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Histological studies on the paraepiglottic tonsil of the sheep

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ABSTRACT

The paraepiglottic tonsil studied in 6 young sheep revealed stratified squamous non-keratinised epithelium having strata basale, spinosum and superficiale which modified into reticular epithelium due to heavy infiltrates of underneath lymphoid tissue. The loose irregular connective tissue of lamina propria submucosa was mainly constituted by lymphoid and glandular tissue. The lymphoid tissue organized in the form of isolated scattered aggregations and the follicles was constituted by lymphocytes, plasma cells and macrophages. The interfollicular areas were rich in lymphocytes, blood capillaries, macrophages and high endothelial venules. The mucous acini showed a strong PAS positive reaction for glycogen, acidic mucopolysaccharides and Alcianophilic mucopolysaccharides. The neutral mucopolysaccharides were localized in smaller concentration only towards the periphery of a few acini. However these reactions were absent in the glandular ducts. In addition, large venous caverns like structures filled with blood, large nerve bundles and fatty tissue were also present.

Key words: Lymphoid tissue, Paraepiglottic tonsil, Reticular epithelium, Sheep

Tonsils not only act as first line of defense but also act as replication site for many pathogens (Timoney and Kumar 2008) including prions and play important role in early pathogenesis. Sheep can be infected both experimentally as well as in natural conditions with prions causing bovine spongiform encephalopathy (Bellworthy *et al*. 2005) and led to accumulation of body-specific prion protein (PrP^c) in almost all the lymphoid tissues (Foster *et al*. 2001) including palatine tonsil (van Keulen *et al*. 2002). A large number of sheep are sacrificed in India for the consumption of their meat including the tonsils so these tonsils are of prime public health importance. The specified risk material to be removed includes brain, spinal cord, eyes, and the tonsils from animals of one year age and the spleen of all age groups (European Commission 2000). The histology of nasopharyngeal and palatine tonsils of sheep have been studied in detail (Kumar and Nagpal 2007, Kumar *et al*. 2008) but data is scarce on the structural details of the paraepiglottic tonsil except its occurrence (Barone 1997, Cocquyt *et al*. 2005). Hence, the present study was envisaged to explore the histoarchitecture of the paraepiglottic tonsil in the sheep.

MATERIALS AND METHODS

Tissues were collected from paraepiglottic tonsils of 6 young sheep (6–9 months age) of either sex from local slaughterhouse immediately after decapitation. The tissues were fixed in 10% neutral buffered formalin and processed for paraffin technique of light microscopy. Paraffin sections (5–6 µ) were stained with routine Harris' hematoxylin and eosin stain, Weigert's method for elastic fibres, Gomori's method for reticulum, Bielschowsky's method for axis cylinder and dendrites, McManus' method for glycogen (PAS), Alcian blue method for muco-substances (pH 2.5) and PAS-Alcian blue method for mucosubstances (pH 2.5), diastase digestion method (Luna 1968) and Crossman's trichrome stain for collagen fibres (Crossman 1937).

RESULTS AND DISCUSSION

The paraepiglottic tonsil (PET) in the form of small elevated nodules was topographically located lateral to the epiglottis at the floor of piriform recess. The tonsil measuring 5–14 mm in length and 2–5 mm in width comprised 1–5 elevated nodular structures in contrast to 1–8 nodules reported earlier (Cocquyt *et al*. 2005). The PET was lined by stratified squamous non-keratinised epithelium and changed to keratinised type in its adjacent area (Fig. 1). The free surface was irregular and presented papillated appearance due to highly folded mucosa but the distinct crypts were not observed. The stratified squamous keratinised epithelium (Fig. 2) having strata basale, spinosum and corneum presented well developed papillary projections towards its deeper surface. The stratum basale had columnar cells with elongated and vertically oriented nuclei. The nuclei were dark due to presence of dense chromatin, which masked the appearance of nucleoli. The cytoplasm of

