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Organization of the Bronchial Associated Lymphoid Tissue (BALT) of The Dromedary Camel *(Camelus Dromedarius)*

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ABSTRACT

The BALT is an organized lymphoid tissue in the mucosa of the bronchi and bronchioles that samples antigens leading to lymphocytes stimulation. This vital structure was studied in different species but not in the camel. Therefore this work studied the bronchi and bronchioles of 14 healthy camels of different ages (10 months - 12 years) using light and transmission electron microscope. BALT was regularly present in all specimens studied. It was variable in locations and structures. BALT was found in the form of patches across the circumference of the bronchial tree in the form of loose lymphocytic aggregations to a well-organized lymphoid tissue with secondary lymphoid follicles. It was localized in the lamina propria and sub mucosa or even the adventitia. At the margin of BALT, several high endothelial venules and lymphatics were localized. The BALT associated epithelium was formed from ordinary pseudo-stratified columnar ciliated epithelium with goblet cells in the bronchi and simple columnar in the bronchioles. Interepithelial lymphocytic infiltration of variable number was observed.

1. INTRODUCTION

The respiratory tract has a large mucosal surface that is continuously exposed to antigens. The local immune system therefore faces continuous challenges (Holt et al., 2008). Numerous lymphocytes are found within the lamina propria and among the epithelial cells of the bronchi and bronchioles. These lymphocytes belong to bronchial associated lymphoid tissue (BALT) (Mescher, 2013). The BALT belongs to the Mucosa-associated lymphoid tissue (MALT) which is defined as an organized lymphoid tissue in the mucosa that samples antigens leading to lymphocytes stimulation (Liebler-Tenorio and Pabst 2006). BALT is a constitutive mucosal lymphoid tissue adjacent to major airway in some mammalian species, including cattle (Anderson et al, 1986), human and occasionally mice (Holt et al. 2008) rats and rabbits (Randall, 2010). BALT was absent in cats (Pabst and Gehrke 1990). It was also found in some birds (Fagerland and Lawrence 1990 and 1993).

BALT acquires antigens from the airways and initiates local immune responses and maintain memory cells in the lungs (Randall, 2010). BALT is made up of a population of lymphocytes covered by a specialized epithelium different from typical pseudostratified ciliated columnar bronchial epithelium. There were distinct age-related differences in BALT structure (Fagerland and Lawrence 1990 and 1993). There is no available data about the BALT in the camel. Therefore the present study aimed to describe BALT in the normal lung of the dromedary camel. This may be useful to understand the histophysiology and immunology of the lung and assist in adjusting nasal vaccination program in the dromedary camel.

2. MATERIAL AND METHODS

2. 1. Samples:

Fresh extrapulmonary bronchi and lung tissue containing intrapulmonary bronchi and bronchioles were obtained from 14 healthy camels of different

ages: 10 months to 12 years old of both sexes. The camels were slaughtered for human consumption according to the rules of the Egyptian Veterinary Authorities in the abattoir of Marsa matrouh, Matrouh, Egypt or kom Hamada, elbehera, Egypt. The samples were prepared for histological and ultrastructure examination as follow:

2. 1. 1. Light microscopy

The Specimens were fixed in 10% phosphate-buffered formaldehyde (Rhodes, 2013). The fixed specimens were processed for paraffin sectioning. Serial sections (5µm) were prepared as outlined by Spencer and Bancroft (2013) and stained using the following stains:

1. Mayer's hematoxylin and eosin (H&E) stain for general studies (Mayer, 1903).
2. Van Gieson's stain, for differentiation between collagen fiber and smooth muscles (van Gieson, 1889).
3. Gordon and sweet's staining method for demonstration of reticular fibers (Gordon and Sweets 1936).
4. Combined alcian blue-PAS technique for demonstration of the full complement of tissue proteoglycans especially goblet cells (Mowry 1956, 1963).

