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Research Article

HISTOLOGY AND HISTOCHEMISTRY OF THE NASOPHARYNGEAL TONSIL OF SHEEP

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ABSTRACT

The nasopharyngeal tonsil of sheep having pseudostratified columnar ciliated epithelium with goblet cells was modified into follicle associated epithelium at places and contained M cells. The lamina propria submucosa had a dense distribution of lymphoid tissue mainly in the form of lymphoid follicles having different sized lymphocytes, plasma cells and macrophages. High endothelial venules were observed towards parafollicular areas. The goblet cells of the respiratory epithelium and mucus glands distributed in the deeper part showed strong PAS and Alcian blue reaction for glycogen, acidic mucopolysaccharides. The morphological and histochemical featutres of the sheep nasopharyngeal tonsil indicates that it is a component of mucosa associated lymphoid tissue.

Key words: Nasopharyngeal tonsil, M cell, FAE, sheep

The lingual, palatine, nasopharyngeal (adenoid) and tubal tonsil and the tonsil of the soft palate collectively known as Waldeyer's ring, form the principal components of the mucosa associated lymphoid tissues (MALTs) of the oro-and nasopharynx in the horse (Kumar *et al*., 2001, Kumar and Timoney, 2001, 2005 a, b, c, d, 2006). The MALT of tonsils is characterized due to presence of a mucosal epithelium being infiltrated and associated by lymphoid tissue especially in crypts called lymphoepithelium. Follicular associated epithelium (FAE) along with mantle zone, interfollicular area and germinal center of the lymphoid follicles acts as lymphoid compartment by contributing to the production of immunocytes and to the protection of the mucosal surface (Kataura *et al*., 1992). The main classes of lymphocytes, T (thymus-dependent) and B (bursa-dependent) cells acquire characteristics during passage through primary lymphoid organs and are responsible for cell mediated and humoral immune responses. B and T lymphocytes localize to specific areas as shown by homing studies and immunohistochemical staining and structural analysis of thymectomized mice, and athymic and normal rats (Fossum, 1980, van der Valk *et al*., 1984). The tonsils are sites of predilection for several non-pathogenic and pathogenic bacteria. These organisms become active and develop mechanisms under certain conditions to use tonsils especially FAE as portal of entry, multiplication, colonization and spread of infection to adjacent organs. These features make the tonsils of prime importance for the purpose of diagnosis of certain infections. Hence, the present study has been envisaged to explore the morphological features of the nasopharyngeal tonsil in the sheep.

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MATERIALS AND METHODS

The present study was conducted on 6 young healthy sheep of local mixed breed. The heads collected immediately after slaughtering were sectioned in mid sagittal plane to collect nasopharyngeal tonsil. The tissues fixed in 10 per cent neutral buffered formalin for 48 h were processed for paraffin technique of light microscopy to obtain sections of 5-6 µ which were stained by routine Harris hematoxylin and eosin stain, Gomori's method for reticular fibres, Weigert's method for elastic fibres, McManus' method for glycogen, PAS-Alcian blue method, Alcian blue (pH 2.5) method (Luna, 1968) and Crossman's trichrome stain for collagen fibres (Crossman, 1937). The observations were recorded and photographs were taken by Olympus trinocular microscope using digital camera.

RESULTS AND DISCUSSION

The nasopharyngeal tonsil (NPT) formed a raised mass with an irregular and deeply furrowed surface caudal to the pharyngeal opening of the Eustachian tube and extended into the pharyngeal recess. The folded mucosa of NPT was lined with pseudostratified columnar ciliated epithelium with goblet cells (Figs 1, 2, 3, 4). The crypts not only increased the lymphoid tissue in a given area but also hypothetically offered a better opportunity for antigen entrapment and attachment as demonstrated earlier (Chen *et al*., 1989). Epithelium varying in its height at different places was generally reduced towards the base of the crypts. It was comprised of 8-10 rows of oval to elongated nuclei distributed mainly towards the basement membrane. The cells were divided into three types on the basis of nuclear morphology. Round to oval nuclei of basal cells were dense because of uniform distribution of chromatin material and located close to the basement membrane. Tall columnar cells had elongated nuclei with less basophilic chromatin material and were distributed throughout the height of the epithelium especially towards the free apical surface. Irregularly distributed goblet cells were strongly PAS positive. The reaction was very strong for glycogen, acidic mucopolysaccharides, (Figs 1, 2, 3, 4) hyaluronic acid, weakly acidic sulfated mucosubstances and sialomucins (Figs 3, 4). The cytoplasm of all the cell types was finely granular and eosinophilic. This epithelium was called as lymphoepithelium (LE) because of its close relation with underneath lymphoid tissue of lamina propria mucosae in the horse (Kumar and Timoney, 2001). A few intraepithelial lymphocytes were also observed.

