Histology, Histochemistry and Scanning Electron Microscopy of Lingual Tonsil of Young Pigs

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Abstract

The present study was conducted on lingual tonsil of 10 young male pigs of 8-10 months age of local mixed breed for light and scanning electron microscopic studies. The lingual tonsil was lined by stratified squamous keratinized to non-keratinized epithelium having different strata. This epithelium modified into reticular epithelium especially toward the crypts which was associated with lymphoid tissue. Propria submucosa had loose irregular connective tissue, glandular tissue, fatty tissue and lymphoid aggregations mainly in the form of follicles. These follicles were separated by interfollicular areas having lymphocytes, plasma cells, macrophages, fine blood capillaries and high endothelial venules. The mucous type of glandular acini presented strong reaction for glycogen, acidic and neutral mucopolysaccharides. The scanning electron microscopy revealed that the surface of the lingual tonsil was having different types of papillae with broader base and pointed tips except a few having blunt tips. The higher magnification revealed flat epithelial cells and different arrangements of microplicae.

Key words: Reticular epithelium, Lingual tonsil, Pigs, High Endothelial Venules.

1. Introduction

The lingual tonsil formed by the collection of tonsillar or crypto-lymphatic units at the base of the tongue (Nair and Rossinsky, 1984) has been termed follicular glands (Bal gadrusen) with structural similarity to tonsil and other lymphoid tissue (Kollikher, 1858). The stratified squamous keratinized or non-keratinised epithelium modifies into reticular epithelium towards crypts which are constantly exposed to alimentary and airborne antigens and acts as first line of defense by neutralizing or containing infectious agents and by initiating and maintaining immune responses (Perry, 1994). It also functions 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 tonsils of pigs have been studied either with preliminary report (Casteleyn et al., 2011) or except the lingual tonsil (Liu et al., 2012). The present study describes in detail the histological architecture, histochemistry and scanning electron microscopy of lingual tonsil in pigs.

2. Material and Methods

2.1 Light Microscopy

The present study was conducted on 10 young male pigs of 8-10 months age of local mixed breed. The heads were procured from local slaughter house immediately after decapitation. The tissues of lingual tonsil for histomorphological and histochemical studies were used from 5 heads and fixed in 10 per cent neutral buffered formalin solution for 48 hours. The fixed tissues were processed for routine paraffin technique for light microscopy. 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), McManus’ method for glycogen (PAS), Alcian blue method for muco-substances (pH 2.5), PAS-Alcian blue method for acidic and neutral mucosubstances (pH 2.5), Meyer’s mucicarmine method for mucin, colloidal iron method for acid mucopolysaccharides (Luna, 1968).

2.2 Scanning Electron Microscopy
Fresh tissues from 5 pig heads were collected for scanning electron microscopy. The tissues were fixed in 2 per cent glutaraldehyde solution for 6 hours after thorough washing in chilled 0.1 M phosphate buffer (pH 7.4). The tissues were again washed twice with 0.1 M phosphate buffer and rest of the procedure was carried out at EM-Laboratory, AIRF, JNU, New Delhi. The processed tissues were viewed in scanning electron microscope (Zeiss EVO-40).

3. Results and Discussion

The surface of lingual tonsil was lined by stratified squamous keratinized and non-keratinized epithelium (Figs 1, 2) in pig as reported earlier (Casteleyn et al., 2011), sheep (Cocquyt et al., 2005, 2008) and goat (Kumar and Kumar, 2005) however it was stratified squamous non-keratinized epithelium in horse (Kumar and Timoney, 2005). The keratinized epithelium was having stratum basale, stratum spinosum, stratum granulosum and stratum cornium while stratified squamous non-keratinized was comprised of strata basale, spinosum and superficiale. The stratum basale cells had oval to elongated nuclei with dense chromatin material localized into smaller irregular clumps with one or more eccentric nucleoli and a slightly basophilic cytoplasm as reported in horse (Kumar and Timoney, 2005a). The stratum spinosum was composed of 3-4 cell rows however it was composed of 8-12 cell rows in horse and goat (Kumar and Timoney, 2005). The nuclei of these cells were oriented vertically towards interpapillary pegs with moderately dense chromatin. These nuclei were round to oval towards the superficial layers and less basophilic. The cytoplasm was eosinophilic with a prickly appearance. The outer most stratum superficiale layer was composed of a varying number of cell layers. The dense elongated nuclei of these cells showed degenerative changes, which masked the nucleoli. The eosinophilic cytoplasm of these cells possessed a few keratohyaline granules in horse (Kumar and Timoney, 2005a) and goat (Kumar and Kumar, 2005) however, in pig the outer most stratum cornium had varying number of cell layers ranging 4-6 to 8-10 at different places. The cytoplasm of all cell layers was eosinophilic in nature.