2. 1. 2. Transmission electron microscopy

Fresh specimens, about 1mm^3 in size, were obtained from the bronchi and bronchioles of the lung of the camels and immediately fixed in 4 FIG (2% formaldehyde, 1.25% gluteraldehyde in 0.1 M sodium cacoddylate, PH 7.2) and stored at 4 ˚C and processed in Electron microscope unite, Faculty of Science, Alexandria university. After fixation, the tissues were washed in several changes of cold (4 ˚C) 0.1 M phosphate buffer every 15 minutes for 2 hours. Then the tissues were post-fixated in 1% solution of phosphate buffered osmium tetroxide (2% osmic acid 5 ml and phosphate buffer 5 ml) for 2 hours at room temperature. Then they were rapidly dehydrated through ascending grades of ethyl alcohol series (30, 50, 70, 90 and 100% for 2 changes) for 30 minutes in each. Then transferred to propylene oxide and placed over night in a 1:1 mixture of propylene and epoxy araldite. Then they were embedded in epoxy araldite (Hayat, 1986). Polymerization of embedding mixture and the tissue blocks was done in an oven for 5 days as following: at 35 ˚C for 24 hrs, at 45 ˚C for 24hrs, and lastly at 60 ˚C for 3 days. Semithin sections (1µm) were cut firstly and stained with toluidine blue and examined with light microscope to select the suitable areas for the electron microscopic examination. Then the ultrathin sections (60-100 nm) were cut by a glass knife, then they were stained with uranyl acetate followed by lead citrate (Hayat, 1986).

The sections were examined with Jeol transmission electron microscope working at 100 cx 80 KVS.

3. RESULTS

The BALT was observed along the bronchial tree including the extrapulmonary bronchi, intrapulmonary bronchi and bronchioles of the dromedary camel. The BALT of this animal was formed from patches of lymphocytic aggregation of variable sizes under the epithelial lining of the bronchi and bronchioles in the lamina propria and submucosa or even in the adventitia at variable locations in the circumference of the bronchi or the bronchioles (Fig. 1).

In the bronchi, the epithelium was formed from ordinary pseudo-stratified columnar ciliated epithelium with goblet cells. Numerous phagosomes were observed in the cytoplasm of the surface epithelium Figs 2-4). In the extrapulmonary bronchus the BALT was a constant structure located in the lamina propria forming dome like region. The BALT was well organized formed from lymphoid follicles with germinal centers supported with reticular network (Figs. 4&5).

In the intrapulmonary bronchus, The BALT of the dromedary camel was formed from patches of lymphocytic aggregation of variable sizes under the epithelial lining of the bronchi in the lamina propria and submucosa or even in the adventitia at variable locations. In the well-developed BALT, A prominent lymphoid follicles with clear germinal centers were observed (Figs. 6-11). In the same time variable numbers of lymphocytes were infiltrating the lining epithelium ranged from few lymphocytic infiltrations to heavy infiltration forming lymphoepithelium or reticuloepithelium respectively (Figs. 10&11).

In the bronchioles the lining epithelium was simple columnar ciliated epithelium with Clara cells. (Fig.12). the lymphoid aggregations ranged from few numbers of lymphocytes in the lamina propria to lymphoid nodules infiltrating the propria, submucosa and adventitia of the bronchioles. (Fig. 13).

High endothelial venules of variable number were distributed at the margin of the BALT and between the lymphoid nodules if present. The number of these venules was related to the size of the BALT.

Variable number of migrating lymphocytes were observed in the wall of these venule (Figs.6, 8, 9 &14).

Lymphatic vessels of variable sizes were extended at the BALT surrounding connective tissue. These lymphatics were present even if the BALT was formed from few lymphocytic aggregations (Figs. 4, 6, 9 &10).

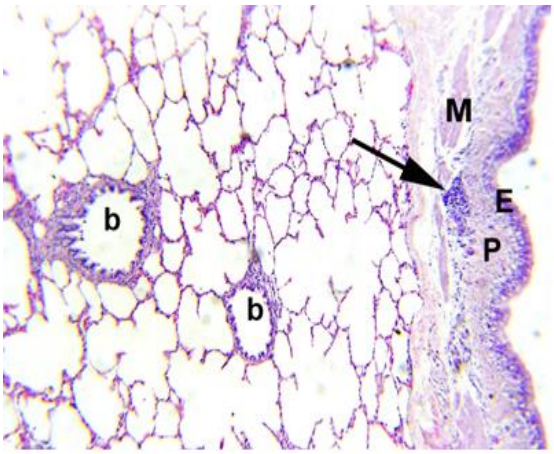

Fig. 1. The lung of the camel showing BALT (arrow) in the lamina propria (P) of the bronchus. The bronchioles (b) has few diffused lymphocytes in the wall. E = Surface epithelium, M=smooth muscles. H&E. X100.

