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Histology, histochemistry and scanning electron microscopic studies on the tubal tonsil of sheep

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Tubal tonsil present around the pharyngeal opening of the eustachian tube, constituted the part of nasal associated lymphoid tissue (NALT) and a component of Waldeyer's ring. M-cells reported in the nasopharyngeal tonsils of the horse and sheep and the tubal tonsil of the horse (Kumar *et al.* 2001, Kumar and Timoney 2005, Kumar *et al.* 2009) were specialized cells involved in active transport of soluble and particulate matter across the epithelium (Gebert *et al.* 1996) by fluid phase or receptor mediated endocytosis at the apical membrane (Neutra *et al*. 1996). Lange *et al*. (2009) reported that cells of immune system and tonsillar epithelial cells recognize pathogens via toll like receptors (TLRs), a group of pattern recognition receptors (PRRs), which were capable of recognizing conserved microbial structures and thus able to discriminate between self and non-self antigens. The light and scanning electron microscopy of the nasopharyngeal tonsil of sheep (Kumar and Nagpal 2007, Kumar *et al*. 2009); and anatomical localization and stereological volume assessment of the tonsils were studied in sheep (Cocquyt *et al*. 2005, Casteleyn *et al*. 2007). A lack of systemic study and prime importance of the tonsils for the purpose of diagnosis of certain infections prompted to envisage light and scanning electron microscopy of the tubal tonsil in the sheep.

The present study was conducted on tubal tonsils of 10 young sheep (6–9 months age) of either sex. The tissues from 5 sheep heads 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, Gomori's method for reticulum, Weigert's method for elastic fibres, McManus' method for glycogen (PAS), Alcian blue method for muco-substances (pH 2.5), PAS-Alcian blue method for mucosubstances (pH 2.5), diastase digestion method (Luna 1968) and Crossman's trichrome stain for collagen fibres (Crossman 1937). The tissues collected from remaining 5 animals were washed thoroughly in chilled 0.1 M phosphate buffer (pH 7.4) and fixed in 2% glutaraldehyde for 8 h followed by dehydration in ascending grades of ethanol, critical point drying and sputter coating with gold. Scanning electron microscopy was performed at EM Laboratory, AIIMS, New Delhi, India using Leo 435–VP (Japan).

The surface of tubal tonsil lined by pseudostratified columnar ciliated epithelium with goblet cells (Fig. 1) showed folds and invaginations which increased the surface area as reported in the horse (Kumar and Timoney 2005) and provided better opportunities for entrapment and attachment of foreign particles including the pathogens. The respiratory epithelium was constituted by 6–10 rows of nuclei of basal, supporting and goblet cells. The elongated nuclei of basal cells were vertically oriented and showed small irregular clumps of fine chromatin at different places (Fig. 1). Each cell possessed mainly one nucleolus which was eccentric in position. The supporting cells had dark round to oval nuclei with dense chromatin material which masked the appearance of nucleoli. The nuclei of goblet cells and a few large sized but less basophilic nuclei were irregularly distributed throughout the epithelium. Cytoplasm of all the cell types was finely granular and eosinophilic. The goblet cells showed strong periodic acid Schiff's (PAS) reaction for glycogen and neutral mucopolysaccharides as compared to acidic mucopolysaccharides (Figs 2, 3) and moderately positive for sialomucins, hyaluronic acid and weakly acidic sulfated mucosubstances. In contrast the goblet cells of nasopharyngeal tonsil of sheep showed predominance of acidic than neutral mucopolysaccharides (Kumar and Nagpal 2007). Diastase treatment reduced the positivity of reaction indicating presence of other mucopolysaccharides in addition to glycogen. The epithelium showed infiltration of isolated lymphocytes. The number of goblet cells was reduced in the epithelium when the latter was associated with

