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Histology, Immunohistochemistry and Ultrastructure of the Equine Palatine Tonsil

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With 11 figures and 2 tables

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Summary

The palatine tonsils of five young horses formed 10–12 cm elongated follicular structures extending from the root of the tongue on either side to the base of the epiglottis and lateral to the glossoepiglottic fold. The stratified squamous non-keratinized epithelium of the outer surface was modified into crypts as reticular epithelium by heavy infiltration of lymphoid cells from underlying lymphoid follicles. In places, lymphoid tissue reaching almost to the surface and with only one to two cell layers intact was identified as the lymphoepithelium. Langerhans cells with Birbeck granules were interspersed between epithelial cells. Lymphoid tissue organized in lymphoid follicles constituted the parenchyma of the palatine tonsil. CD4-positive cells were more numerous and CD8-positive lymphocytes less numerous compared with their distribution in the lingual tonsil. B cells and macrophages were also more numerous than in the lingual tonsil and lectins showed a different pattern of attachment. M cells were not observed. High endothelial venules with well-developed vesiculo-vacuolar organelle had structural evidence of transendothelial and interendothelial migration of lymphocytes. Striated muscles as seen in the deeper lamina propria mucosae of the lingual tonsil were absent. The immunohistological and ultrastructural characteristics of the equine palatine tonsil are similar to those of humans but differ from those of the lingual tonsil and are consistent with a role as an effector and inductor immunological organ.

Introduction

The palatine tonsil, a lymphoepithelial organ, is a component of the integrated mucosal immune system of the pharynx (Ogra, 2000) and is a major element of Waldeyer's ring. Its anatomical topography favours exposure to exogenous material including microbial pathogens and their transport to lymphoid cells. Crypts formed by invaginations of the outer surface stratified squamous epithelium are characterized by the association of epithelial and non-epithelial cells. In places, the latter predominate with disruption of the basement membrane (Kumar and Timoney, 2005a). This epithelium together with the mantle zone, interfollicular area and germinal centre of the lymphoid follicles function as an immune organ by production of immunocytes (Kataura et al., 1992). The lymphoepithelial barrier samples and translocates antigens to the underlying lymphoid tissue (Perry and Whyte, 1998). The tonsils consist of follicles, T-dependent zones, and areas through which antigens can pass and effector cells accumulate (Velinova et al., 2001). Extravasation of lymphocytes from the blood to the palatine tonsils and back is essential for the immunological competence of these organs (Baekkevold et al., 1999). This traffic is essential because few immunocompetent cells specific for an individual antigen are available in a non-infected tonsil (Pabst et al., 1998). Cell to cell interactions between lymphocyte and high endothelial venule cells in various lymphoid organs or inflamed tissues account for the peculiar roles of circulating lymphocytes during a local immune response (Fischer et al., 1993). It is known that extravasation of lymphocytes from a vessel involves tethering of lymphocytes by lectin-mediated interaction, activation of integrin by chemotactins and cytokines, and tight adhesion of the lymphocyte to the vessel wall via interaction of integrin and IgG superfamily molecules of the vessel (Sasaki et al., 1996).

The nasopharyngeal, lingual and tubal tonsillar components of Waldeyer's ring have been studied in detail in the horse (Kumar and Timoney, 2001, 2005a,b,c). The palatine tonsil of the horse has been classified as tonsilla folliculata aggregata disjuncta impressa, whereby aggregated lymph nodules are situated around crypts (Kleinschmidt, 1939). Elias (1946) concluded that the equine palatine tonsil was a haemolymphatic organ and differed from the tonsil of other species in that the tunica propria formed lamellae rather than papillae. More recent studies on the mucosal lymphoid tissue of the equine upper respiratory tract did not include the palatine tonsil (Mair et al., 1987, 1988). This study focuses on features of the palatine tonsil that may contribute to its function.

