Histology and Histochemistry of Tonsil of Soft Palate of the Sheep (*Ovis aries*)

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ABSTRACT

Soft palate of the sheep having nasal and oral surfaces was studied in six young sheep of local mixed breed. The nasal surface was lined by pseudostratified columnar ciliated epithelium with goblet cells. The respiratory epithelium was modified at places into the follicle associated epithelium due to infiltration of lymphoid tissue from the propria. The goblet cells presented a strong PAS positive reaction for glycogen, acidic, neutral and weakly sulfated mucopolysaccharides. Isolated patches of mucosa also showed presence of stratified squamous non-keratinised epithelium. Propria submucosa had loose irregular connective tissue, small aggregations of lymphoid tissue, isolated lymphoid follicles and clusters of mucous glandular acini. The glandular acini and their ducts also presented a strong PAS positive reaction similar to that of goblet cells.

**Key words:** Follicle associated epithelium, Respiratory epithelium, Sheep, Soft palate tonsil

MATERIALS AND METHODS

The heads of 6 adult sheep (6-9 months age) were procured from local abattoir immediately after decapitation. The tissues were collected from the soft palate having nasal and oral surfaces, fixed in 10 per cent neutral buffered formalin for 48 hours and processed for routine paraffin embedding technique. The paraffin sections of 5-6 µ were cut and stained with routine Harris’ hematoxylin and eosin stain, Gomori's method for reticulum, Weigert's method for elastic fibres (Luna, 1968), Crossman's trichrome stain for collagen fibres (Crossman, 1937). For histochemical studies, the sections were stained by Alcian blue method for mucopolysaccharides (pH 2.5), McManus' method for glycogen (PAS), PAS-Alcian blue method for mucopolysaccharides (pH 2.5), Meyer's mucicarmine method and colloidal iron method for acid mucopolysaccharides (Luna, 1968).

RESULTS AND DISCUSSION

The soft palate towards nasal surface was lined by pseudostratified columnar ciliated epithelium. The epithelium was comprised of 6-8 rows of nuclei placed at varying heights and comprised of basal, ciliated and goblet cells (Fig. 1A). The nuclei of basal cells were round to oval shaped, vertically oriented and contained larger clumps of chromatin material which were irregularly distributed throughout the nucleoplasm. The cells contained generally one nucleolus which was eccentric in position. Supporting cells had narrow, elongated and cylindrical nuclei oriented towards mid portion of the epithelium. A few oval to irregular shaped nuclei were also present towards free surface. Cytoplasm of basal and supporting cells was finely granular and eosinophilic. Goblet cells were interspersed in between other cells and presented vacuolated appearance due to washing of the mucin during processing. The goblet cells were strongly PAS positive for glycogen (Fig. 1B). The concentration of neutral mucopolysaccharides was more than acidic (Fig. 1C). These cells showed positive

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affinity for Alcian blue indicating presence of weakly sulfated mucopolysaccharides, hyaluronic acid and sialomucins (Fig. 1D). A strong reaction was also demonstrated for acid mucopolysaccharides and mucins by colloidal iron and Meyer's mucicarmine methods, respectively (Figs. 1E, F). In contrast, a stratified squamous non-cornified epithelium lining the nasal and oral surfaces of the soft palate has been reported in the dog (Arrighi et al., 2011).

The majority of the respiratory epithelium was called as lymphoepithelium (LE) because of infiltration of lymphocytes and its close relation with underlying lymphoid tissue (Fig. 1A). The LE was further modified into small patches of follicle associated epithelium (FAE) which was distorted due to profuse infiltration of lymphocytes, reduced epithelial height, absence of goblet and ciliated cells (Figs. 1D, 2A). The regions of FAE were comparatively fewer in number however, the histoarchitecture features were similar to those of LE and FAE of nasopharyngeal tonsil of sheep and goats (Kumar and Nagpal, 2007; Kumar et al., 2006). Isolated microvillus cells distributed amongst ciliated respiratory epithelial cells were intimately associated with lymphocytes without presence of lymphoid follicles in the lamina propria. These cells were comparable to M cells present in intestinal villi in areas other than Peyer’s patches (Jang et al., 2004) and tubal tonsil of the horse (Kumar and Timoney, 2005). These cells have been reported to function as an independent gateway for antigen sampling and induction of immune responses. The lamina epithelialis mucosae towards lateral sides transitioned into patches of stratified squamous non-keratinised epithelium (Fig. 2B).

