Histology and immunohistochemistry of the palatine tonsil in goats

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ABSTRACT

Histological studies were conducted on the palatine tonsil of six male crossbred goats of six months of age. The tonsils were lined by a stratified squamous non-keratinized surface epithelium which continued into the crypts forming the non-reticular epithelium. At some areas in the crypts, the non-reticular epithelium associated with lymphoid follicles showed a great reduction in height with only one to two intact cell layers and were called reticular epithelium or lymphoepithelium. Propria-submucosa of the palatine tonsil was characterized by dense irregular connective, lymphoid, glandular, adipose and muscular tissues. Lymphoid tissue constituted majority of the palatine tonsil and was organized into primary and secondary lymphoid nodules and dense diffuse lymphatic tissue. Average diameter of lymphoid nodules was 684.17±6.88µm while the lymphocyte count in the nodules was 28826.54±236.25. The average number of lymphatic nodules counted per field under low power magnification of microscope was 2.67±0.42 and the internodular distance was 34.67±1.41. Glandular tissue was present in the deeper areas of propria-submucosa. A well developed connective tissue capsu le separated the lymphoid and glandular tissues of the palatine tonsil. In the immunohistochemical staining technique strong positive reaction for cytoplasmic IgG bearing B-lymphocytes was noticed within the germinal centre of lymphoid nodules, towards the base of the FAE and some cells even infiltrated the crypt epithelium. In the mantle zone and internodular area, reaction was very mild indicating that T-lymphocytes predominated in these areas. It was concluded that the palatine tonsils were histologically mature as a local defence mechanism against the harmful substances to be encountered from the environment after birth.

Key words: Goats, Histology, Immunohistochemistry, Palatine, Tonsil.

INTRODUCTION

The location of the tonsils at the entrance of respiratory and alimentary tracts makes them the gate keepers to mucosal immunity. The size and location of tonsils varies in different animals (Tenorio and Pabst, 2006). A perusal of literature revealed only few studies on the palatine tonsils in goats and hence the present work was undertaken.

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 rinsed in tap water. Tissue pieces were collected from the region of the palatine tonsils, fixed in 10 per cent neutral buffered formalin and processed routinely to obtain 5-6µm thick serial paraffin sections. The sections were stained by Haematoxylin and Eosin (Luna, 1968), Gomori’s rapid one step trichrome method for collagen fibres (Luna, 1968), Verhoeff’s method for elastic fibres (Singh and Sulochana, 1996), Gordon and Sweet’s method for reticular fibres (Bancroft and Gamble, 2003) and Unna’s method for mast cells (Luna, 1968).

The tonsils not more than 2 to 3 mm thick was fixed in pre-cooled Carnoy’s fluid at 4°C for immunochemical method of unlabelled antibody technique using Peroxidase anti-peroxidase (PAP) soluble complex, to detect the areas of IgG bearing B-cells as per Sternberger (1986). Tissue was then processed and embedded in soft paraffin having melting point 52°C to 54°C. Three to five mm thick tissue sections were cut, deparaffinized and transferred to alcohol. The sections were then immersed in 0.3 per cent hydrogen peroxide (H2O2) in Tris buffer saline (pH 7.6, 0.05 M)(TBS) for 30 min at room temperature to block endogenous peroxidase activity, washed in running water for 10 min, incubated at 37°C in distilled water and then passed through two changes of TBS containing 1 per cent Triton–100 detergent to reduce the nonspecific background. Thereafter, sections were treated with 3 per cent rabbit serum for 30 min at room temperature for background blocking. Sections were then immersed in primary antibody i.e. Anti-goat IgG raised in rabbit at a concentration of 1: 1000 in TBS containing 1 per cent rabbit serum for 24 hours at 4°C. Thereafter sections were washed in three successive changes of TBS for 30 min each and incubated in secondary antibody i.e. Anti-rabbit IgG raised in goat-HP conjugate at 1: 100 dilution in TBS for 45 min at room temperature. After

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incubation sections were washed as above and then incubated in 0.05 per cent solution of 3’3’Diaminobenzidine tetrahydrochloride and 0.01 per cent of H₂O₂, in TBS for 5 min. Then they were washed and counterstained in sections with one per cent Methyl green, dehydrated, cleared and mounted in DPX as per the routine procedures.