Materials and Methods

Histomorphology

The study was conducted on five 1–2-year-old horses of either sex, raised on the farm of the University of Kentucky. The palatine tonsils were removed immediately after killing and aliquots fixed in 10% neutral-buffered formalin, and embedded in paraffin. Sections (5–6 μm) were stained with routine Harris haematoxylin and eosin, Mallory's trichrome stain for collagen, Gomori's method for reticulum, Weigert's stain for elastic fibres, McManus' periodic acid Schiff (PAS) method for glycogen, PAS Alcian blue method for acidic and neutral mucopolysaccharides, and Alcian blue (pH 2.5) for weakly sulphated mucosubstances (Luna, 1968).

Lectin histochemistry

Sections were incubated with biotinylated lectins, SBA (Soya bean), WBA 1 (*Phosphocarpus tetragonolobus* 1), UEA (*Ulex*

europaeus) and GS 1-B4 (*Griffonia simplicifolia* 1 isolectin-B4), diluted to 10 μg/ml in 0.2% gelatin PBS for 60 min at room temperature as described earlier (Kumar and Timoney, 2005a). Slides were screened by epifluorescence microscopy (Axioscope-20, Zeiss, Thornwood, NY, USA) and image analysis was carried out using the QUIPS-XL and QUIPS-AKS system (Vysis, Downer's Grove, IL, USA).

Vimentin

Frozen sections were treated with mouse antivimentin (mAb V9) diluted 1:40 in phosphate-buffered saline (PBS) for 1 h followed by fluorescein-conjugated antimouse IgG 1:100 for 1 h as reported earlier (Kumar and Timoney, 2005a).

Immunohistochemistry

Sections of frozen palatine tonsil were processed and incubated separately with mouse monoclonal antibodies specific for equine IgA, IgGb (undiluted) and CD4 (1:100), CD8 (1:50), CVS-38 (1:100), CD172a (1:50) as described earlier (Kumar and Timoney, 2005b).

Scanning and transmission electron microscopy

Fresh tissues were thoroughly washed and processed for scanning and transmission electron microscopy (Kumar and Timoney, 2005b).

Results and Discussion

The palatine tonsils were elongated and follicular structures, approximately 10–12 cm long and 1.5–2 cm wide, extending on either side from the root of the tongue and lateral to the glossoepiglottic fold to the base of the epiglottis. The outer surface was lined by stratified squamous non-keratinized epithelium (Fig. 1) comprised of strata basale, spinosum and superficiale as reported in the lingual tonsil (Kumar and Timoney, 2005a). The oval to elongated nuclei of the stratum basale were vertically oriented on a uniform basement membrane of reticular fibres and were strongly basophilic because of the condensation of chromatin towards the inner nuclear membrane. Their cytoplasm was slightly basophilic. A variable number of rows of lightly basophilic nuclei of varied shape with fine chromatin formed the stratum spinosum. Nuclei towards the surface were round to oval, vacuolated, and intermingled with those of the stratum superficiale. The nuclei of the latter were elongated, basophilic and showed signs of degeneration towards their free surfaces. Their cytoplasm was eosinophilic and finely granular.

The epithelium within the crypt was designated reticular epithelium (Figs 1 and 2) because of its heavy infiltrate of lymphoid cells and proximity to underlying lymphoid follicles. This epithelium with fewer rows of cells lacked distinct strata. An early study (Elias, 1946) concluded, without evidence, that this epithelium was the end result of a destructive process. Nuclei of the stratum basale on the interrupted basement membrane were less basophilic than those of the outer epithelium. Other nuclei were round to oval with fine chromatin and one to two distinct nucleoli. The most superficial cells had either flat or round to oval nuclei. Small patches of reticular epithelium one to two cell layers thick were called lymphoepithelium (Fig. 2). Cells in the crypt lumen appeared to be shed from this zone. The intact layers had squamous to cuboidal cells. The direct transepithelial access of antigens may result in greater influx and recruitment of

