Haryana Vet. **47** (December, 2008), pp 15-18

Research Article

LIGHT MICROSCOPIC STUDIES ON THE PALATINE TONSIL OF SHEEP

PAWAN KUMAR[1], R. MAHESH, GURDIAL SINGH and S. K. NAGPAL
Department of Veterinary Anatomy and Histology, College of Veterinary Sciences
CCS Haryana Agricultural University, Hisar -125 004

ABSTRACT

The palatine tonsil of sheep showed stratified squamous keratinized epithelium towards outer surface which was modified into reticular epithelium without keratinization in crypt. The crypt appeared to be single but it had branching pattern in the deeper part where the reticular epithelium associated with lymphoid tissue changed into lymphoepithelium just comparable to follicle associated epithelium seen in the nasopharyngeal tonsil. The lymphoid tissue was mainly seen in the form of lymphoid follicles along with a few isolated aggregations. The lymphoid follicles having lightly stained germinal centre, darkly stained corona and parafollicular area were constituted by lymphocytes, plasma cells, macrophages and high endothelial venules. The latter were also observed in isolated aggregations of lymphoid tissue in subepithelial propria submucosa, a feature not reported earlier in tonsils. The mucous glandular acini present in propria submucosa showed strong PAS positive reaction for glycogen, acidic, and weakly sulfated muco-polysaccharides.

Key words: Palatine tonsil, crypt epithelium, lymphoepithelium, sheep

Palatine tonsil, a lymphoepithelial organ, constitutes a component of Waldeyer's ring which is an integrated mucosal immune system of the pharynx (Ogra, 2000). The anatomical topography and presence of crypts in palatine tonsil favors its exposure to exogenous material including microbial pathogens and their transport to lymphoid tissue in horse (Kumar and Timoney, 2005). The crypt epithelium along with mantle zone, interfollicular area and germinal center of lymphoid follicle functions as an immune organ by production of immunocytes (Kataura *et al.*, 1992). The tonsils as first line of defence against foreign antigens have been explained by high incidence of infectious diseases in young cattle during first months of life when the tonsils are not well developed (Menesse *et al.*, 1998). The present study on the palatine tonsil of sheep explores its histoarchitecture for better understanding of early pathogenesis of different respiratory diseases.

MATERIALS AND METHODS

Palatine tonsils were collected from heads of 6 young sheep (6-9 months age) procured from local slaughter house immediately after decapitation. The tissues were fixed in 10 per cent neutral buffered formalin and processed for light microscopy. Paraffin sections (5 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, Ayoub-Shklar's method for keratin and pre-keratin (Luna, 1968) and Crossman's trichrome stain for collagen fibres (Crossman, 1937). The sections were also stained by McManus' method for glycogen (PAS), Alcian blue method for muco-substances (pH 2.5), PAS-Alcian blue method for mucosubstances (pH 2.5) and diastase digestion method (Luna, 1968).

RESULTS AND DISCUSSION

The palatine tonsil of sheep was lined by stratified squamous keratinized epithelium towards outer surface which was irregular at places (Figs 1, 2). Whereas, the deeper surface was uneven due to presence of papillary pegs which were deeply indented reaching almost to the level of stratum corneum. The epithelium was comprised of strata basale, spinosum, granulosum and corneum as reported in horse and goat (Kumar and Timoney, 2005, Kumar *et*

[1]Corresponding author

al., 2006). The stratum basale was comprised of simple columnar cells having elongated darkly stained nuclei and finely granular eosinophilic cytoplasm. Several layers constituted the stratum spinosum and its cells shared the histological features similar to those of stratum basale except that the cells of its superficial layers had oval to elongated nuclei which were horizontally oriented. Stratum granulosum constituted by several layers of cells possessing narrow, elongated and basophilic nuclei which were horizontally placed. The nuclei of the superficial layers were reduced in dimensions. Stratum corneum was thick having most of its nuclei showing degenerative changes whereas the cytoplasm was more eosinophilic and homogeneous. Keratin was observed as demonstrated by special stain.

The stratified squamous keratinized epithelium became non-keratinized in the crypts and had strata basale, spinosum and superficiale. This epithelium decreased in height due to reduced number of cell layers and was called as reticular epithelium because of its association with lymphoid tissue and lacked distinct strata (Fig 3). At places, the reticular epithelium was drastically reduced with only 1-2 cell layers with predominance of lymphoid cells and this modification was called as lymphoepithelium (Fig 3) which was considered as an equivalent to follicle associated epithelium reported in nasopharyngeal tonsil (Kumar and Nagpal, 2007). At places the reticular epithelium not associated with lymphoid tissue was also observed (Fig 4). This type of epithelium has not been reported earlier in tonsils of domestic animals. The infiltration of lymphoid cells, mainly the lymphocytes, plasma cells and macrophages was so extensive in reticular and lymphoepithelium that it obscured the presence of epithelial cells and reached to the free surface of the crypt. The superficial layers of reticular and lymphoepithelia contained vacuolated cells.