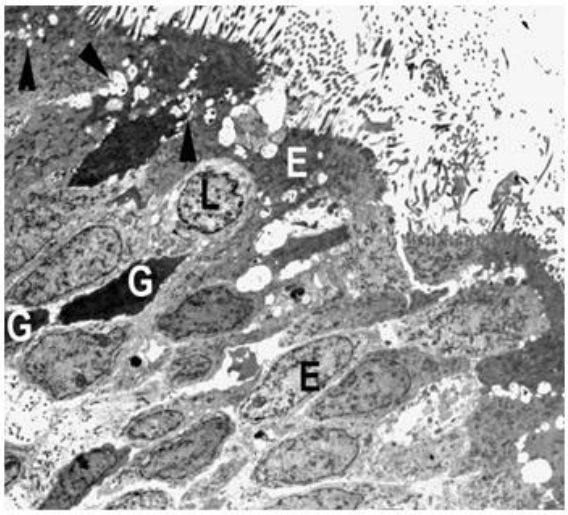

Fig. 2. Electron micrograph of the ciliated pseudostratified columnar bronchial epithelium (E) with goblet cells (G). Several phagosomes (arrowheads) are found in the cytoplasm of epithelial cell. L = migrating lymphocyte. X1000.

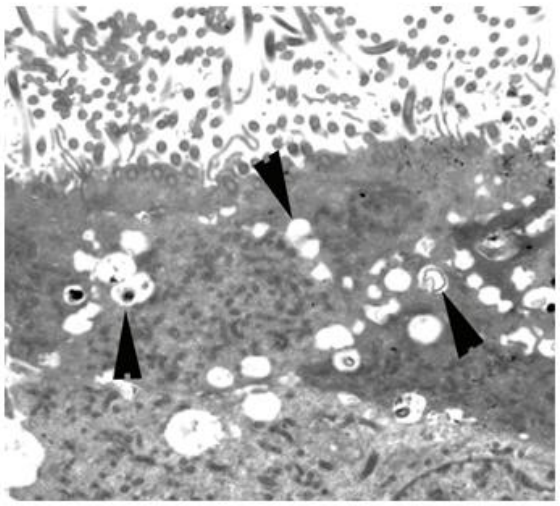

Fig. 3. Electron micrograph of surface epithelium showing several phagosomes (arrowheads) of variable nature in their cytoplasm. X3000.

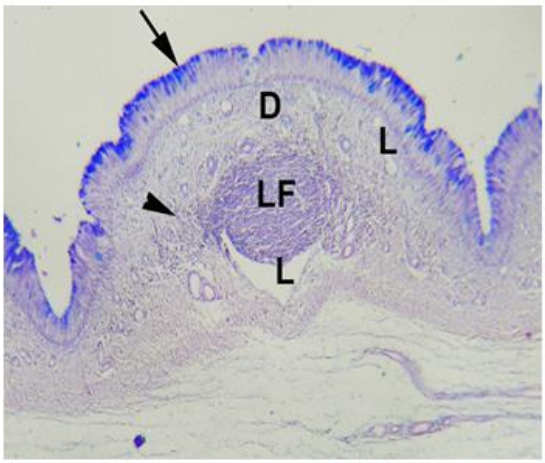

Fig. 4. The BALT of the extrapulmonary bronchus is localized in dome like area (D) and formed from lymphoid follicle (LF) and diffused lymphocytes (arrowhead). The covering epithelium (arrow) is rich in goblet cells. L= lymphatics. Alcian blue- PAS stain.