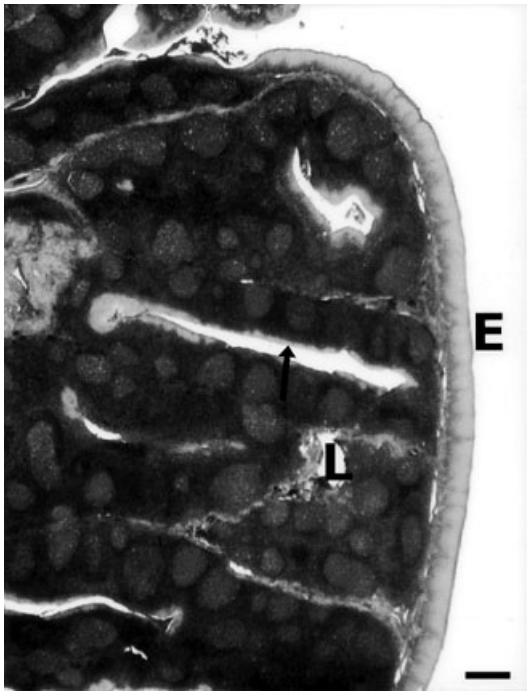

Fig. 1. Palatine tonsil of the horse showing outer surface epithelium (E), reticular epithelium (arrow) and lymphoid tissue (L). H&E, ×10 (bar 450 μm).

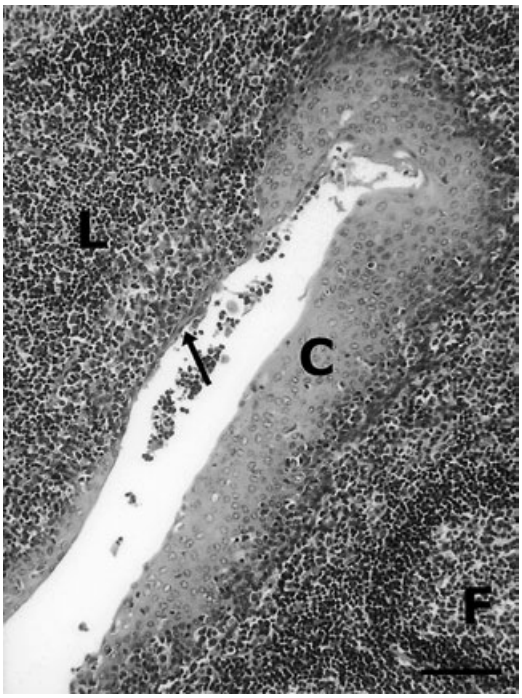

Fig. 2. The reticular epithelium (C) and associated lymphoid follicles (F) with their corona facing the epithelium. Note few cell layers of lymphoepithelium (arrow) with associated lymphoid tissue (L). H&E, ×100 (bar 100 μm).

non-epithelial cells in a particular patch, in contrast to a less strongly stimulated area which may have retained stratified squamous non-keratinized cover (Perry, 1994). The appropriate balance of epithelial and non-epithelial cells is required to preserve efficient mucosal protection as locally produced IgA secretory component is transported by these cells. The secretory component is not recycled, but is continually synthesized by the epithelial cells (Perry, 1994). Small amounts of glycogen and acidic mucopolysaccharides were present between the epithelial cells of the outer surface and those of the crypt. Tonsillar epithelial cells are known to exhibit energy demanding absorptive and secretory functions and so the stored glycogen in the upper strata of the epithelium may form an energy source (Perry, 1994).

Several adhesion molecules, cytokines and chemokines are necessary for entry of lymphocytes into the tonsils. Chemokines produced in the tonsillar crypts attract memory B cells and play a role in retaining them in the crypt (Casamayor-Palleja et al., 2001). Lamellated structures associated with the surface epithelium of the lingual and palatine tonsils (Elias, 1946; Kumar and Timoney, 2005a) were seen in the crypt epithelium of the palatine tonsil.