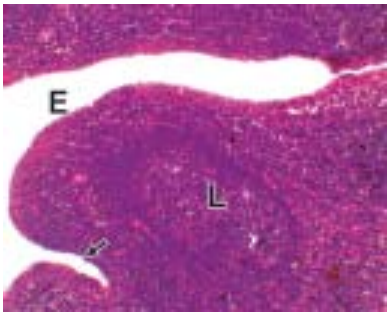

Fig 1. Photomicrograph of the NPT showing pseudostratified columnar ciliated epithelium (E), follicle associated epithelium (arrow) and lymphoid tissue (L). (H. & E. x 40)

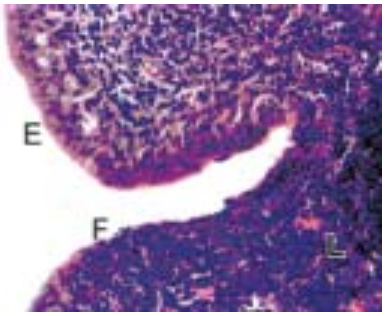

Fig 2. Photomicrograph showing respiratory epithelium (E) and FAE (F) of the NPT along with associated lymphoid tissue (L). (H. & E. x 400)

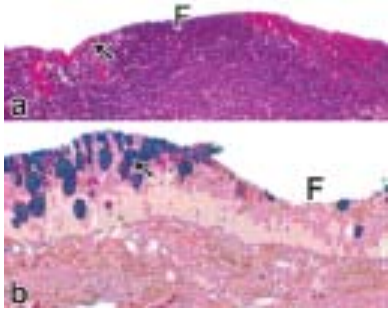

Fig 3 a. Photomicrograph of the NPT showing PAS positive goblet cells (arrow) in the respiratory epithelium. Note absence of PAS reactivity in the FAE (F). (McManus PAS method x 100)
b. Photomicrograph of the NPT showing predominance of acidic mucopolysaccharides in the goblet cells (arrow) of the respiratory epithelium. Note absence of positive reactivity in the FAE (F). (PAS Alcian blue method x 400)

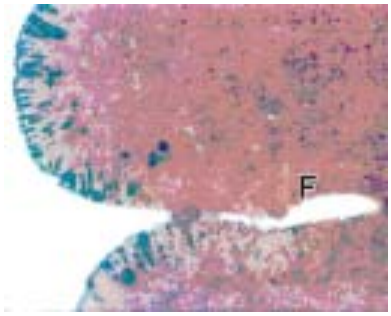

Fig 4. Photomicrograph of the NPT showing Alcianophilic positive goblet cells in the respiratory epithelium (blue colour). Note absence of positive reactivity in the FAE (F). (Alcian blue method pH 2.5 x 400)

The respiratory epithelium modified irregularly at places into FAE having low epithelial height, reduced number of rows of nuclei, absence of goblet cells, loss of cilia and infiltration of lymphocytes (Figs 5, 6). The absence of goblet cells had been associated to reduce the thickness and/or modify the

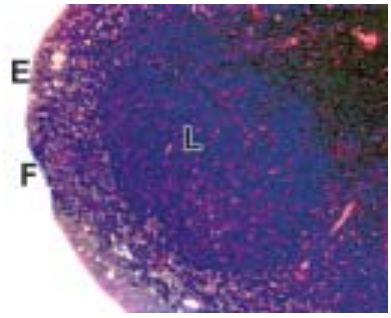

Fig 5. Photomicrograph of the NPT showing respiratory epithelium (E) and FAE (F) of the NPT along with associated lymphoid tissue (L). (H. & E. x 400)

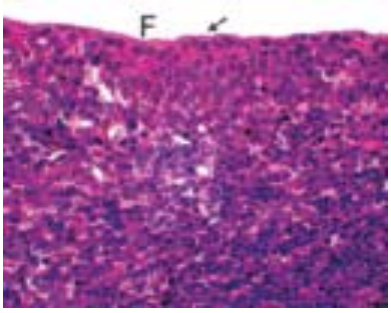

Fig 6. Photomicrograph of FAE (F) at higher magnification showing M cells (arrow) associated with lymphoid tissue. (H. & E. x 400)