The crypt usually appeared as a single opening towards the free surface but in the deeper part it was divided and contained varying number of lymphoid cells and cell debris. The crypts increased the epithelial surface area in contact with the tonsillar parenchyma and thus, ingested material might be carried by penetration of crypt system into proximity with the substantially larger volume of lymphoid tissue. These factors indicated a specific functional relationship between environment of oral cavity and tonsillar lympho-epithelial tissue (Koburg 1967). The desquamation and loss of mesenchymal cells into lumen might have eliminated most of the material taken up by the tonsil and permitted only a sample to reach the lymphoid tissue (Trautmann and Fiebiger, 1952, Williams and Rowland, 1972). The crypt region of human palatine tonsil was involved in the uptake of antigen and their presentation to lymphocytes (Howie, 1980). The lamellated structures resembling Pacinian's corpuscles reported in stratified squamous epithelium of palatine tonsil of horse (Kumar and Timoney, 2005) and goat (Kumar *et al.*, 2006) were not observed in present study.

Propria submucosa having loose irregular connective tissue and large number of small blood capillaries was mainly occupied by the lymphoid tissue towards the crypt (Figs 1, 2, 4) and glandular tissue adjacent to the crypt. The reticular fibres in addition to formation of the basement membrane were few in number. The concentration of collagen and elastic fibres was more towards the superficial part of propria submucosa which was lacking the lymphoid tissue (Fig 4) and in the portion separating the lymphoid and glandular tissues.

The lymphoid tissue was mainly organized into lymphoid foillicles along with isolated small aggregations without follicle in the subepithelial propria submucosa (Figs 1, 3). The follicular lymphoid tissue encapsulated by bundles of collagen and elastic fibres was separated from adjacent one by trabeculae like structure which was mainly formed by fine collagen and elastic fibres along with few reticular fibres. The follicles of different size and shape stacked one above the other were separated from each other by interfollicular areas (Fig 1). Most of follicles had darkly stained corona facing the crypt epithelium, parafollicular area and lightly stained germinal centre which were constituted by small and large lymphocytes, plasma cells, macrophages, high endothelial venules (HEVs) and blood capillaries

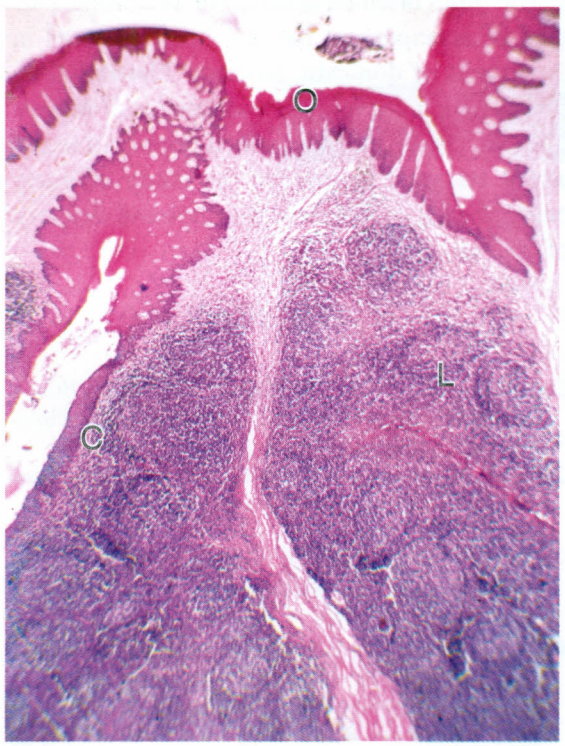

Fig 1. Stratified squamous keratinized epithelium towards outer surface (O), reticular epithelium (C) along with crypt and dense arrangement of lymphoid tissue (L) in the propria submucosa of palatine tonsil of sheep. (H. & E. x 40)