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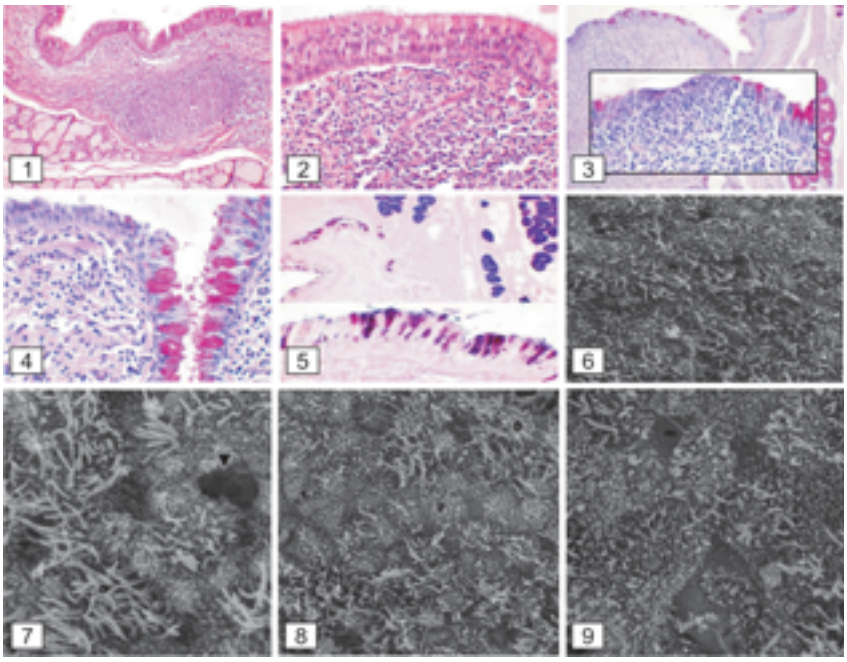

Figs 1-3. Photomicrograph of tubal tonsil showing: **1.** pseudostratified columnar-ciliated epithelium with goblet cells and scattered lymphoid tissue in the superficial lamina propria submucosa. H & E. ×400. **2.** A small modified patch of respiratory epithelium showing microvillus cells with absence of goblet and ciliated cells and the underlying lymphoid tissue. McManus' PAS. ×400. **3.** Predominance of neutral mucopolysaccharides in goblet cells and acidic mucopolysaccharides in mucous glandular acini. PAS Alcian blue. ×100 (a). ×400 (b). Scanning electron micrograph showing: **4.** A patch of FAE sandwiched in between the respiratory portion. Note: ciliated (D), type-I (1) and II (2) microvillus cells and isolated M cell. ×1960. **5.** type-I MV, intermediate (3) and M (m) cells; Note: reduced size of microvilli and depressed appearance of the M cells. × 3580.

underlying lymphoid follicle. A similar type of epithelium was called lymphoepithelium in the horse because of association of large amount of lymphoid tissue (Kumar and Timoney 2005).

At places the pseudostratified columnar ciliated epithelium was modified into follicle associated epithelium (FAE) characterized by absence of goblet cells, reduced number of cell layers and a large amount of infiltration of lymphoid cells due to interrupted basement membrane. The cells of FAE lacked cilia and contained microvillous (MV) cells some of which with very small sized microvilli and close association with lymphocytes were identified as M cells. These cells had been shown to possess epitopes on their apical surfaces reactive with lectin GS1-B4 (*Griffonia simplicifolia* 1 isolectin-B4) specific for α (1–3) linked galactose (Giannasca *et al.* 1997, Kumar *et al*. 2005). In some places an excessive lymphoid infiltration into the FAE obscured the occurrence of epithelial cells as reported in the horse (Kumar and Timoney 2005). Lack of goblet cells and mucous may facilitate direct contact of microorganisms with the FAE including M-cells. The topographical, structural and biochemical features of apical membrane of M-cells may enhance attachment and sampling of microorganisms as part of an immune surveillance mechanism (Giannasca *et al.* 1997). The poorly developed carbohydrate coat on the latter may also facilitate antigen uptake (Gebert *et al.* 1996).