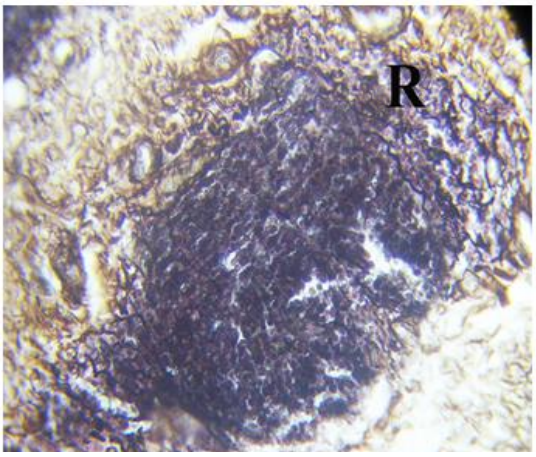

Fig. 5. The Lymphoid nodule of BALT of the extrapulmonary bronchus is enclosed with a reticular network (R). Gordon & Sweet's reticulin stain. X400.

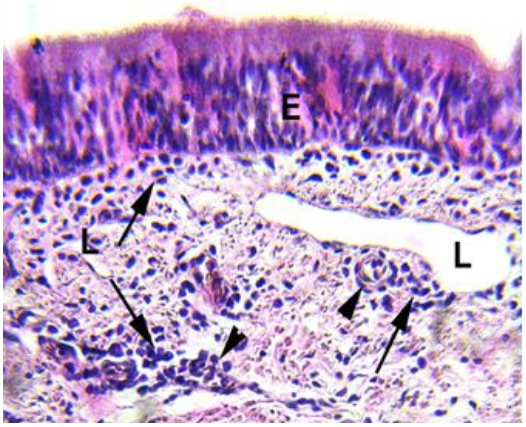

Fig. 6. A few aggregations of lymphocytes (arrows) in the lamina propria and beneath the epithelium (E) of the bronchus. Note the presence of lymphatics (L) and high endothelial venules (arrow heads). H&E. X400.

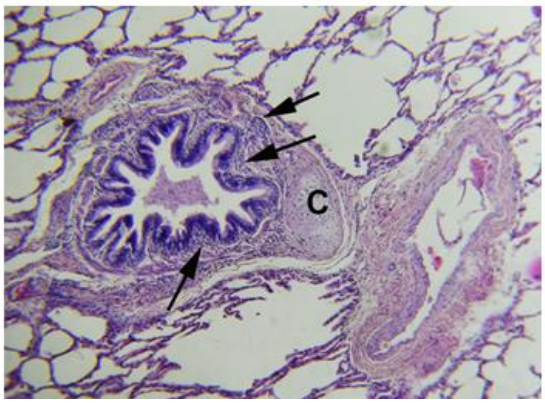

Fig. 7. A small BALT in the propria and submucosa (arrows) of camel bronchus. C= cartilage. H&E. X100

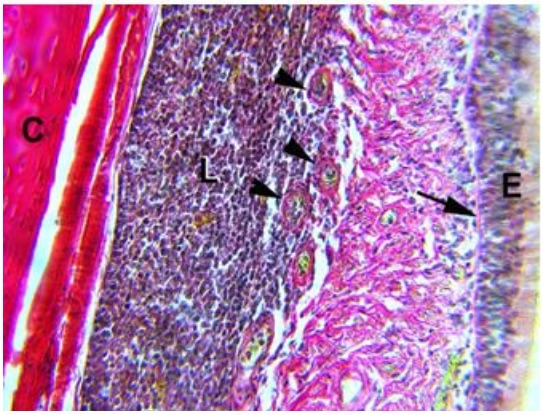

Fig. 8. Intrapulmonary bronchus lined with pseudo stratified columnar epithelium (E) resting on basement membrane (arrow) supported with loose connective tissue containing BALT (L) with high endothelial venules (arrowheads). C = hyaline cartilage plate. Van Gieson's

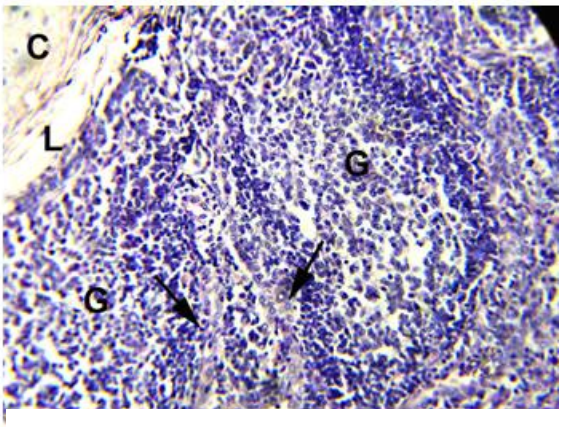

Fig. 9. A well-developed BALT made up of two active lymphoid nodules with germinal centers (G). High endothelial venules (arrows) extended between the follicles and lymphatic vessels (L) observed at the margin of the BALT. C=cartilage. H&E. X400.