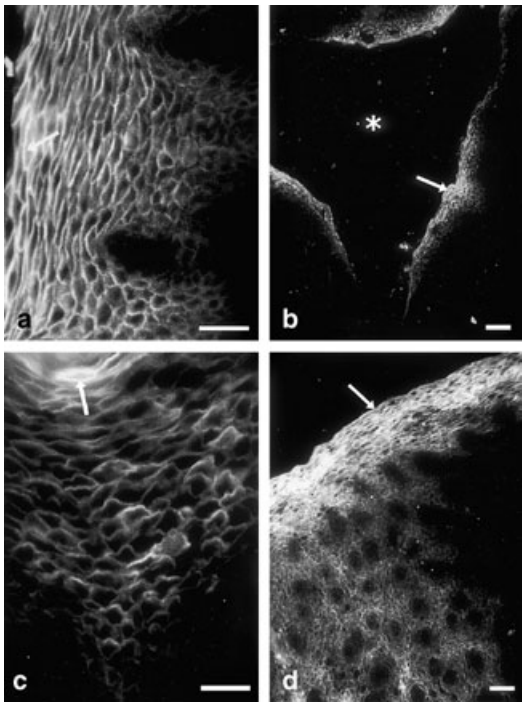

Fig. 3. (a) Outer surface epithelium showing binding of lectin SBA; (b) WBA to the crypt epithelium; (c) UEA to the outer surface epithelium; and (d) GS1-B4 to the outer surface epithelium. Note crypt (*) in (b) and comparatively more attachment toward the arrows (bar 100 μm).

The lamina propria mucosae consisted of connective, lymphoid and glandular tissues. The subepithelial portion had a uniform thin layer of densely arranged collagen fibres and a few reticular fibres arranged vertically in the form of septae to divide the lymphoid tissue into compartments, each of which had reticular epithelium in the centre. The collagen fibres extended to the entire height of the lymphoid tissue and merged with collagen bundles encircling the periphery of the lymphoid tissue. The reticular crypt epithelium was closely related with a fine meshwork of reticular fibres but collagen fibres were lacking in this area. Lymphoid tissue constituted the majority (approximately 90%) of the palatine tonsil (Fig. 1) and was organized into lymphoid follicles, which were vertically stacked and separated by interfollicular areas. Lymphoid follicles characteristically extended uniformly without interruption into the subepithelial lamina propria mucosae. Unlike the lingual tonsil, there were no isolated aggregations of lymphoid tissue (Kumar and Timoney, 2005a). Lymphoid follicles consisted of a germinal centre (Elias, 1946), parafollicular area and darkly stained corona, which generally faced the crypt epithelium (Fig. 2). The germinal centre was populated with lymphocytes, plasma cells, follicular dendritic cells (FDC) and few macrophages. The parafollicular and interfollicular regions were rich in lymphocytes, plasma cells, macrophages, interdigitating cells, blood capillaries, venules and high endothelial venules (HEVs) along with a meshwork of fine reticular fibres. HEVs were greater in number, possibly because of the heavy concentration of lymphoid cells. Efferent lymphatics were also evident.

Small clusters of mucus glandular acini were strongly positive for glycogen and neutral mucopolysaccharides. Acidic mucopolysaccharides were located near the luminal surface. The acini were weakly positive for sulphated mucosubstances unlike those of the lingual tonsil (Kumar and Timoney, 2005a). These mucopolysaccharides were absent in the intra- and interglandular ducts lined by simple to stratified epithelia except for a few cells of the intraglandular ducts. The dense arrangement of collagen fibres and striated muscles reported in the lingual tonsil were not present in the deeper part of the lamina propria mucosae of the palatine tonsil (Kumar and Timoney, 2005a).

The attachment patterns of different lectins (Fig. 3a,b,c,d) are summarized in Table 1. Soya bean lectin showed comparatively more binding to the superficial layers of the surface and

Table 1. Attachment patterns of different lectins in the palatine tonsil of the horse

	Outer surface epithelium			Reticular epithelium of crypt				
Lectin	Stratum basale	Stratum spinosum	Stratum superficiale	Stratum basale	Stratum spinosum	Stratum superficiale	Connective tissue	Glandular tissue
SBA	+ +	+ + +	+ + + +	+ +	+ + +	+ + +	+	+ + +
WBA	+	+ +	+ + +	+	+ +	+ + +	–	–
UEA	+	+	+ +	+	+ +	+ +	+	+ + + +
GS1-B4	+ +	+ + +	+ + + +	+ +	+ + +	+ + + +	+ +	–

–, negative; +, weak; + +, moderate; + + +, Strong; + + + +, very strong reaction.
SBA, soya bean specific for GalNAc; WBA, *Phosphocarpus tetragonolobus* I specific for terminal GalNAc; UEA, *Ulex europaeus* specific for fucose; GS1-B4, *Griffonia simplicifolia* 1 isolectin-B4 specific for terminal galactose.