Small patches of the respiratory epithelium shared the characteristics of FAE in the absence of underlying lymphoid tissue (Fig. 2). A few MV cells of this region were also associated with intra-epithelial lymphocytes in the absence of underlying lymphoid tissue as reported in the horse (Kumar and Timoney 2005) similar to intestinal-villous M-cells which were not associated with Peyer's patches (Jang *et al.* 2004). Isolated patches of stratified squamous non-keratinised epithelium were interspersed in between the respiratory epithelium.

The lymphoid tissue mainly presented the scattered distribution in the superficial lamina propria along with a few lymphoid follicles (Fig. 1). Cocquyt *et al.* (2005) reported 41–150 nodules on each side in sheep of which several primary and secondary lymph nodules were present towards the medial nasopharyngeal part of the auditory tube (Casteleyn *et al*. 2007). However the lateral mucosa contained less and mostly diffused lymphoid tissue. The concentration of these follicles was comparatively less as compared to those of nasopharyngeal tonsil in the sheep (Kumar and Nagpal 2007). These follicles were constituted by small, medium and large sized lymphocytes, macrophages and plasma cells. The high endothelial venules as specialized vessels were distributed only towards the parafollicular area that supported active lymphocyte migration from peripheral blood to the secondary lymphoid organs as reported in the

horse (Kumar and Timoney 2005). These were comparatively less in distribution as compared to those of the nasopharyngeal tonsil of the sheep (Kumar *et al.* 2009).

Longitudinally oriented dense elastic fibres separated the lymphoid follicles and the glandular tissue. The mucous type of acini was strongly PAS positive and had more distribution of acidic as compared to neutral mucopolysaccharides (Fig. 3). This was interesting to note as the goblet cells of the surface epithelium in contrary showed predominance of neutral mucopolysaccharides. The glandular acini also showed moderate positive reaction for sialomucins, hyaluronic acid and weakly acidic sulfated mucosubstances, whereas the glandular ducts showed positive reaction for glycogen, acidic and neutral mucopolysaccharides. Small nerve bundles and fatty tissue was observed irregularly in between clusters of glandular acini.

Scanning electron microscopy of the tubal tonsil revealed a dense mat of ciliated cells with interspersed microvillus and goblet cells. Majority of the ciliated cells possessed tufts of uniform sized cilia with bulbous endings and these masked the appearance of other cells. The microvillus (MV) cells with large microvilli were discernible where the density of ciliated cells was less. At places a few goblet cells and the glandular duct opening on the surface were also visible. The region of FAE possessed microvillus cells having uniform small sized and the large sized microvilli which were considered as type-I and type-II MV cells (Fig. 4), respectively as reported earlier in the horse (Kumar and Timoney 2005). Isolated MV cells interspersed in between the other types of MV cells, with much small sized microvilli and depressed centre were identified as M-cells (Fig. 5). The M-cells occurred singly however small clusters of these cells were observed in the nasopharyngeal tonsil of the sheep (Kumar *et al.* 2009). Cells with fewer cilia towards the periphery and small sized microvilli towards the centre were considered as intermediate cells (Fig. 5). Cells with microplicae representing the stratified squamous epithelium were sparsely distributed. The histoarchitecture and cellular components led the tubal tonsil as part of mucosa associated lymphoid tissue and it appeared as to be an inductive site for processing of antigens entering the respiratory system.

SUMMARY

The present light and scanning electron microscopic study conducted on tubal tonsils of young sheep revealed a pseudostratified columnar-ciliated epithelium with goblet cells which irregularly modified at places into follicle associated epithelium. The 2 epithelia differed significantly of which the latter was characterized by absence of goblet and ciliated cells, reduced number of cell layers along with their height and a close association with infiltrated lymphocytes. Scanning electron microscopy delineated 2 types of microvillus cells, transitional and the characteristic M-cells in the follicle associated epithelium on the basis of distribution of microvilli. The lamina propria submucosa had large amount of lymphoid tissue along with few lymphoid follicles which were constituted by different sized lymphocytes, plasma cells, macrophages and high endothelial venules. The goblet cells were strongly PAS positive for neutral whereas the glandular acini had a predominance of acidic mucopolysaccharides.

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