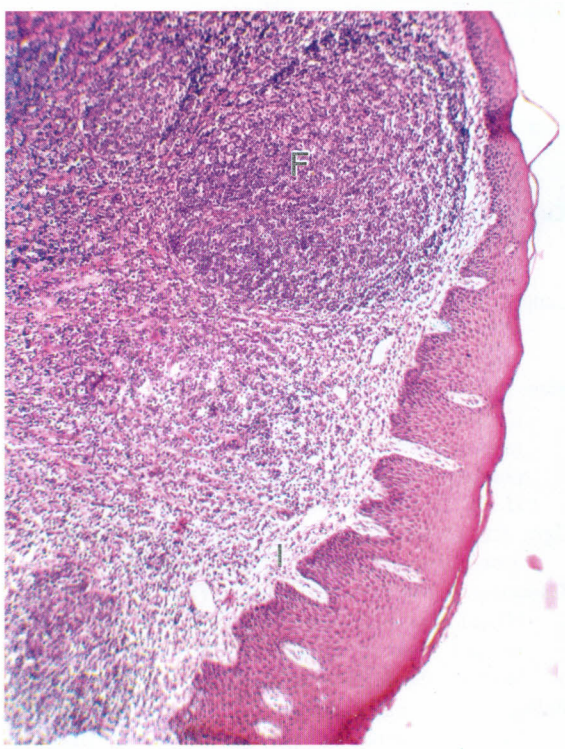

Fig 2. Outer surface epithelium, lymphoid tissue arranged in follicles (F) and isolated aggregate (I) of palatine tonsil of sheep. (H. & E. x 100)

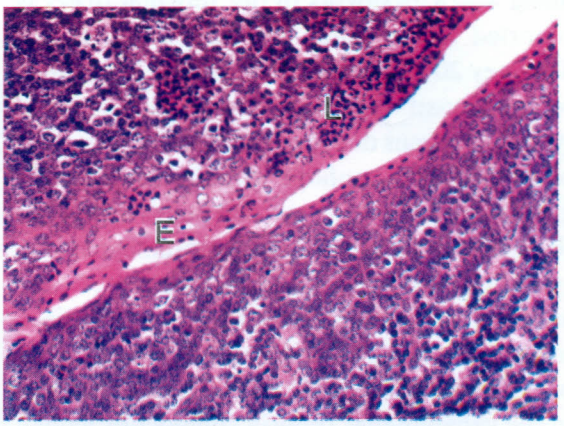

Fig 3. Palatine tonsil showing modification of reticular epithelium into lymphoepithelium (E). Note higher concentration of lymphocytes linearly arranged in subepithelial portion (L). (H. & E. x 100)

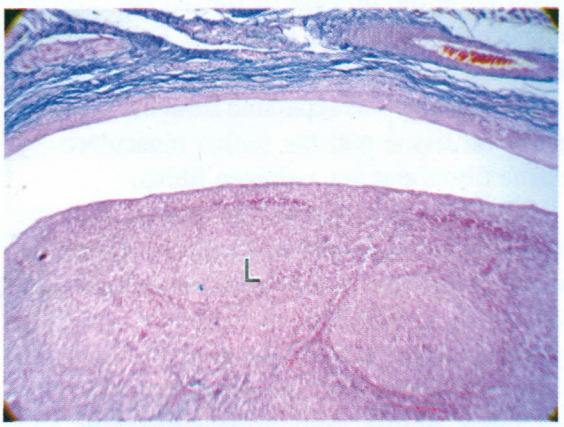

Fig 4. Palatine tonsil showing distribution of collagen fibres just below reticular epithelium in superficial part of propria submucosa (blue colour). Note absence of fibres in the area of lymphoid tissue (L). (Crossman trichrome x 100)

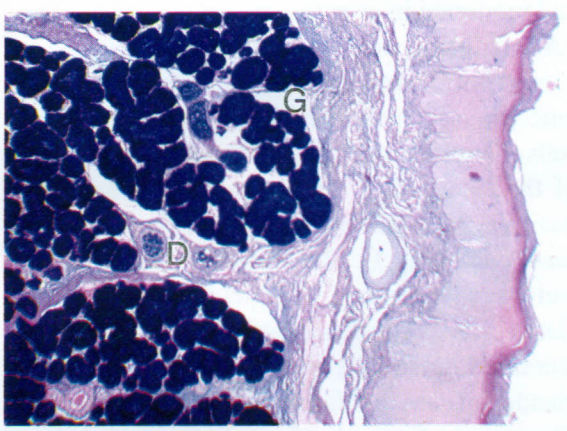

Fig 5. Photomicrograph of palatine tonsil showing more distribution of acidic than neutral mucopolysaccharides in glandular acini (G). Note absence of reaction in inter and intraglandular ducts (D). (PAS AB x 100)

