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Ultramicroscopic Examination of the Ovine Tonsillar Epithelia

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ABSTRACT

As solid morphological knowledge of ovine tonsillar epithelia might contribute to a better understanding of the pathogenesis of several diseases including prion diseases, the epithelia of all tonsils of 7 one-year-old Texel sheep were examined using scanning and transmission electron microscopy. Major parts of the pharyngeal and tubal tonsils were covered by pseudostratified columnar ciliated epithelia that were interrupted by patches of epithelium containing cells with densely packed microfolds or microvilli, and cells with both microvilli and cilia. Smaller parts were covered by either flattened polygonal cells with densely packed microvilli or microfolds, squamous epithelial cells, or patches of reticular epithelium. The palatine and paraepiglottic tonsils were mainly lined by squamous epithelial cells with apical microplicae or short knobs. Additionally, regions of reticular epithelium containing epithelial cells with apical microvilli were seen. The lingual tonsil was uniformly covered by a keratinized squamous epithelium and devoid of microvillous cells and patches of reticular epithelium. The rostral half of the tonsil of the soft palate was lined by a pseudostratified columnar ciliated epithelium with characteristics of the pharyngeal and tubal tonsils. The epithelium of the caudal part resembled the epithelia of the palatine and paraepiglottic tonsils. Putative M cells, mainly characterized by apical microvilli or microfolds and a close association with lymphoid cells, seem manifestly present on the nasopharyngeal tonsils. The reticular epithelium of the palatine and paraepiglottic tonsils also harbor cells with small apical microvilli. The exact nature of these presumptive M cells should, however, be elucidated in functional studies. Anat Rec, 293:879–889, 2010. © 2010 Wiley-Liss, Inc.

Key words: sheep; tonsillar epithelium; lymphoid tissue; electron microscopy; M cell

INTRODUCTION

Tonsils are major components of the pharyngeal mucosa-associated lymphoid tissue representing a first line of defense against ingested and inhaled foreign antigens (Gebert et al., 1995; Caramelli et al., 2003). The "Waldeyer ring" of sheep consists of six tonsils (von Waldeyer-Hartz, 1884). The pharyngeal tonsil (tonsilla pharyngea), the tubal tonsil (tonsilla tubaria) and the tonsil of the soft palate (tonsilla veli palatini) are located

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in the nasopharynx, the lingual tonsil (tonsilla lingualis) and the palatine tonsil (tonsilla palatina) in the oropharynx, and the paraepiglottic tonsil (tonsilla paraepiglottica) in the laryngopharynx (Cocquyt et al., 2005; Casteleyn et al., 2007, 2008).

As tonsils lack afferent lymphatics (Mair et al., 1987; Chen et al., 1989) a direct local interaction of the tonsillar lymphoid tissue with hazardous antigens is a prerequisite for initiating and maintaining immune responses (Kumar and Timoney, 2005a). M cells rank among the most important epithelial cell types that facilitate the adherence, uptake, and sampling of foreign antigens and micro-organisms (Kraehenbuhl and Neutra, 2000; Claeys and De Belder, 2003). They transport soluble macromolecules, particles, and entire micro-organisms via endocytosis at the apical membrane and exocytosis to the basolateral membrane where both T and B lymphocytes are present in a basolateral pocket (Gebert, 1997; Kraehenbuhl and Neutra, 2000; Yamanaka et al., 2001). As such, M cells are ports of entry for invasive pathogens (Sansonetti and Phalipon, 1999; Kraehenbuhl and Neutra, 2000). They might be of particular importance in sheep since prions exploit M cell dependent transcytosis to gain access to the immune system in which they replicate (van Keulen et al., 1996, 2002; Aguzzi et al., 2003; Huang and MacPherson, 2004; Brayden et al., 2005; Davies et al., 2006; Niess and Reinecker, 2006). As a result, they play a pivotal role in the development of scrapie and bovine spongiform encephalopathy which can cause the new variant of Creutzfeldt-Jakob disease (nvCJD) in humans (Bruce et al., 1997; Hill et al., 1997; Zeidler and Ironside, 2000; Narang, 2001).