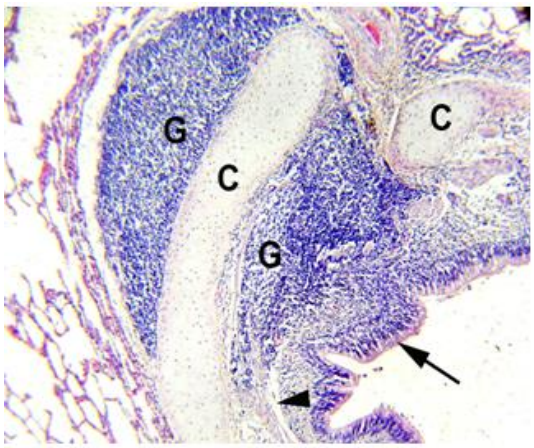

Fig.10. A prominent BALT in the wall of bronchus with clear germinal center (G) interrupted with a cartilaginous plates (C). The associated epithelium is highly infiltrated with lymphocytes forming lymphoepithelium (arrow). Lymphatic vessel (arrowhead) found at the margin of the BALT. H&E X100.

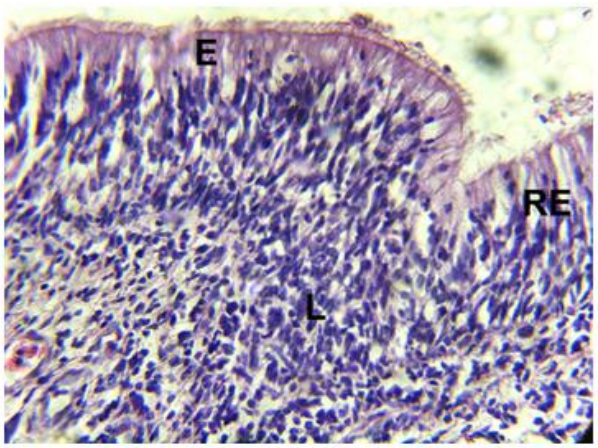

Fig. 11. The BALT (L) associated epithelium is highly infiltrated with lymphocytes forming lymphoepithelium (E) or reticuloepithelium (RE). H&E. X400.

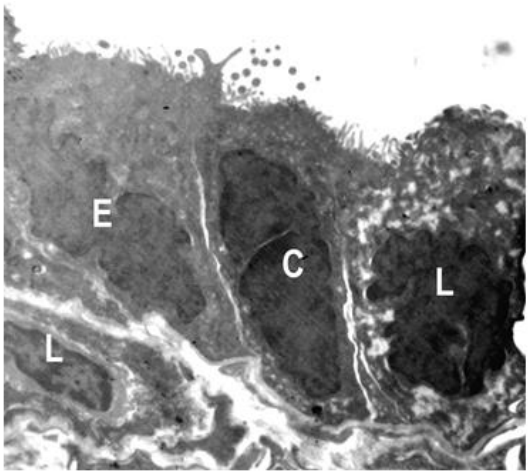

Fig. 12. Electron micrograph showing the surface epithelium of bronchiole is formed from ciliated simple columnar epithelium (E) with Clara cells (C). L = lymphocyte. X1500.

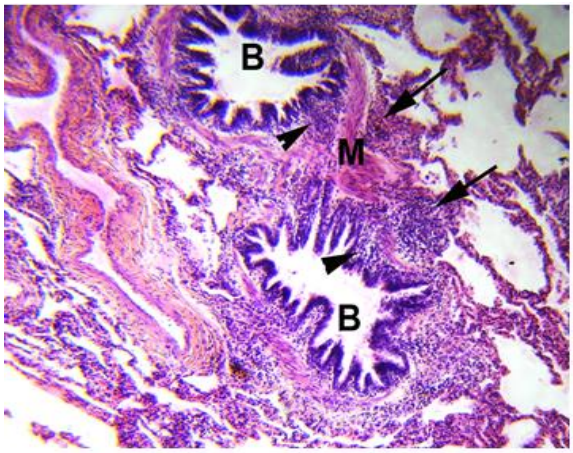

Fig. 13. Two bronchioles (B) with BALT in the lamina propria (arrowheads), and in the submucosa and adventitia (arrows). M = smooth muscles. H&E. X 100.

