding capability as these vessels may differ within lymphoid tissue and among species.

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