Despite their importance in ovine infectious diseases, M cells are very poorly documented in this species (Chen et al., 1990, 1991; Stanley et al., 2001). The correct ultramicroscopic identification of M cells is, however, not evident since these cells exhibit considerable site- and species-related variation (Gebert and Pabst, 1999; Clark et al., 2001). In general, they are characterized by sparse irregular microvilli or microfolds on their apical surfaces, and their apical cytoplasm is less electron dense and possesses numerous electron-lucent vesicles. Additionally, a basolateral pocket which often harbors a lymphocyte is present (Neutra et al., 1999; Kraehenbuhl and Neutra, 2000; Clark et al., 2001; Koshi et al., 2001). The correct identification of M cells is further hampered by reversible transition forms between mature M cells and epithelial cells through a wide range of intermediate cell types which bear certain characteristics of mature M cells (Savidge, 1996).

M cells are, however, not the only sampling cells. Dendritic cells, which are confined to the lower epithelial strata and possess long dendritic processes, possibly act synergistically with M cells (Niess and Reinecker, 2006). They open the tight junctions between epithelial cells, send dendrites outside the epithelium and directly sample foreign antigens (Koshi et al., 2001; Milling et al., 2005). The integrity of the epithelial barrier is preserved as dendritic cells express tight-junction proteins (Rescigno et al., 2001). For this reason, the presence of dendritic cells was also examined in our study. However, according to Man et al. (2004), M cells certainly remain the most important antigen-sampling cells to be investigated.

The aim of this study was to describe the ultramicroscopic characteristics of the epithelia of all six ovine tonsils, paying special attention to the potential presence of M cells and dendritic cell processes. To our knowledge, the epithelial linings of the lingual, palatine and paraepiglottic tonsils and of the tonsil of the soft palate have never been investigated on the ultrastructural level. In contrast, a few ultramicroscopic studies have been performed on the ovine pharyngeal and tubal tonsils by Chen et al. (1990, 1991) and Stanley et al. (2001), respectively. The results presented in our study could possibly contribute to a better understanding of the initiation of immune responses in the ovine tonsils.

MATERIALS AND METHODS

Scanning Electron Microscopy

The heads of 5 freshly slaughtered one-year-old Texel sheep were collected at a commercial slaughterhouse and perfused within 1 hr with 1 L saline solution (0.9% NaCl) followed by 1 L of a HEPES (4-(2-hydroxyethyl)piperazin-1-ethanesulonic acid) (Sigma Aldrich, Steinheim, Germany) buffered solution of 2% paraformaldehyde and 2.5% glutaraldehyde through cannulas that were inserted into both common carotid arteries. The latter solution was simultaneously dripped onto the tonsils to initiate fixation prior to removal. After perfusion was completed all tonsils were excised and gently rinsed with saline solution to wash away overlying mucus.

The samples were processed based on the protocol described by De Spiegelaere et al. (2008). In brief, samples of the mucosal surfaces of each tonsil were taken and stored for 24 hr in the HEPES buffered fixative. They were subsequently washed three times with distilled water, post-fixed for 2 hr in 1% OsO_4, washed again and dehydrated in an increasing alcohol series followed by an increasing ethanol-acetone series up to pure acetone. The samples were then dried at critical point (Balzers CPD 030, Sercolab, Merksem, Belgium), mounted on metal bases and coated with platinum (JEOL JFC 1300 Auto Fine Coater, Jeol, Zaventem, Belgium). Finally, they were examined with a scanning electron microscope (JEOL JSM 5600 LV, Jeol).

Transmission Electron Microscopy

Two one-year-old Texel sheep were culled by exsanguination after they were stunned using a captive bolt. Immediately after decapitation their tonsils were fixed and dissected in a similar way as for scanning electron microscopy (SEM).