The stratified squamous epithelium at some places towards outer surface and mainly towards the crypts was modified into reticular epithelium (Figs 2, 3, 4) or lymphoepithelium (Perry and Whyte, 1998; Cocquyt et al., 2008) which was similar to the outer surface epithelium except for its association with lymphoid tissue, absence of interpapillary pegs and fewer cell layers that lacked distinct strata and interrupted basal lamina as reported in horse (Kumar and Timoney, 2005a) and goat (Kumar and Kumar, 2005). The stratum basale was heavily infiltrated with lymphoid cells and numerous blood capillaries (Figs 2, 3, 4). At some places, the lymphoid tissue extended to the crypt surface so that the internal environment of the tonsil and lumen of the crypt were separated by a layer of only one to two cells thickness. This arrangement has been termed a lymphoepithelial symbiosis (Fioretti, 1957) because of its lymphoid appearance and absence of classic epithelial features. Characteristic of reticular epithelium was coexistence of epithelial and non-epithelial cells and, sometime there was predominance of the later (Figs 3, 4) as reported in human (Nave et al., 2001) and horse (Kumar and Timoney, 2005a). The non-epithelial elements in the reticular epithelium included a varying number of lymphocytes, macrophages, plasma cells and dendritic cells (Perry, 1994; Kumar and Timoney, 2005a; Kumar and Kumar, 2005). The presence of these 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 (von Gaudecker and Muller-Hermelink, 1982a, b) was a normal developmental process. Reticulation was life long and the presence of non-epithelial cells was considered a physiological characteristic (Perry, 1994). Lamellated structures resembling Hassall’s corpuscle occasionally observed towards the outer surface epithelium in horse (Kumar and Timoney, 2005a) could not be observed during present study in pigs.

The propria submucosa was characterized by loose irregular connective, lymphoid, glandular and muscular tissues as reported in horse (Kumar and Timoney, 2005a), goat (Kumar and Kumar, 2005) and sheep (Cocquyt et al., 2005; 2008). Reticular fibres basement membrane at the base of the outer surface epithelium except for those areas where lymphoid infiltration extended to the crypt surface as reported in the horse (Kumar and Timoney, 2005a) and goat (Kumar and Kumar, 2005). A dense arrangement of collagen fibres (Fig 5) was intermingled with a fine meshwork of reticular fibres and fine blood capillaries in the subepithelial portion however, lymphoid follicles were separated from each-other by a dense meshwork of reticular fibres and a few collagen, elastic and nerve fibres as reported in goat (Kumar and Kumar, 2005). The stratified squamous epithelium and most of the propria submucosa showed negative PAS reaction by McManus’ method.

Lymphoid tissue (Figs 2, 3, 4) was distributed in the form of isolated clusters towards the outer surface epithelium, and lymphoid follicles of different dimension which generally possessed of germinal -

Figs 10-12: SEM of lingual tonsil showing 10. Different types of papillae at higher resolution x 59 (Bar = 200 μ). 11. Different arrangements of microplicae at higher magnification x 12740 (Bar = 2 μ). 12. Various patterns of microplicae still at higher magnification x 17350 (Bar = 2 μ).
centre and darkly stained corona as reported in horse (Kumar and Timoney, 2005b), goat (Kumar and Kumar, 2005) and sheep (Cocquyt et al., 2008). The interfollicular areas were having the lymphocytes, plasma cells, macrophages, blood capillaries, high endothelial venules (HEVs) and fine reticular fibres. The HEVs were specialized vessels which supported active migration of lymphocytes from peripheral blood to secondary lymphoid organs. Cytoplasmic processes of the endothelial cells increased the surface area and may converted a laminar flow into a turbulent one and thus increased probability of interaction between circulating lymphocytes and the endothelial surface (Stan, 2002).

The lobes of glandular tissue were separated from each other by bundles of striated muscles. In addition, the fatty tissue and small to medium sized blood vessels were also observed in goat (Kumar and Kumar, 2005). A connective tissue capsule consisting of a dense arrangement of collagen and fine reticular fibres separated the lymphoid follicles. Mast cells irregularly distributed in the propria submucosa were more numerous in the vicinity of blood vessels in horse (Kumar and Timoney, 2005b). The glandular tissue was having mucus acini which were comprised of pyramidal cells however interglandular and intraglandular ducts had simple to stratified cuboidal epithelium, generally of two cell layers thickness. Mucus glandular acini showed a positive reaction for glycogen (Fig 6), acidic and neutral mucopolysaccharides, weakly sulfated mucopolysaccharides, hyaluronic acid and sialomucins (Fig 7) as reported in goat (Kumar and Kumar, 2005). Neutral mucopolysaccharides were more abundant than acidic as reported in horse (Kumar and Timoney, 2005b). The interglandular ducts were devoid of Alcianophilic reaction as reported in goat (Kumar and Kumar, 2005).

Scanning electron microscopy of lingual tonsil revealed an irregular surface presenting papillae of varying shapes and size (Figs 8, 9, 10) as reported in horse (Kumar and Timoney, 2005a) and goat (Kumar and Kumar, 2005). The higher magnification revealed flat squamous epithelial cells forming a sheath like structure as reported in horse (Kumar and Timoney, 2005a). Further higher magnification showed different arrangements of microplicae (Figs 11, 12) resembling to finger prints of humans as reported in the horse (Kumar and Timoney, 2005a) and goat (Kumar and Kumar, 2005). At sites of desquamation, the microplicae of underlying and superficial cells were continuous with each other as reported in goat (Kumar and Kumar, 2005).

4. Conclusion
The modification of stratified squamous epithelium into reticular epithelium especially towards crypts suggested its involvement in processing of antigen because of its close association with diffuse lymphoid tissue and follicles and also presence of high endothelial venules.

References