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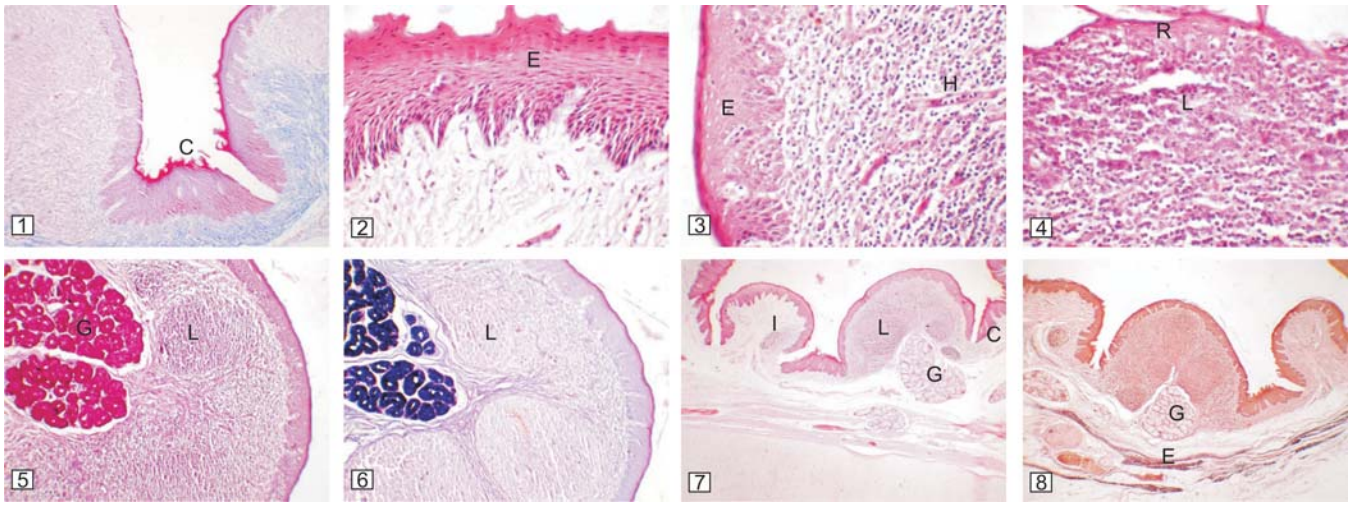
these cells was finely granular and eosinophilic. The stratum spinosum with varying number of rows of cells had nuclei characteristics similar to those of basale cells except in the superficial portion where the nuclei were horizontally oriented and became more irregular and elongated with tapering free ends. These cells possessed cytoplasmic processes which were distinct and undulating having eosinophilic cytoplasm and also had wide intercellular spaces. The stratum corneum comprised irregular shaped, pyknotic nuclei with degenerative changes. Their cytoplasm was strongly eosinophilic. This layer constituted almost 1/3 of total epithelial height and was desquamating at places.

This epithelium was modified into reticular epithelium due to intimate association of the lymphoid tissue present in the lamina propria submucosa and fewer cell layers lacking distinct strata (Figs 3, 4). The cells of stratum basale were having histological features similar to those described earlier except that wide gaps were observed because of infiltration of lymphoid cells. The cells of stratum spinosum had large, oval vertically oriented nuclei with large clumps of chromatin material. The superficial nuclei were comparatively lightly stained and some of these presented vacuolated appearance. However, the cytoplasm of these cells was eosinophilic. The stratum superficiale cells had darkly stained narrow, elongated nuclei with degenerative changes. Their cytoplasm was strongly eosinophilic. These cells showed a moderate positive reaction for glycogen and neutral mucopolysaccharides (Figs 5, 6). Tonsillar epithelial cells exhibit energy demanding absorptive and secretory functions and so the stored glycogen in the upper strata of the epithelium may form an energy source (Perry 1994).