Again, a protocol used by De Spiegelaere et al. (2008) was applied. Samples of the mucosal surfaces of each tonsil were placed overnight in Karnovsky's fixative (2% formaldehyde and 2.5% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.4) on a rotor at 4°C. Subsequently, the specimens were washed for 8 hours in 0.1 M cacodylate buffer, replacing the solution three times. After washing, the specimens were post-fixed overnight in 1% reduced OsO_4 on a rotor at 4°C. They were then washed three times with 0.1 M cacodylate buffer for 15 min and automatically dehydrated in an increasing alcohol series up to 100% ethanol (Leica EM TP, Leica Microsystems, Groot-Bijgaarden, Belgium). The specimens were stored overnight in alcohol/Spurr's resin (1/3, v/v) at 4°C, then

for 8 hr in alcohol/Spurr's resin (2/3, v/v) at 4°C and finally overnight in 100% Spurr's resin at 4°C. The resin was allowed to polymerize at 70°C for 9 hr. Semi-thin section of 1 μm were first made and stained with toluidine blue to select the most appropriate regions using light microscopy. After trimming (Leica EM TRIM, Leica Microsystems), sections of 60 nm were made at random positions using a Leica EM UC6 ultramicrotome (Leica Microsystems) on pioloform-coated single slot copper grids (Laborimpex, Brussels, Belgium). They were poststained (Leica EM stain, Leica Microsystems) for 20 min in uranyl acetate at 40°C and for 5 min in lead citrate at 20°C. For examination a JEOL 1200 EXII electron microscope (Jeol) was used.

RESULTS

Pharyngeal Tonsil

On SEM, the pharyngeal tonsil was mainly covered by a ciliated epithelium which was randomly interrupted by round or oval patches, 100–400 μm in diameter, of follicle associated epithelium consisting of cells with densely packed short (±0.3 μm) microvilli (Microvillous type 1 cells, Mv1) or medium-sized (±0.8 μm) microvilli (Microvillous type 2 cells, Mv2), intermediate cells with both microvilli and cilia, and some nonkeratinized squamous cells with irregular microplicae (Fig. 1). Small round cells with large widely arranged membranous folds (Membranous cells) were sometimes observed. Some small parts of the tonsil were lined by flattened polygonal cells which had ledge-like cytoplasmic elevations around their borders. Their surfaces contained either densely packed microfolds (Microfold cells), very short microvilli (Mv1 cells) or microvilli of intermediate length (Mv2 cells). Additionally, some patches of reticular epithelium exposing the underlying lymphoid tissue were observed. These epithelia consisted of either loosely arranged squamous epithelial cells with densely packed microvilli or small knobs, or columnar epithelial cells lacking cilia.

Transmission electron microscopy (TEM) revealed that the pharyngeal tonsil was mainly lined by a pseudostratified columnar ciliated epithelium. In many smaller regions the pseudostratified epithelium consisted of microvillous cells instead of ciliated cells. The nuclei of the Mv1 cells were located more apically than those of the Mv2 cells. The latter cells bulged into the pharyngeal lumen due to apical constrictions of Mv1 cells. Mv1 cells had shorter and more densely packed microvilli, a more electron dense cytoplasm and less endoplasmic reticulum and apical mitochondria than the Mv2 cells. Intermediate cells covered with both microvilli and cilia, and containing numerous mitochondria were also observed. Apical mitochondria were also abundantly present in some specific cells with a bulging apical surface which possessed few slender microvilli or irregular membrane ruffles (Membranous cells). Additionally, the latter cell type contained many apical vacuoles and was characterized by its intimate contact with intraepithelial lymphoid cells which were located manifestly closer to the pharyngeal lumen than the subepithelial lymphoid cells.

Tubal Tonsil

The tubal tonsil shared many characteristics with the pharyngeal tonsil (Fig. 2). On its surface that was mainly covered by ciliated cells, randomly distributed patches of epithelium containing many Mv1, Mv2, and intermediate cells, and sparse membranous, microfold and nonkeratinized squamous cells were visible on SEM. A reticular epithelium consisting of loosely arranged epithelial cells with interspersed superficial lymphoid cells was seen in some places. The reticular epithelial cells were either squamous epithelial cells with densely packed microvilli or small knobs, or columnar epithelial cells lacking cilia.