Lamina propria mucosae having loose irregular connective tissue was comprised of reticular, collagen and elastic fibres along with large number of blood capillaries and lymphoid tissue (Figs. 2C, D). The lymphoid tissue scattered irregularly throughout in the form of small clusters and a few lymphoid follicles, was constituted mainly by lymphocytes of varying dimensions, plasma cells and macrophages as reported in the nasopharyngeal tonsil of sheep and goat (Kumar and Nagpal, 2007; Kumar et al., 2006). Only few follicles presented a darkly stained corona towards periphery. Parafollicular and interfollicular areas were having connective tissue fibres, plasma cells, macrophages, blood capillaries, venules and high endothelial venules (HEVs) as reported in the nasopharyngeal tonsil of the sheep and goat (Kumar and Nagpal, 2007; Kumar et al., 2006).

Lamina muscularis mucosae was present in the form of an irregular layer of smooth muscles which separated the lymphoid and glandular tissue except at few places where it was interrupted and the glandular tissue extended into the connective tissue of propria. Submucosa was large and presented clusters of sero-mucous alveoli which were separated from each other by bundles of smooth muscles, connective tissue fibres especially the elastic and collagen fibres (Figs. 2C, D). Densely arranged elastic fibres were oriented horizontally at the junction of lymphoid and glandular tissue (Fig. 2D). A few elastic fibres were also vertically arranged in between the glandular alveoli. Predominantly occurring mucous alveoli had strongly basophilic and elongated nuclei which were pushed towards the basement membrane. Their cytoplasm was finely granular, lightly eosinophilic and had a vacuolated appearance. The mucous alveoli were strongly positive for PAS (Figs. 1B, C). Similar type of observations have been reported in nasopharyngeal tonsil of sheep and goat (Kumar and Nagpal, 2007; Kumar et al., 2006) and soft palate of dog (Arrighi et al., 2011). However, acidic and neutral mucopolysaccharides were almost equally distributed (Fig. 1C). The intra and inter glandular ducts were lined by simple cuboidal to stratified cuboidal epithelium and opened towards free surface of the epithelium. Some cells of the ducts were also PAS positive and presented an interesting pattern of distribution. Ducts of superficial portion had a predominance of neutral whereas the ducts of deeper portion had a dominance of acidic mucopolysaccharides. These ducts also presented a positive reaction for Alcian blue, colloidal iron and Meyer’s mucicarmine methods (Figs. 2D, E, F). An increased vascularity in the form of large sized venous caverns and blood vessels of varying dimensions along with large amount of fatty tissue was observed between glands and tunica muscularis towards lateral surface of the soft palate.

The oral surface was lined by stratified squamous epithelium (Fig. 3) having varying rows of nuclei of different strata as reported in the horse (Kumar and Timoney, 2006). The basal surface was uneven due to formation of papillary pegs. The stratum basale had oval nuclei which were oriented perpendicular to longitudinal axis of epithelium. Most of the nuclei of stratum spinosum were oriented horizontally and presented a prickly cell appearance. Stratum granulosum layer was constituted by cells having nuclei which were comparatively smaller but with more tapering ends and darkly stained chromatin material.
Fig. 1. Photomicrograph of nasal surface of the soft palate lined by pseudostratified columnar ciliated epithelium with goblet cells showing A. Lymphoepithelium and high endothelial venules (arrow). H. & E. × 400; B. Strong PAS positive reaction in goblet cells of the epithelium (arrow) and glandular acini. McManus' PAS method × 100; C. Presence of acidic and neutral mucopolysaccharides in the goblet cells of the epithelium, glandular acini and the glandular ducts (arrow). PAS Alcan blue method × 100; D. Alcianophilic positive reaction in goblet cells of the epithelium and glandular acini but absence in FAE (arrow). Alcian blue method × 100; E. Presence of acidic mucopolysaccharides in the goblet cells of the epithelium, glandular acini and the glandular ducts (arrow). Colloidal iron method × 100; F. Presence of mucins in the goblet cells of the epithelium, glandular acini and the glandular ducts (arrow). Meyer's mucicarmine method × 100.