along with a meshwork of fine reticular fibers as reported in the horse and goat (Kumar and Timoney 2005, Kumar et al., 2006). The human palatine tonsil was unique among the peripheral lymphoid organs due to presence of large number of secondary lymphoid follicles with active germinal centres (Curran and Jones 1978). At few places in the subepithelial propria submucosa, lymphocytes were densely populated and arranged in a linear pattern. The HEVs localized to parafollicular area contained varying number of lymphocytes. The HEVs were also observed in the subepithelial propria submucosa having isolated aggregations of lymphoid tissue, a site which had not been reported earlier. These HEVs also had large number of lymphocytes which might be in the process of trafficking through inter or intra-endothelial migration. The lymphoid tissue extended up to the deeper part of propria submucosa and was separated from the adjacent glandular tissue and the tunica muscularis by elastic fibres and few collagen fibres.

The glandular tissue was localized in the deeper propria submucosa adjacent to lymphoid tissue. However, it was most superficial just below the epithelium in the portion where lymphoid tissue was absent (Fig 5). The acini showed strong PAS positive reaction for glycogen, acidic mucopolysaccharides and weakly sulphated mucosubstances. The concentration of neutral mucopoly-saccharides was very less (Fig 5). Alcianophilic reaction was weak in the horse (Kumar and Timoney, 2005) and moderate in goat (Kumar *et al.*, 2006). The PAS reaction was moderate after diastase digestion indicating presence of mucopolysaccharides other than glycogen. The cells of intra and inter glandular ducts were devoid of the PAS positive reaction though the PAS positive material was noticed in the lumen of these ducts (Fig 5). The mucous glandular acini were surrounded by fine reticular, collagen and elastic fibres. The concentration of elastic fibres increased drastically in the area separating the glandular tissue and fasciculi of striated muscles. The muscle fasciculi of striated muscles were oriented in different directions and were separated by loose connective tissue having large elastic fibres oriented in different directions. This type of arrangement has not been reported in the tonsils of domestic animals. The immuno-histological features of the palatine tonsil suggest it as part of mucosa associated lymphoid tissue and a targeted organ for the study of early pathogenesis of different diseases and delivery of oral vaccines.

REFERENCES

Crossman, G.A. (1937). A modification of Mallory's connective tissue stain with a discussion of principles involved. *Anat. Rec.* **69**: 33-38.

Curran, R.C. and Jones, E.L. (1978). The lymphoid follicles of the human palatine tonsil. *Clin. Exp. Immunol.* **31**: 251-259.

Howie, A.J. (1980). Scanning and transmission electron microscopy of the epithelium of human palatine tonsil. *J. Pathol.* **130**: 91-97.

Kataura, A., Harabuchi, H., Matsuyama, H. and Yamanaka, N. (1992). Immunohistological studies on immunocompetent cells in palatine tonsil. *Adv. Oto-Rhino-Laryngol.* **47**: 97-100.

Koburg, E. (1967). Cell production and cell migration in the tonsil. In: Germinal Centre in Immune Response. Odartchenko, N, Schindler R and Congdon, C.C. (Edts.), Berlin, Springer Verlag. (cited by Williams, D.M. and Rowland, A.C. 1972).

Kumar, P., Kumar, Pawan and Kumar, Suraj. (2006). Light and scanning electron microscopic studies on the palatine tonsil of the goat. *Indian J. Anim. Sci.* **76**: 1004-1006.

Kumar, Pawan and Nagpal, S.K. (2007). Histology and histochemistry of the nasopharyngeal tonsil of sheep. *Haryana Vet.* **46**: 75-79.

Kumar, Pawan and Timoney, J.F. (2005). Histology, immunohistochemistry and ultrastructure of the equine palatine tonsil. *Anat. Histol. Embryol.* **34**: 192-98.

Luna, L.G. (1968). Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology. (3rd edn.), McGraw-Hill Book Co., New York.

Menesse, M., Delverdier, M., Bourges, N.A., Sautet, J., Cabanie, P. and Schelcher, F. (1998). An immunohistochemical study of bovine palatine and pharyngeal tonsils at 21, 60 and 300 days of age. *Anat. Histol. Embryol.* **27**: 179-185.

Ogra, P.L. (2000). Mucosal immune response in the ear, nose and throat. *Pediatr. Infect. Dis. J.* **19**: 4-8.

Trautmann A. and Fiebiger J. (1952). Fundamentals of the Histology of Domestic Animals. (Translated and revised from the 8th and 9th German edn., 1949). Bailliere, Tindall and Co., London.

Williams, D.M. and Rowland, A.C. (1972). The palatine tonsils of the pig-an afferent route to the lymphoid tissue. *J. Anat.* **113**: 131-37.