TEM demonstrated that, in regions without exposed lymphoid cells, the pseudostratified columnar ciliated epithelium was often infiltrated by lymphoid cells, causing a reduction of the distance between the lymphoid tissue and the pharyngeal lumen. Moreover, this type of epithelium was often transformed into a single layered epithelium consisting of large, flattened Mv1 cells with interspersed cylindrical Mv2 cells. The cytoplasm of the Mv1 cells was most electron dense. Tonsillar lymphoid cells could also get access to the nasopharyngeal cavity through very small epithelial channels that were lined by electron dense cuboidal Mv1 cells. Such channels were, however, not that frequently seen on TEM and hardly visible on SEM.

Palatine Tonsil

The palatine tonsil contained a quite uniform epithelial cell population (Fig. 3). SEM revealed that the epithelium covering its crypts consisted of nonkeratinized squamous cells with loosely arranged microplicae (Squamous epithelial cell type 1, S1), densely packed microplicae (Squamous epithelial cell type 2, S2) or densely packed short knobs (Squamous epithelial cell type 3, S3). Patches of reticular epithelium containing detached epithelial cells, interspersed lymphoid cells and debris were randomly distributed throughout the crypt surfaces. On TEM it was observed that the thickness of the epithelium varied from one to more than 10 cell layers. In the regions with heavy lymphoid cell infiltration, the epithelial cells were rounded and contained small microvilli on their apical surfaces. These epithelial cells were, however, not all completely detached, but many were still connected with neighboring epithelial cells via tight junctions. Some penetrating cytoplasmic processes from cells located underneath the covering squames were additionally observed using TEM. These were electron dense and contained no obvious organelles.

Paraepiglottic Tonsil

The paraepiglottic tonsil shared many characteristics with the palatine tonsil (Fig. 4). On SEM it was visible as one to four round or oval tonsillar nodules which protruded into the pharyngeal lumen. These were mainly covered by a nonkeratinized squamous epithelium consisting of cells of which the apical surfaces showed similar membrane differentiations as those of the palatine tonsil (S1, S2, and S3 cells). TEM revealed that the nonkeratinized squamous epithelium was mainly stratified consisting of more than 10 layers of squamous cells. Only in the depth of the invaginations between the