RESULTS AND DISCUSSION

In histological sections the palatine tonsils were lined by a stratified squamous non-keratinized epithelium with irregular finger-like projections towards the lamina propria submucosa called interpapillary pegs. In between the pegs dense irregular connective tissue called papillae protruded into the surface epithelium with a hairpin loop of capillar in it. These observations tally with that made in pigs (Anderson, 1974), dog (Belz and Heath, 1995), bovines (Velinova et al., 2001) and sheep (Cocquyt et al., 2005 and Raju et al., 2012).

The stratified squamous epithelium comprised of stratum basale, stratum spinosum and stratum superficiale. Number of cell layers in the surface epithelium ranged from twelve to fifteen. The cells of stratum basale were columnar, with oval to elongated strongly basophilic nuclei and slightly basophilic cytoplasm. The stratum spinosum consisted of six to twelve layers of irregularly polyhedral cells with lightly basophilic nuclei of different shapes. The stratum superficiale consisted of six to twelve layers of irregularly polyhedral cells with lightly basophilic nuclei of different shapes. The stratum superficiale consisted of horizontally oriented squamous cells with eosinophilic and finely granular cytoplasm and basophilic nuclei showing signs of degeneration towards their free surface (Fig.1). These observations are in accordance with the reports in goat (Kumar et al., 2006) and sheep (Kumar et al., 2008). Mean height of the surface epithelium of palatine tonsils in the present study was 691.67±10.14µm. According to Raju et al. (2012) in sheep, the height of epithelium in the palatine tonsils was 360.00±9.31µm in young and 795.50±5.24µm in adult age groups.

The surface epithelium invaginated into the lymphoid tissue below it to form primary tonsillar crypts, which in turn formed secondary crypts. Studies in dogs (Baykan et al., 2001), ruminants, horses and swine (Casteleyn et al., 2007) and sheep (Kumar et al., 2008 and Raju et al., 2012) led to similar conclusions. The deep branching of the crypts markedly increased the contact area between the external environment and lymphoid tissue as reported by Salles and Middleton (2000) in pigs.

The crypts were lined by two types of epithelium viz., reticular and non-reticular in all the animals under study. Continuation of the tonsillar surface epithelium into the crypts formed the non-reticular epithelium and was comprised of strata basale, spinosum and superficiale. In the deep regions, the crypt epithelium associated with lymphoid follicles showed a great reduction in height with only one to two intact cell layers and were called reticular epithelium or lymphoepithelium. It was heavily infiltrated by lymphocytes, plasma cells and a few macrophages and formed a lymphoepithelial symbiosis (Fig.2). These observations are in accordance with the reports of Kumar and Timoney (2005a) in horse. Belz and Heath (1995) opined that reticular epithelium was a specialised region of the canine tonsillar epithelium for ingestion of the foreign particles.

**Fig 1:** C.S. of palatine tonsil showing surface epithelium. H&E x 400
1. Stratum superficiale
2. Stratum spinosum
3. Stratum basale
4. Interpapillary pegs
5. Papillae
6. Capillaries

**Fig 2:** C.S. of palatine tonsil showing lymphoepithelial symbiosis. H&E x 200
1. Non-reticular epithelium
2. Reticular epithelium
3. Lymphoepithelial symbiosis
4. Stratum superficiale
5. Stratum spinosum
6. Stratum basale
Mean height of the reticular epithelium was 229.5±5.95µm in the present study. Small blood vessels were also seen within the reticular epithelium. Lamellated structures resembling Pacinian corpuscles could be located in the reticular epithelium of palatine tonsil as reported in horse (Kumar and Timoney, 2005a) and goat (Kumar et al., 2006). Kumar and Timoney (2005a) in horse suggested that the occlusion and interruption of crypts might lead to isolation of islands of epithelial cells, its degeneration and formation of onion-like corpuscles.

Propria-submucosa of the palatine tonsil was characterized by dense irregular connective, lymphoid, glandular, adipose and muscular tissues. The basement membrane was uniform below the base of the outer surface epithelium and consisted of reticular fibres. However, below the reticular epithelium, it was fragmented due to heavy infiltration of lymphoid tissue. These observations concur with the findings of Kumar et al. (2008) in sheep. The fenestrated basement membrane of the reticular epithelium helped in the passage of either antigens or their processed products to the deeper regions populated by the lymphoid cells (Belz and Heath, 1995).

Beneath the reticular epithelium, where lymphoid tissue was dense, the reticular fibres formed a thick meshwork and collagen and elastic fibres were sparsely distributed. Mast cells were also seen in the propria-submucosa of palatine tonsil as noticed by Ramos et al. (1992) in the tonsils of pigs. At the region of reticular epithelium, reduced amount of collagen fibres and increased number of blood capillaries compensated for the increased metabolic needs of the epithelium and increased the interaction between endothelial cells and leucocytes, and transport of immunoglobulins and other molecules across the vessel walls (Perry and Whyte, 1998).