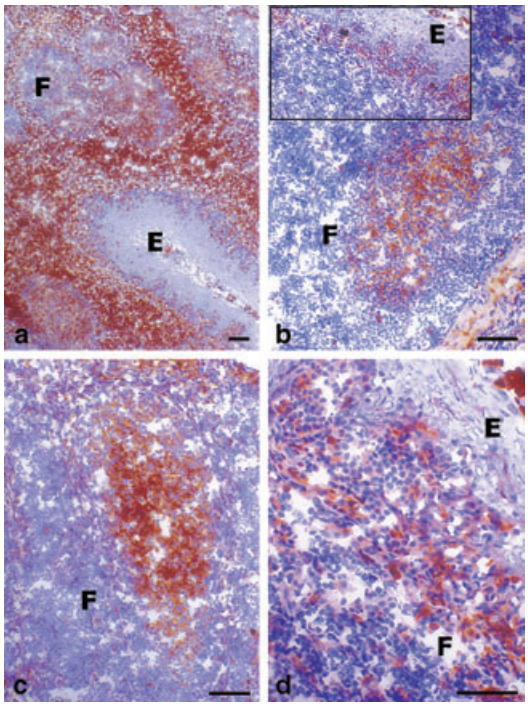

Fig. 4. (a) Distribution of CD4+, (b) IgA+, (c) IgGb+ B lymphocytes and (d) macrophages in the epithelium (E) and follicles (F) of the palatine tonsil (bar 100 μm).

to the crypt epithelium than to those regions of the lingual tonsil (Kumar and Timoney, 2005a). Binding of lectin WBA was similar to that of the lingual tonsil. The lectin UEA bound weakly to the strata basale and spinosum, although showing no binding in the lingual tonsil. The stratum superficiale of the outer surface showed a strong binding of lectin GS1-B4 when compared with the weak binding observed in the lingual tonsil (Kumar and Timoney, 2005a). Vimentin filaments could not be demonstrated in the outer surface and reticular epithelia consistent with the absence of M cells as reported in the lingual tonsil (Kumar and Timoney, 2005a).

The distribution of lymphoid cells by phenotype (Fig. 4a,b,c,d) is summarized in Table 2. CD4+ lymphocytes formed dense populations in the interfollicular area followed by the parafollicular area, germinal centre and the crypt epithelium and were more numerous (Fig. 4a) than in the lingual tonsil (Kumar and Timoney, 2005b). CD8+ cells were much fewer than CD4+ cells. The sparsity of CD8+ cells contrasts with their much greater numbers in the lingual tonsil. CD4+ and CD8+ lymphocytes in the crypt epithelium reflect their involvement in cellular and humoral immune responses (Salles and Middleton, 2000). IgA+, IgGb, IgG(T)+ B cells and macrophages (Fig. 4b,c,d) were fewer in number than in the lingual tonsil (Kumar and Timoney, 2005b). T and B cells, macrophages, dendritic cells and plasma cells have previously been reported in all regions of the palatine epithelium (Nave et al., 2001).

Scanning electron microscopy revealed an irregular outer surface with deep branching crypts (Fig. 5), extending almost to the entire depth of the tonsil. The cut surface showed lymphoid tissue and intervening collagen fibres (Fig. 6). The deep branching of the crypts markedly increases the contact area between the external environment and lymphoid tissue (Salles and Middleton, 2000). Higher magnification images of the outer and crypt epithelia revealed patterns of microplicae (Fig. 6) as reported in the lingual tonsil (Kumar and Timoney, 2005a).