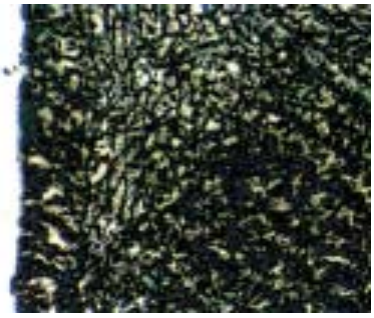

Fig 7. Photomicrograph showing interrupted basement membrane in the region of FAE (arrow) and fine reticular fibres extending in the lymphoid tissue. (Gomori's method x 400)

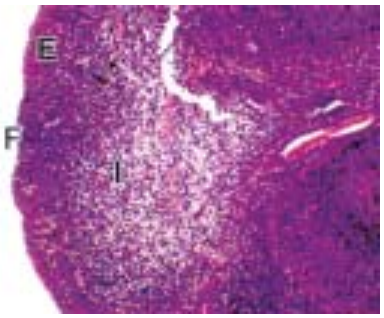

Fig 8. Photomicrograph of respiratory epithelium (E) and FAE (F) associated with isolated aggregation of lymphoid tissue (I). (H. & E. x 100)

composition of the mucous layer over the FAE. The most superficial cuboidal cells of the FAE with round to oval nuclei presenting flat surface and associated with lymphocytes had been identified as M (membranous/microvillus) cells (Fig 6) as they had been shown to possess epitopes on 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*., 2001). The flattened apical surface of M cell facilitated close contact with particulate antigens trapped in mucous blanket and not cleared by the mucociliary system of the respiratory epithelium (Kuper *et al.*, 1992). The topographical, structural and biochemical features of the apical membrane of M cells may enhance attachment and sampling of micro-organisms as part of an immune surveillance mechanism (Giannasca *et al*., 1997). The presence of intraepithelial small to medium lymphocytes was a regular feature but their number increased drastically towards the base of the FAE as reported in the goats (Kumar *et al*., 2006). The irregular and interrupted basement membrane in the FAE region led to infiltration of lymphoid cells (Fig 7). The free surface had fine cilia, irregular clusters of free lymphocytes and cell debris. The regions of FAE amongst the respiratory epithelium were comparatively more as compared to those of horse and goats (Kumar *et al*., 2001, Kumar *et al*., 2006). The FAE shared histological features with bronchus associated lymphoid tissue and other MALTs in different species of domestic and laboratory animals (Mair *et al*., 1987, Chen *et al*., 1989, Giannasca *et al*., 1997, Kumar *et al*., 2001, 2006, Kumar and Timoney, 2001).

The lamina propria mucosae had loose irregular connective tissue along with dense aggregates of lymphoid tissue and clusters of mucous acini. The lymphoid tissue was mainly arranged in the form of lymphoid follicles (Figs 1, 5). At few places small aggregations of isolated lymphoid tissue were also seen in the subepithelial portions (Fig 8). The lymphoid follicles having parafollicular and central nodular areas were separated from each other by interfollicular areas. A few follicles also showed darkly stained corona towards the dome facing the epithelium. The lymphoid tissue was comprised of small, medium and

large lymphocytes, plasma cells, interdigitating cells and macrophages. The interfollicular areas separating the lymphoid follicles were constituted mainly by macrophages, interdigitating reticular cells and a few high endothelial venules (HEVs). The reticular fibres surrounding the lymphoid follicles in addition penetrated the latter (Fig 7) which otherwise had been reported to be absent in other species (Kumar *et al*., 2001, Kumar *et al*., 2006). The location of these nodules and the topography of associated FAE had been the basis of the suggestion that they acted as mechanisms for trapping and sampling antigens in the air stream and might have helped to guard against infections spreading from pharynx towards the middle ear (Mair *et al*., 1987) and may be source of immunoglobulin producing for other mucosal sites like tracheo-bronchial tree in cattle (Anderson *et al*., 1986). Prominence of lymphoid tissue in the NPT was related to increased deposition of air-borne particles due to bending of air stream from the nasal passages and convergence of multiple streams of mucous carrying particles from all parts of nasal passages in the nasopharynx (Morgan *et al*., 1984). The HEVs were mainly localized to parafolliclular areas along with a few blood capillaries and venules. The HEVs appeared to be in greater numbers as compared to those of goats (Kumar *et al*., 2006). The mucous acini present in the deeper part of the lamina propria mucosae were strongly PAS positive for glycogen, acidic mucopolysaccharides, weakly acidic sulfated mucosubstances, sialomucins and hyaluronic acid as reported in goats (Kumar *et al*., 2006). The concentration of neutral mucopolysaccharides was dominated by the acidic ones. The PAS reaction was very mild to absent in the rest of the propria mucosae.

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