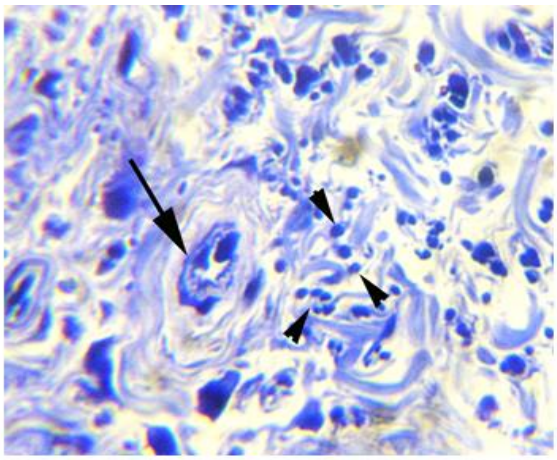

Fig. 14. Semithin section showing high endothelial venule (arrow) associated to loosely arranged lymphocytes (arrowheads). Toluidine blue. X1000.

4. DISCUSSION

The present study to the best of our knowledge is the first work describes the localization and organization of the BALT in the camel (*Camelus dromedarius*). The bronchi and bronchioles of the camels of different ages were studied in this work which revealed that, the BALT was regularly present in all specimens studied. Therefore the camel, is similar to other mammals in possessing the BALT (Anderson et al, 1986, Holt et al, 2008 and Randall, 2010) and opposed to mice and pig in which BALT is only occasionally observed (Pabst and Gehrke 1990 and Holt et al, 2008).

In spite of that, the BALT was observed in all specimens, bronchi or bronchioles without any BALT were observed. This indicates that the BALT is localized in the form of patches across the circumference and the pathway of the bronchial tree to be different from that of rabbits and rat which were distributed along the bronchial tree and cats which were absent (Pabst and Gehrke 1990).

The present study showed that the BALT associated epithelium may be infiltrated by lymphocytes forming lymphoepithelium or even reticuloepithelium. The same was observed in human (Stephen and Isaacson 1993). This characteristic epithelium was described in buffalo and camel palatine tonsils (Zidan and Pabst 2009, 2011). The presence of lymphoepithelium and reticuloepithelium may be due to antigen stimulation (Perry, 1994 and Zidan and Pabst 2011). This lympho-epithelial barrier samples and translocates antigens to the underlying lymphoid tissue (Perry and Whyte, 1998). This was documented with the presence of several phagosomes in the BALT associated epithelium in the bronchi. This makes it very likely that the camel BALT plays a continuous role in immunity during the animal's life and encourages the aerosol vaccination programs. Throughout this study no specialized cells like M cell were observed.

The BALT in the camel was variable from few lymphocytes to well- organized lymphatic tissue with clear germinal center. The development of several secondary lymphoid follicles is explained by the exposure of the camel to unlimited number of antigens by inhalation from the surrounding environment. The presence of secondary lymphoid follicles in the BALT indicates their role in lymphocyte and antibody production in a response to antigenic stimulation (Press and Landsverk 2006) this indicate that aerosol vaccination could be applied in the camel.

The BALT follicles of the camel possess high endothelial venules in the periphery. This arrangement was observed in the camel lymph nodes (Zidan and Pabst 2012). Recirculating lymphocytes may migrate across the wall of these venules from the blood to the BALT similar to other lymphoid tissue (Gowans and Knight 1964, Anderson and Anderson 1976 and Zidan et al, 2000) and filtered again into the associated lymphatic vessels which were observed in the margin of all BALT of the camel. This mechanism of lymphocytes recirculation were described in several lymphoid structure (Williams et al, 1995, Zidan et al, 2000 and Zidan and Pabst 2008, 2012).

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