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Microscopic and Histoenzymic Studies on the Lymphoid Tissue in Pharyngeal Tonsil of Goats

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A B S T R A C T

Microscopic and histoenzymic studies were conducted on the lymphoid tissue in pharyngeal tonsil of six male crossbred goats of six months of age. The tonsil was lined by pseudostratified ciliated columnar epithelium which was transformed at places into follicle-associated epithelium (FAE). Propria-submucosa comprised of a central axis of loosely arranged connective tissue with dense aggregates of lymphoid tissue in the form of cryptolymphatic units and tonsillar nodules, fine blood capillaries and few nerve fibres folded around it. The average diameter of lymphoid nodules was 921.67±8.72µm and the lymphocyte count per nodule was 32233.23±324.24. The average number of lymphatic nodules counted per field under low power magnification of microscope was 2.5±0.43 and the internodular distance was 29.83±1.40µm. The fibroblastic reticulum cell (FRC) in lamina propria were acid-phosphatase (ACP) and alkaline phosphate (ALP) positive and gave a reticular reaction in the parafollicular and internodular regions and linear reaction in the capsule of lymphatic nodules. The FRC around the lymphatic nodules and internodular regions, the follicular dendritic cells (FDC) in dome, corona and FAE and the B-cell area lymphocytes showed ATPase activity. Alpha naphthyl acetate esterase (ANAE) activity was seen in the cytoplasm of T-lymphocytes and macrophages in the intercellular spaces between FAE and the basement membrane, internodular area and mantle zone of the lymphoid nodules.

Introduction

Pharyngeal tonsils were located at the posterior part of the nasopharynx in animals and formed the first line of defense against potential invasive microorganisms passing through both the respiratory and digestive tracts (Baykan *et al.,* 2001; Palmer *et al.,* 2011). The lymphoid tissue in nasopharyngeal tonsil served as an important source of immunoglobulin production for other mucosal sites like tracheo-bronchial tree.

A perusal of literature revealed only few studies on the microstructure and histochemistry of lymphoid tissue in pharyngeal tonsils of goats and hence the present work was undertaken to gain a better understanding of their immune functions.

Materials and Methods

Six crossbred male goats of six months of age were used for the present study. The heads collected were sectioned in median plane and tissue pieces were collected from the region of the pharyngeal tonsils and fixed in 10 per cent neutral buffered formalin, Baker's formal calcium solution at 4ºC, Carnoy's fluid and processed for histological and histoenzymic studies.

The materials were processed routinely to obtain 5-6µm thick serial paraffin sections and were stained by Haematoxylin and Eosin (Luna, 1968) for histological studies. The histoenzymic studies conducted were - azo dye coupling method using α naphthyl phosphate for acid and alkaline phosphatases (Bancroft and Stevens, 1996), Lead method for adenosine triphosphatase activity (Bancroft and Gamble, 2003) and acid alpha naphthyl acetate (ANAE) technique for histological identification of T-lymphocytes (Ranki *et al.,* 1976)

Results and Discussion

The pharyngeal tonsil was lined by pseudostratified ciliated columnar epithelium consisting of basal, supporting and goblet cells with 8-14 rows of oval to elongated nuclei. At various places, the epithelium was modified into stratified cuboidal. In between the cells numerous small to medium sized intraepithelial lymphocytes were seen. These observations confirmed the earlier descriptions given by Kumar and Timoney (2001) in equines, Kumar and Kumar (2004) in goats and Kumar *et al.,* (2006) and Kumar *et al.,* (2011) in sheep.

The epithelium of pharyngeal tonsil showed a large number of primary and secondary folds that formed crypts within the lymphoid tissue. This epithelium was transformed at places into simple columnar or stratified cuboidal reticular epithelium or follicle-associated epithelium (FAE). This was characterized by decreased height of the epithelial cells, absence of cilia and goblet cells and heavy infiltration of lymphocytes through the interrupted basement membrane. These observations are in accordance with the findings of Kumar and Timoney (2001) in horse, Kumar and Kumar (2004) in goats, Kumar and Nagpal (2007) in sheep and Palmer *et al.,* (2011) in bovines. Absence of mucus helped direct contact of microorganisms and their antigens to the FAE in pharyngeal tonsils as reported by Kumar and Timoney (2001) in horse. Toppets *et al.,* (2011) suggested that the pharyngeal tonsils were perfectly adapted to sample foreign antigens due the massive intraepithelial lymphocyte infiltration. The lymphoepithelial barrier sampled and transferred antigens to the underlying lymphoid tissue (Perry and Whyte, 1998).