The ultrastructure of cells of the stratum basale revealed large numbers of polyribosomes, mitochondria and tonofilaments, which were even more numerous in the cells of the stratum spinosum. The irregular, polyhedral nuclei of the latter with very small clumps of chromatin were more electronlucent than those of the lingual tonsil. However, the distribution of their cell organelles was similar (Kumar and Timoney, 2005a). Langerhans cells interspersed between these cells contained large numbers of phagosomes, Golgi bodies, mitochondria, vacuoles, endoplasmic reticulum and characteristic dumb-bell-shaped Birbeck granules (Fig. 7), which are formed by langerin, a C-type lectin responsible for the uptake of mannosylated bacterial antigens (Valladeau et al., 2000). These cells along with monocytes and macrophages are involved in phagocytosis, processing and presentation of foreign antigens (Yamamoto et al., 1988). The tonofilaments were further decreased in the cells of the stratum superficiale, which, however, were comparatively more abundant than those of the lingual tonsil (Kumar and Timoney, 2005a). Other cell organelles were reduced in proportion to the stage of nuclear degeneration. However, glycogen granules, cytoplasmic filaments and small-sized vesicles were increased in number. Small spine-like structure projected from the surface of the stratum superficiale. These cells in the crypt were closely associated with lymphocytes, macrophages and plasma cells and were of different shapes, their free surfaces possessing small microvilli-like structures (Fig. 8). These may be the precursors of M cells, which are capable of pinocytosis in the lymphoepithelial tissue (Olah et al., 1988). A few Langerhans cells were also observed in this area. FDC, plasma cells,

Table 2. Relative numbers of lymphoid cells and macrophages in different regions of the palatine tonsil of the horse

Phenotype	Outer epithelium	Crypt epithelium	Interfollicular area	Parafollicular area	Germinal centre
CD4	*	+ +	+ + +	+ + +	+ +
CD8	*	+	+ +	+ +	+
IgA	+	+	–	+	+ + +
IgG(T)	–	+	+	+	+ + +
IgGb	–	+	+	+	+ + +
CD172a	–	+	+ +	+ +	+

–, not detected; *Scarce; +, few; + +, moderately frequent; + + +, very frequent.
Monoclonal antibodies HB61 (CD4), HT14A (CD8), DH59B (CD172a) were obtained from VMRD, Pullman, WA and those specific for IgA (BVS2), IgG(T) (CVS38), IgGb (CVS39) were obtained as described elsewhere (Lunn et al., 1998; Sheoran et al., 1998).

Fig. 5. Scanning electron micrograph showing the irregular outer surface and crypts (arrow) of the palatine tonsil ×20 (bar 1500 μm).

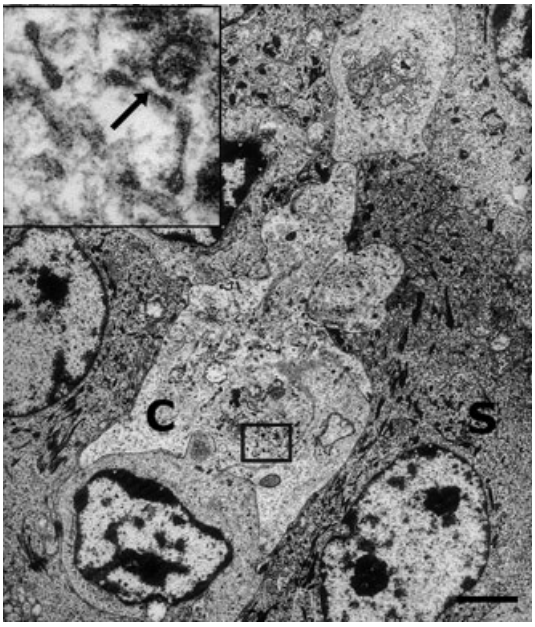

Fig. 7. Transmission electron micrograph showing Langerhans (C) and stratum spinosum cells (S). The dumbbell-shaped Birbeck granules (arrow) are shown in the inset at higher magnification ×4000 (bar 2.25 μm).