Lymphoid tissue constituted majority of the palatine tonsil and was organized into lymphoid nodules and dense diffuse lymphatic tissue. Large number of capillaries and lymphatics were seen in the subepithelial areas and in the lymphoid nodules. At some regions, cryptolymphatic units (CLU) or tonsillar follicles were seen with lymphatic nodules organized in aggregations around an epithelial crypt and encapsulated by thin connective tissue (Fig 3&4). Williams and Rowland (1972) demonstrated that this arrangement transported the foreign particles through the crypt epithelium into the subepithelial tissue and to the macrophages in the interfollicular lymphoid tissue in the tonsils of pigs. The close association between the lumen of the crypt and the tonsil provided an important role for tonsils in the immunological response against antigens which entered the body by oral cavity. Lymphoid aggregations without crypts called tonsillar nodules were also noticed in the present study. The lymphoid nodules were round or oval in shape and were vertically piled up with their axes perpendicular to the basement membrane of the epithelium. Kelly et al. (1983) made similar observations in the palatine tonsils of pigs.

The present study revealed that lymphoid nodules were of primary and secondary types. The primary nodules were highly basophilic because of the presence of densely packed small lymphocytes and were devoid of germinal

Fig 3: C.S. of palatine tonsil showing cryptolymphatic units (CLU) and tonsillar nodules. Gomori’s one step trichrome X 40
1. Collagen fibres in capsule
2. Septa
3. Cryptolymphatic units (CLU)
4. Tonsillar nodules
centre. The secondary lymphoid nodules consisted of a germinal centre, parafollicular area and darkly stained corona or mantle zone, which generally faced the crypt epithelium. In the light zone lymphocytes were more loosely arranged and the developing lymphocytes had larger and lighter staining nuclei with more cytoplasm. In the dark zone, numerous small lymphocytes and macrophages were densely packed. The mantle zone consisted of small darkly stained lymphocytes, numerous macrophages and reticular cells. The same features were also observed in goats (Kumar et al., 2006) and sheep (Kumar et al., 2008).

Parafollicular region present between and beneath the follicles showed more of medium-sized lymphocytes than small lymphocytes, plasma cells, macrophages, blood capillaries, venules and high endothelial venules (HEVs) along with a meshwork of fine reticular fibres. The HEVs were lined with cuboidal endothelium and contained varying number of lymphocytes. Observations similar to this were made in horse (Kumar and Timoney, 2005a), goats (Kumar et al., 2006) and sheep (Kumar et al., 2008).

Average diameter of lymphoid nodules was 684.17±6.88µm while the lymphocyte count in the nodules was 28826.54±236.25. The average number of lymphatic nodules counted per field under low power magnification of microscope was 2.67±0.42 and the internodular distance was 34.67±1.41.

Glandular tissue was present in the deeper areas of propria-submucosa adjacent to the lymphoid tissue and was lined by pyramidal cells. These observations tally with the reports made in horse (Kumar and Timoney, 2005a), goats (Kumar et al., 2006) and sheep (Kumar et al., 2008). A well developed connective tissue capsule separated the lymphoid and glandular tissues of the palatine tonsil. The capsule was mainly composed of collagen and reticular fibres with a few smooth muscle fibres. Thick bundles of elastic fibres and nerve bundles were also seen in it. One to two connective tissue septa arose from the capsule and divided the tonsil into lobes. Numerous arteries, veins and lymphatics were observed in the septa. Similar observations were recorded by Belz and Heath (1995) in canines and Casteleyn et al. (2007) and Raju et al. (2012) in sheep.

In the immunohistochemical staining technique strong positive reaction for cytoplasmic IgG bearing B-lymphocytes was noticed within the germinal centre of lymphoid nodules, towards the base of the FAE and some cells even infiltrated the crypt epithelium. In the mantle zone and internodular area, reaction was very mild indicating that T-lymphocytes predominated in these areas (Fig. 5). These results were in accordance with the findings of Ramos et al. (1992) in pigs and Breugelmans et al. (2011) in bovine tonsils.

It was concluded that the palatine tonsils were histologically mature as a local defence mechanism against the harmful substances to be encountered from the environment after birth.

CONCLUSION

In goats, lymphoid tissue was well developed in the palatine tonsils suggesting that they could be exploited as targets for oral or nasal vaccines for the induction of mucosal immune response in this species.

REFERENCES