Fig. 2. Photomicrograph of nasal surface of the soft palate lined by pseudostratified columnar ciliated epithelium with goblet cells showing A. Follicle associated epithelium (arrow) due to heavy infiltration of lymphocytes (arrow). H.E. stain × 400; B. Transition of respiratory epithelium into stratified squamous non-keratinised epithelium and presence of acidic and neutral mucopolysaccharides in glandular acini and the glandular ducts (arrow). PAS Alcan blue method × 100; C. Distribution of collagen fibres (arrow). Crossman's trichrome stain × 100; D. Horizontally arranged elastic fibres (arrow) separating lymphoid and glandular tissues. Weigert's method × 100.

Fig. 3. Photomicrograph of oral surface of the soft palate lined by stratified squamous non-keratinised epithelium showing A. Different strata of the epithelium and a lamellated structure (arrow). H. & E. × 100; B. Higher magnification of lamellated structure (arrow) in the epithelium. Crossman's trichrome stain × 400; C. Distribution of collagen fibres (arrow). Crossman's trichrome stain × 100; D. Strong PAS positive reaction in glandular acini (arrow). McManus' PAS method × 100; E. Presence of weak reaction for mucins in the glandular acini and absence in glandular ducts (arrow). Meyer's mucicarmine method × 100; F. Presence of weak reaction for acidic mucopolysaccharides in the glandular acini and absence in the glandular ducts (arrow). Colloidal iron method × 100.
However, eccentric nucleoli were discernible. Size and number of nuclei kept on reducing towards stratum corneum and became irregular shaped with pyknotic appearance. In addition, a few very large sized nuclei were also observed. The cells had long cytoplasmic process having slightly basophilic cytoplasm. Stratum corneum also possessed varying number of cell layers having only a few strongly basophilic nuclei with darkly stained chromatin material masking the presence of nucleoli. The cytoplasm of these cells was finely granular and strongly eosinophilic.

The epithelium did not exhibit modification in the form of reticular epithelium as reported in palatine tonsil of the sheep (Kumar et al., 2008). The cells of stratum basale and stratum spinosum were loosely arranged in a spongiform network with finger-like projections into the lymphoid tissue in the tonsil of soft palate of the horse (Kumar and Timoney, 2006). Similar structure was not observed during present study however, lamellated structures with concentric arrangement of nuclei were observed at the junction of stratum corneum and granulosum (Figs. 3A, B). These structures having lightly stained nuclei were comparable to Pacinian corpuscles.

Lamina propria mucosae was having loose irregular connective tissue and fine blood capillaries. The lymphoid tissue as present towards the nasal surface was not observed. In contrast, lymphoid tissue in the form of small aggregates and lymphoid follicles had been reported in the tonsil of soft palate of the horse (Kumar and Timoney, 2006) and the palatine tonsil of the sheep (Kumar et al., 2008). Lamina muscularis mucosae was constituted by a few layers of smooth muscles. Tunica submucosa had predominance of mucous alveoli being separated by collagen fibres (Fig. 3C). The glandular alveoli had pattern of distribution of PAS positive material similar to that of nasal surface as demonstrated by different histochemical techniques (Figs. 3D, E). However, overall intensity of acid mucopolysaccharides seen by colloidal iron method was comparatively reduced (Fig. 3F). The intra and interglandular ducts were devoid of PAS activity (Figs 3D, E, F). At places, bundles of striated muscles interspersed in between these glands were also observed. Tunica muscularis was common of oral and nasal surfaces and had bundles of striated muscles which were oriented in different profiles.

Nasopharynx associated lymphoid tissue has been reported to be a preferred site for intranasal route of vaccination due to induction of B cell responses and generation of plasma cells. The histoarchitectural features suggest that the nasal surface of soft palate is part of mucosa associated lymphoid tissue which may be exploited as a targeted organ for intranasal vaccines.

REFERENCES