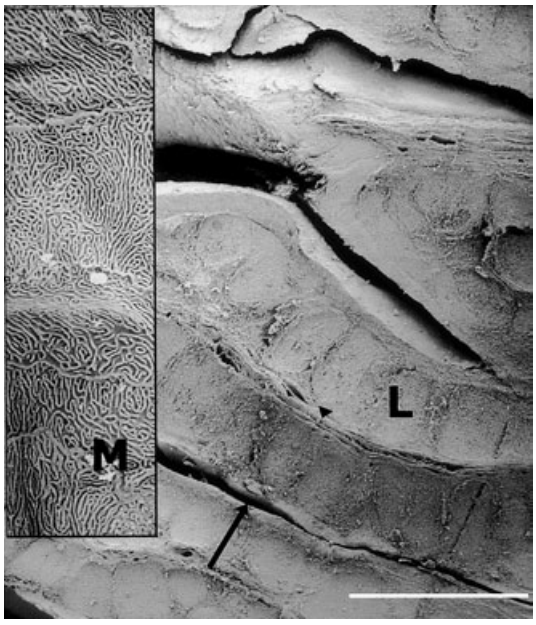

Fig. 6. Scanning electron micrograph of cut surface of the palatine tonsil showing crypt (arrow), lymphoid tissue (L) and collagen fibres (arrowhead). Microplicae (M) are shown in the inset at higher magnification of the epithelium ×20 (bar 1500 μm).

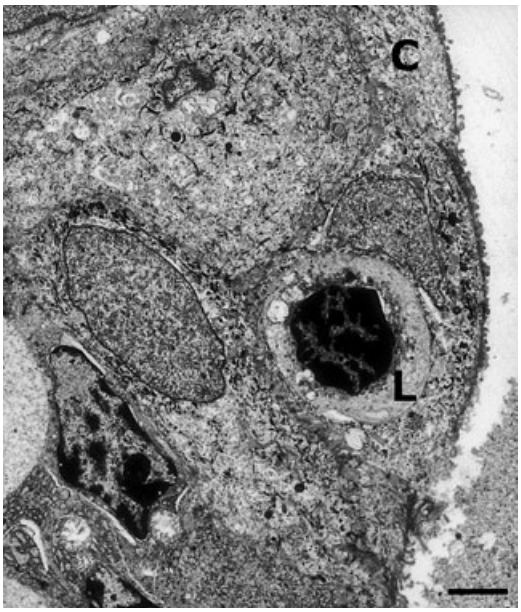

Fig. 8. Transmission electron micrograph showing varied shaped nuclei of the reticular epithelium (C) and an infiltrated lymphocyte (L) ×4000 (bar 2.25 μm).

lymphocytes, interdigitating cells and macrophages of characteristic morphology were identified in the lymphoid compartments (Figs 9 and 10). Electron-dense deposits on the plasma membranes of some FDC were identified as immune complexes (Velinova et al., 2001).

The specialized vessels termed HEVs had high, irregularly shaped, light staining endothelial cells with distinct basal lamina, and a pericytic sheath with lymphocytes migrating through the endothelial wall (Fig. 10). The basal lamina showed a few peripheral weaker layers surrounding the HEV that provided circumferential compartments for migrating lymphocytes (Sunami-Kataoka et al., 2001). Sulphated glycosaminoglycan was rich in chondroitin sulphate, which has ligand potential for some adhesion molecules, including L-selectin in the HEVs (Sunami-Kataoka et al., 2001). The high cuboidal to low columnar endothelial cells of HEVs were shorter than those of the lingual tonsil (Kumar and