Propria-submucosa of pharyngeal tonsil comprised of a central axis of loosely arranged connective tissue with dense aggregates of lymphoid tissue in the form of secondary lymphoid nodules and internodular lymphoid tissue, fine blood capillaries and few nerve fibres folded around it. This is in accordance with the descriptions given by Casteleyn *et al.,* (2011) and Toppets *et al.,* (2011) in sheep.

The cryptolymphatic units and tonsillar nodules of varying shape and dimensions constituted the majority of the lymphoid tissue. The lymphoid nodules were dome shaped towards the epithelium and consisted of a parafollicular area and central nodular area. These nodules were separated from each other by internodular areas. Most of the nodules had darkly stained corona formed by large number of small lymphocytes and a germinal centre (Fig. 1).

Fig.1 C.S. of pharyngeal tonsil showing lymphoid tissue. H&E x 100
1.Epithelium; 2.Germinal centre; 3.Corona; 4.Parafollicular area; 5.Internodular area and 6.Lymph vessel

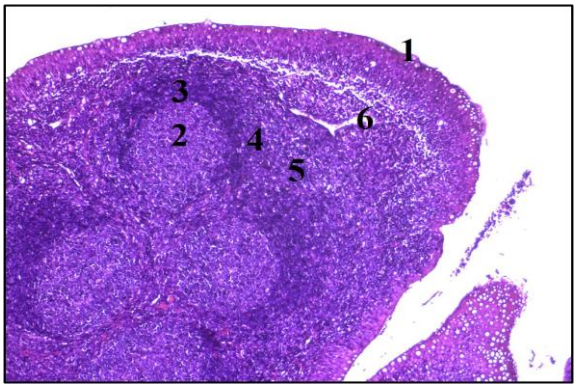


Fig.2 C.S. of pharyngeal tonsil showing reticular reaction of acid phosphatase. Azo dye coupling method x 100
1. Lymphoid nodules and 2. Internodular area

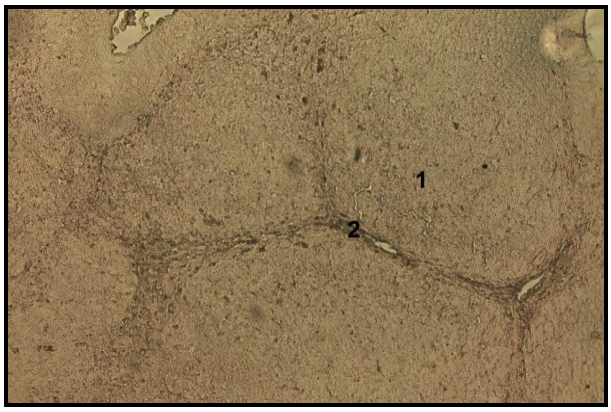


Fig.3 C.S. of pharyngeal tonsil showing showing alkaline phosphatase activity. Azo dye coupling method x 200
1. Lymphoid nodule and 2. Corona