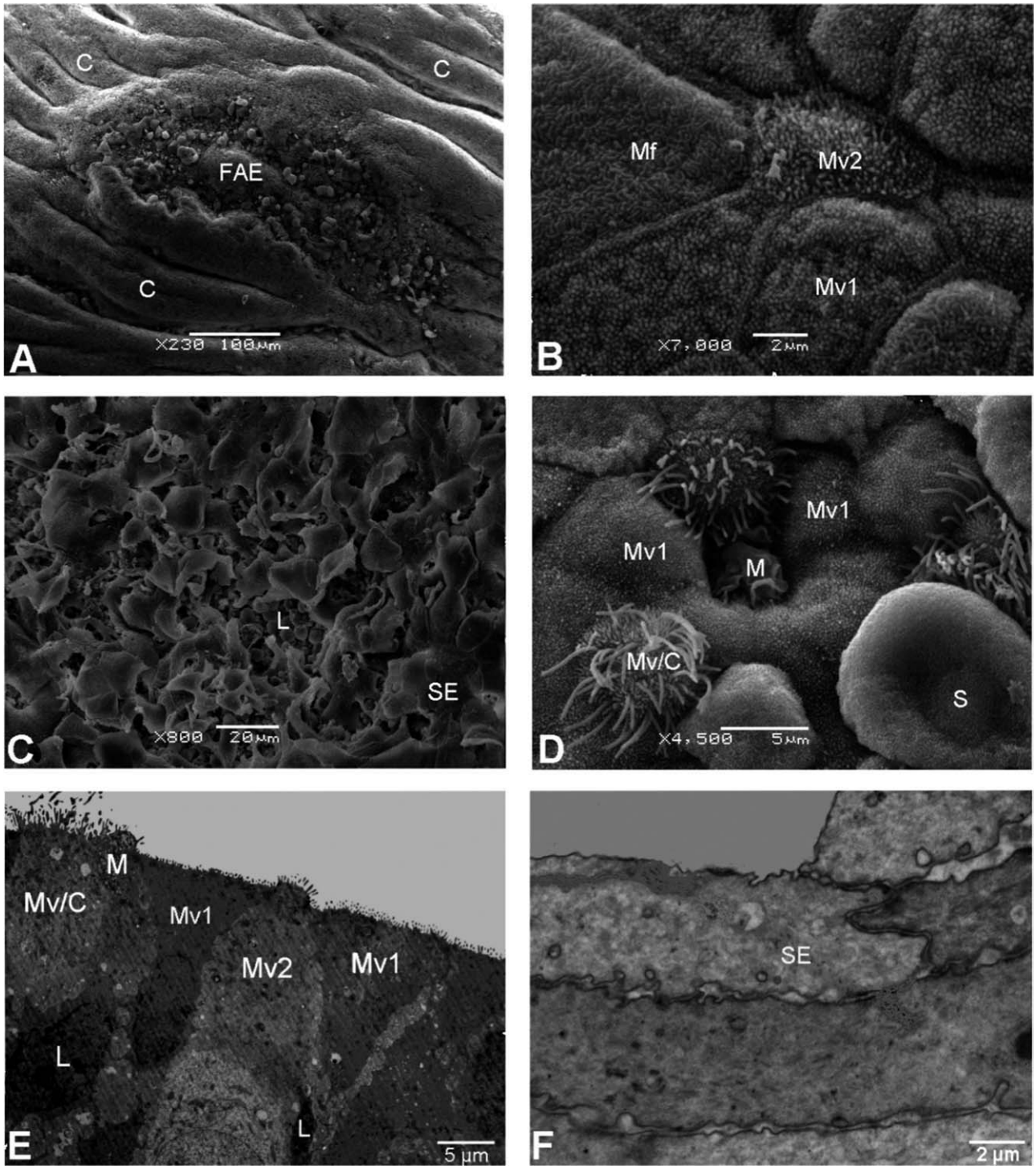

Fig. 1. Ultramicroscopic views of the ovine pharyngeal tonsil. **A**: The pharyngeal tonsil is mainly covered by a ciliated epithelium (C) that is interrupted by patches of follicle associated epithelium (FAE) (SEM view). **B**: Minor parts of the tonsil are lined by a flattened type of epithelium consisting of microfold cells (Mf) and cells with short (Mv1) or medium-sized (Mv2) microvilli (SEM view). **C**: Patches of reticular epithelium with disrupted squamous epithelial cells with densely packed microvilli or short knobs on their apical surfaces (SE), exposing the underlying lymphoid cells (L), are seldom observed (SEM view). **D**: Higher magnification of a patch of FAE showing a small, round membranous cell (M) with membranous ruffles, microvillous cells type 1 (Mv1) with very short microvilli, intermediate cells (Mv/C) with both microvilli and cilia, and a squamous cell (S) with microplicae (SEM view). **E**: TEM view of the FAE showing an intermediate cell (Mv/C) with both microvilli and cilia, a membranous cell (M) with some apical membrane ruffles and many cytoplasmic electron-lucent vesicles, microvillous cells type 1 (Mv1) with very short microvilli, and a microvillous cell type 2 (Mv2) with medium-sized microvilli. Some lymphoid cells (L) are located in close association with the epithelial cells. **F**: TEM view of squamous epithelial cells (SE) covering small parts of the pharyngeal tonsil.