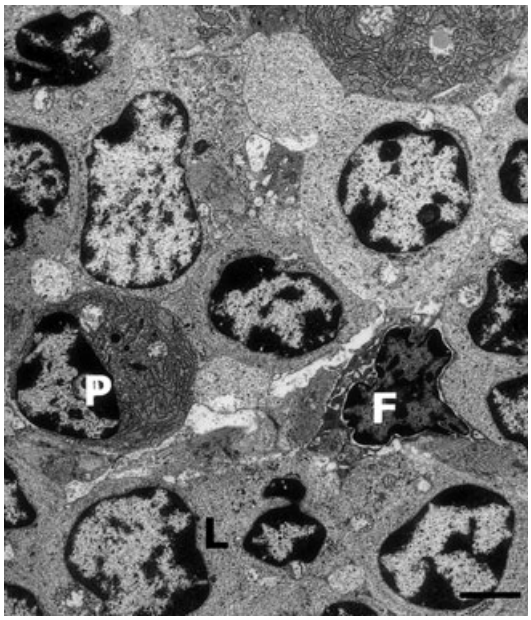

Fig. 9. Transmission electron micrograph showing a follicular dendritic cell (F), plasma cell (P) and lymphocytes (L) ×4000 (bar 2.25 μm).

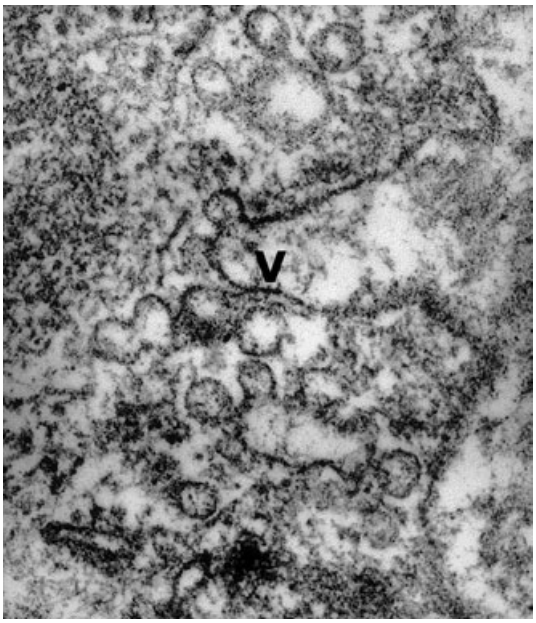

Fig. 11. Transmission electronmicrograph showing a vesiculo-vacuolar organelle (V) in the cytoplasm of the endothelial cells ×60 000 (bar 0.1 μm).

Fig. 10. Transmission electron micrograph showing a high endothelial venule with high endothelial cells (E) and lymphocyte (L) attached to the luminal surface. Note lymphocyte in process of transendothelial migration (arrow) and an interdigitating cell (I) adjacent to the venule ×3000 (bar 2.75 μm).

Timoney, 2005b) and with relatively narrow bases, and blunt luminal free surfaces with small cytoplasmic processes. Adjacent endothelial cells were joined by desmosomes at irregular intervals. The chromatin of the irregular nuclei of endothelial cells was uniformly distributed with eccentric nucleoli. The distribution of smooth and rough endoplasmic reticulum, mitochondria, Golgi, multivesicular and membrane-bound bodies and large numbers of lysosome-like membrane bound bodies, phagosomes, filaments, storage granules, vacuoles and vesiculo-vacuolar organelles in their cytoplasm was almost identical to that of the lingual tonsil (Kumar and Timoney, 2005b). Caveolae as spherical invaginations of the plasma membrane and associated vesicles were either single or in clusters in the cytoplasm and attached to the endothelial cell surface. The vesiculo-vacuolar organelle (VVOs) in the form of grape-like clusters (Fig. 11) were well developed in the cytoplasm of endothelial cells especially toward their lateral borders as reported in the lingual tonsil (Kumar and Timoney, 2005b). In places, larger vesicles and vacuoles were also observed. VVOs communicated with each other and with endothelial plasma membranes by means of stomata controlled by small, thin diaphragms. Endothelial pores distributed in the cytoplasm had a distribution similar to that of VVOs and a tendency to open towards intercellular clefts, luminal and abluminal surfaces. Evidence of transvascular and inter-endothelial migrations of lymphocytes from the HEVs to lymphatic parenchyma were also visible (Fig. 10).

The immunohistological and ultrastructural characteristics suggest that the palatine tonsil of the horse is similar to that of humans. Although generally similar to the lingual tonsil it has unique histochemical and immunological features that may reflect biases in function.

Acknowledgements

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