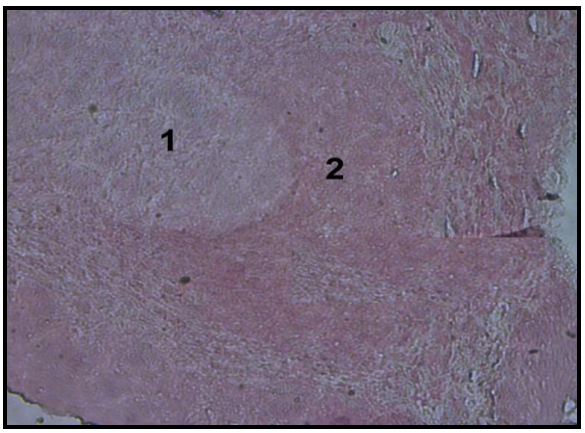
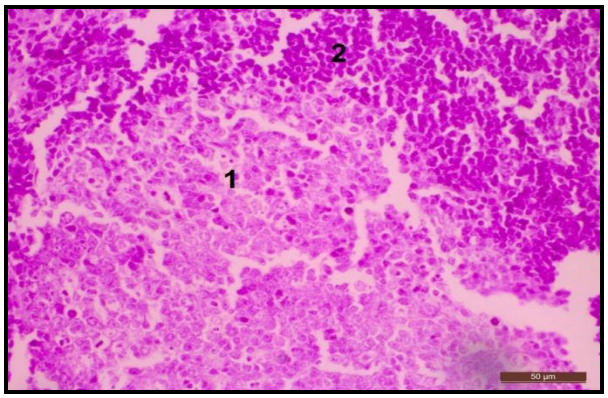


Fig.4 C.S. of pharyngeal tonsil showing alpha naphthyl acetate esterase activity (arrows). ANAE x200
1. Germinal centre and 2. Internodular area



These observations confirmed the reports of Kumar *et al.,* (2001) in horse, Kumar and Kumar (2004) in goats, Billen *et al.,* (2006) in dogs, Kumar *et al.,* (2011) in sheep and Liu *et al.,* (2012) in pigs. The lymphoid tissue comprised of small, medium and large lymphocytes, plasma cells and macrophages. The average diameter of lymphoid nodules was 921.67±8.72µm and the lymphocyte count per nodule was 32233.23±324.24. The average number of lymphatic nodules counted per field under low power magnification of microscope was 2.5±0.43 and the internodular distance was 29.83±1.40µm. Similar reports on the micrometry of lymphoid tissue of pharyngeal tonsils are not available for comparison.

Large number of plasma cells was seen beneath the epithelium, in both follicular and diffuse lymphoid tissue and in the glandular tissue. The average number of plasma cells counted per field under high power magnification within the pharyngeal tonsils was 63.50±2.89.

Histoenzymic studies revealed the presence of acid-phosphatase (ACP) positive fibroblastic reticulum cell (FRC) that gave a reticular reaction in the parafollicular and internodular regions and linear reaction in the capsule of lymphatic nodules. ACP reaction was not seen in the lymphocytes (Fig. 2). According to Heusermann *et al.,* (1982) the FRCs were mesenchymal cells which formed a special arrangement with reticular fibres for placement of lymphocytes and macrophages.

The FRC also gave a strong alkaline phosphatase (ALP) activity in the form of reticular staining pattern in the centre of lymphatic nodules and internodular area and linear reaction in the capsule of lymphatic nodules. The activity was more intense over the corona and domes of lymphoid nodules (Fig. 3). This is in agreement with the observations made by Landsverk (1984) in calves.

In pharyngeal tonsils, the FRC around the lymphatic nodules and internodular regions and the B-cell area lymphocytes showed ATPase activity. Lymphocytes in T-cell area showed a weak reaction. The follicular dendritic cells (FDC) in dome, corona and FAE also gave reticular reaction suggesting differentiation of FDC in the secondary lymphoid nodules. According to Ramos *et al.,* (1992) FDC were fundamental in the development of secondary follicles. All these observations tally with the reports of Halleraker *et al.,* (1990) in goats and Raju *et al.,* (2012) in sheep.

Presence of fine-granular alpha naphthyl acetate esterase (ANAE) activity was seen in the cytoplasm of T-lymphocytes and macrophages in the intercellular spaces between FAE and the basement membrane, internodular area and mantle zone of the lymphoid nodules. Nearly all lymphocytes in the germinal centres were ANAE negative. The macrophages in lymphoid nodules, corona, dome, lamina propria, T-cell area and muscularis mucosa were positive for ANAE (Fig. 4). These observations tally with the reports of Ramos *et al.*, (1992) in pigs, Baykan *et al.*, (2001) in dogs and Halleraker *et al.*, (1990) in calves.

It was concluded that lymphoid tissue was abundant in the pharyngeal tonsils of goats, suggesting that they could be exploited as targets for nasal vaccines for the induction of mucosal immune response in this species.

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