The infiltration of lymphoid tissue in the reticular epithelium was so much extensive at places that it was difficult to discern epithelial cells and the later formed only a single layer of epithelium where the basement membrane was interrupted as by Nave *et al*. (2001). The direct transepithelial access of antigens may result in greater influx and recruitment of non-epithelial cells in a particular patch, in contrast to a less strongly stimulated area which may have retained stratified squamous non-keratinised epithelium. An appropriate balance of epithelial and non-epithelial cells is required to preserve efficient mucosal protection as locally produced IgA secretory component is not recycled, but is continually synthesized by the epithelial cells (Perry 1994). The presence of non-epithelial cells as early as the 15th week of gestation in the human suggested that reticulation along with the formation of primary follicles, interfollicular areas and high endothelial venules was a normal developmental process (von Gaudecker and Muller-Hermelink 1982). The reticular epithelium also acted as an additional lymphoid compartment by contributing to the production of committed immunocytes and to the protection of the mucosal surface (Brandtzaeg and Halstensen 1992).

The lamina propria submucosa had loose irregular connective tissue comprising connective tissue cells, fine blood capillaries, bundles of collagen along with fine reticular and elastic fibres, lymphoid and glandular tissue. A dense



Figs 1–8. Photomicrograph of paraepiglottic tonsil showing: **1.** stratified squamous epithelium (C), lymphoid follicle (L), isolated aggregation of lymphoid tissue (I) and mucous glandular acini (G). H & E × 40. **2.** stratified squamous keratinised epithelium (E) in the region lacking the lymphoid tissue. H & E × 400. **3.** stratified squamous non-keratinised epithelium (E), lymphoid tissue and high endothelial venule (H). H & E × 400. **4.** reticular epithelium (R) with only few cell layers being infiltrated by lymphoid cells and lymphoid tissue (L). H & E ×400. **5.** lymphoid tissue (L) and a strong PAS positive reaction in mucous glandular acini (G). McManus' PAS method. ×100. **6.** lymphoid tissue (L) and a strong reaction for acidic mucopolysaccharides (blue colour) in glandular acini. Note a moderate reaction for neutral mucopolysaccharides (pink colour) in stratum superficiale of epithelium and at the periphery of some glandular acini. PAS Alcian blue × 100. **7.** epithelium (C) and a more distribution of collagen fibres (blue colour) in the lamina propria submucosa where lymphoid tissue was absent. Crossman trichrome ×100. **8.** epithelium lymphoid tissue, glandular tissue (G) and a layer of elastic fibres (E). Weigert's method × 40.

arrangement of collagen along with fine reticular and isolated elastic fibers was observed in the subepithelial region where the lymphoid tissue was lacking and also surrounding the glandular acini (Figs 7, 8). A uniform layer of elastic fibers surrounded the glandular tissue and separated the later from the nerve bundles and blood vessels (Fig. 8). The collagen and elastic fibers also surrounded the fatty tissue and their concentration increased in the deeper propria where the glandular tissue was absent. The lymphoid tissue was organized in the form of isolated scattered aggregations and the primary and secondary follicles (Figs 1, 5, 6, 8) being encapsulated by connective tissue especially the collagen fibres. The secondary follicles were only a few and were constituted by different sized lymphocytes, plasma cells and few macrophages. The parafollicular areas of the follicles being separated by interfollicular areas were rich in lymphocytes, blood capillaries, macrophages, venules and high endothelial venules (HEVs). The HEVs were comparatively fewer in comparison to the palatine tonsil of the sheep (Kumar *et al*. 2008) and these were found involved in trafficking of T and B lymphocytes from blood to tonsil and back to the circulation via lymph under the influence of adhesion molecules, cytokines and chemokines (Schaerli *et al*. 2000).

Below lymphoid tissue, clusters of mucous acini with serous demilunes were observed. These glandular acini showed a strong PAS-positive reaction for glycogen, acidic (Figs 5, 6) weakly acidic sulfated mucopolysaccharides, sialomucins and hyaluronic acid. The neutral mucopolysaccharides were localized in smaller concentration towards the periphery of some acini (Fig. 6). Ducts lined by simple cuboidal epithelium did not exhibit reaction for the mucopolysaccharides. Large venous caverns like structures filled with blood, blood capillaries, large nerve bundles and fatty tissue were also present (Fig. 1). The connective tissue mainly the collagen fibres became denser in the deeper portion close to the elastic cartilage. The reticular epithelium and lymphoid tissue of the PET of the sheep suggests that the tonsil is a component of mucosa associated lymphoid tissue.

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