Larynx-Associated Lymphoid Tissue (LALT) in Young Yak (Bos grunniens)

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ABSTRACT

The distribution of lymphoid tissue in the mucosa of the yak larynx was observed. A total of 10 yak larynges were examined both macroscopically after tissue fixation in acetic acid and microscopically using histology. It was found that no paraepiglottic tonsil was present in yak, although a few lymphoid follicles with immunopositive reactions for follicular dendritic cells were present in the mucosa at the base of the epiglottis and the corniculate processes of the arytenoid cartilages. Organized lymphoid tissue was identified in the mucosa of vestibulum laryngis and cavum infraglotticum. The results suggest that larynx-associated lymphoid tissue is a ubiquitous finding in yak. Although the yak larynx is devoid of a proper tonsil, it can probably still organize a local immune response due to the presence of LALT.

Key words: Histology, Larynx-associated lymphoid tissue, Yak

Larynx-associated lymphoid tissue (LALT) is an organized lymphoid tissue associated to the mucosa, characterized by the existence of a germinal center and a mantle zone (Kutta et al., 2003). The tonsils are major components of the mucosa-associated lymphoid tissue (MALT). They are secondary lymphoid organs consisting of accumulations of lymphocytes which are often organized in lymphoid follicles (Ogra, 2000). The follicular dendritic cells (FDC) are typical, specific, histological components of lymph nodules in secondary lymph organs. The FDC have been chosen as an identification parameter for tonsils, because FDC characterize the tonsillar lymph nodules and distinguish them structurally from unspecific accumulations of lymphocytes (Rebmann and Gasse, 2008). The aims of the present study were to investigate whether microscopic paraepiglottic lymphoid tissue and paraepiglottic tonsil are present in yak (Bos grunniens). This information will be helpful to understand the pathogenesis of microbial infection in the larynx of yak.

MATERIALS AND METHODS

Ten larynges from 1-year-old yaks without symptoms of clinical disease (inspection by a veterinarian) were collected from the yak slaughter house, Tianzhu, Gansu, China, after the animals had been killed by exsanguination for human consumption. All larynges were inspected macroscopically for the presence of visible lymphoid tissue. From seven larynges, the mucosa at the base of the epiglottis was fixed in 10% formaldehyde for 24 h, dehydrated, cleared in xylene and embedded in paraffin. Serial tissue sections (8 μ thick) with a 100 μ interval were made from all tissues. The slides were stained with hematoxylin and eosin. All sections were examined for the presence of lymphoid tissue with light microscope. Three larynges were immersed in 2% acetic acid for 24 h to macroscopically visualise subepithelial lymphoid nodules. When these were observed, photographs were taken using a digital camera and tissue samples were collected, fixed and processed for light microscopic analysis as described above.

The sections were processed and incubated with mouse monoclonal anti-human CNA.42 (DAKO, Glostrup, Denmark) antibody as described earlier (Rebmann and Gasse, 2008). The specimens were then counterstained with hematoxylin. Negative controls were prepared by incubating the sections in 0.01M phosphate buffer solution (pH 7.4). Sections from yak pharyngeal tonsils served as positive controls to confirm the specificity of staining for anti-human CNA.42 in the yak lymphoid nodules. All sections were viewed in a light microscope equipped with a micrometer scale in one of the oculars.

RESULTS AND DISCUSSION

No obvious lymphoid tissue was observed on fresh larynges. After fixation with 2% acetic acid, a few lymphoid nodules appeared macroscopically as opaque white spots
under the mucosal surface of the epiglottis (Fig. 1-A). The diameters of these nodules ranged from 0.5 to 2.5 mm. The lymphoid tissue had a conventional follicular structure in the subepithelial lamina propria of the larynx mucosa (Fig. 1-B) as described earlier by Casteleyn et al. (2008) in LALT in young cattle. Organized lymphoid tissue was identified in the mucosa of vestibulum laryngis and cavum infraglotticum (Fig. 2-A). Numerous scattered lymphocytes were present in the mucosa of false vocal cords (Fig. 2-B), which was similar to the lymphoid tissue associated to the respiratory mucosa (Rossi-And-Silva et al., 2009). The paraepiglottic tonsil was bilaterally present at the base of the epiglottis in small ruminants (Cocquyt et al., 2005). Since a tonsil is defined as an aggregation of lymphoid follicles (lymphonoduli aggregati), the term paraepiglottic tonsil cannot be used when referring to the observed disseminated lymphoid follicles. Therefore, it should be concluded that no paraepiglottic tonsil was present in yak, which was in accordance with previous reports in bovines (Casteleyn et al., 2008).

LALT was an organized lymphoid tissue associated to a mucosa, characterized by the existence of a germinal center and a mantle zone (Kutta et al., 2003). Microscopically visible primary and secondary lymphoid follicles were present in the mucosa of vestibulum laryngis and cavum infraglotticum of all 10 examined yaks. Serial sections demonstrated that follicle-associated epithelium (FAE) was separated from the follicle by the subepithelial dome region. FAE was attenuated, non-ciliated and heavily infiltrated by lymphocytes (Fig. 2-B) as reported in tonsil of soft palate of the sheep (Ovis aries) (Kumar and Singh, 2014). The observed lymphoid follicles were similar to isolated lymphoid follicles (ILFs) that were found in the murine and human gut in addition to Peyer’s patches (Cesta, 2006). ILFs have recently been recognised as members of the mucosal immune system and have been defined as tertiary lymphoid structures as their occurrence was inducible by antigen stimulation (McDonald et al., 2005). The results revealed that LALT was common in yak, and was part of the normal histological structures of the organ.

LALT was absent in the subglottis of the human larynx (Kutta et al., 2003), which was in contrast to our results. The individuals were relative young in our experiment. It is possible that the laryngeal lymphoid nodules found in the present study were induced by invading pathogens. This larynx associated lymphoid tissue may be typical for young individuals as these frequently cope with primary exposure to antigens (Kracke et al., 1997). Consequently, LALT could be used as an entry site for aerosolized vaccines in young individuals (Hiller et al., 1998).
The FDC were typical, specific, histological components of lymph nodules in secondary lymph organs (Kumar and Timoney, 2006). The recognition of FDC helped to emphasize that lymph nodules were not only present in the proper, i.e. the macroscopically visible part of the tonsil (Rebmann and Gasse, 2008). CNA.42 showed positive labeling of FDC in specimens from the mucosal of the epiglottis (Fig. 3-A). Immunoreactions were localized in the cytoplasm of FDC of a lymphoid follicle. Additional sections from yak pharyngeal tonsils were used as positive controls also reacted with monoclonal antibody, while no positive labelling was observed in any of the negative control sections (Fig. 3-B). However, no paraepiglottic tonsil was present in yak. So the observed disseminated lymphoid follicles with immunopositive reactions for FDC were only secondary lymphoid follicles. They shouldn’t be referred to as the disseminate part of the ‘paraepiglottic’ tonsil in yak.

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REFERENCES