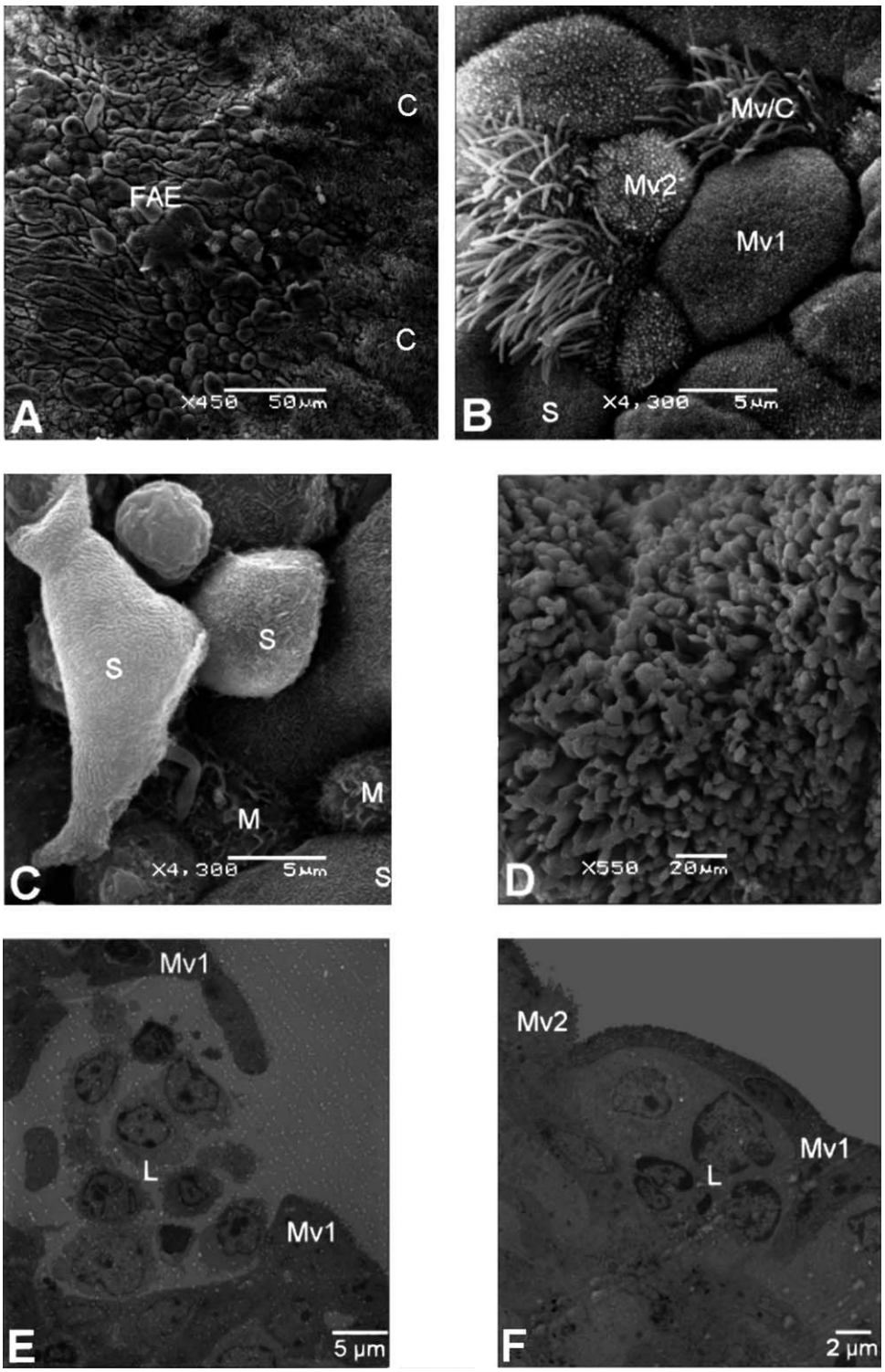

Fig. 2. Ultramicroscopic views of the ovine tubal tonsil. **A**: The epithelial lining of the tubal tonsil consists of a ciliated epithelium (C) that is interrupted by regions of follicle associated epithelium (FAE; SEM view). **B**, **C**: Higher magnifications of the FAE showing microvillous cells type 1 (Mv1) and type 2 (Mv2) with short and medium-sized microvilli, respectively, intermediate cells (Mv/C) with both microvilli and cilia, membranous cells (M) with membranous ruffles, and squamous cells (S) with microplicae (SEM views). **D**: Patches of reticular epithelium with disrupted columnar epithelial cells which lack cilia, exposing the underlying lymphoid cells, are seldom observed (SEM view). **E**: TEM view of an epithelial channel lined by microvillous cells type 1 (Mv1) through which the underlying lymphoid cells (L) get access to the nasopharyngeal cavity. **F**: TEM view of the FAE showing lymphoid cells (L) that are present within the epithelium that consists of large, flattened microvillous type 1 cells (Mv1) and smaller round microvillous type 2 cells (Mv2).

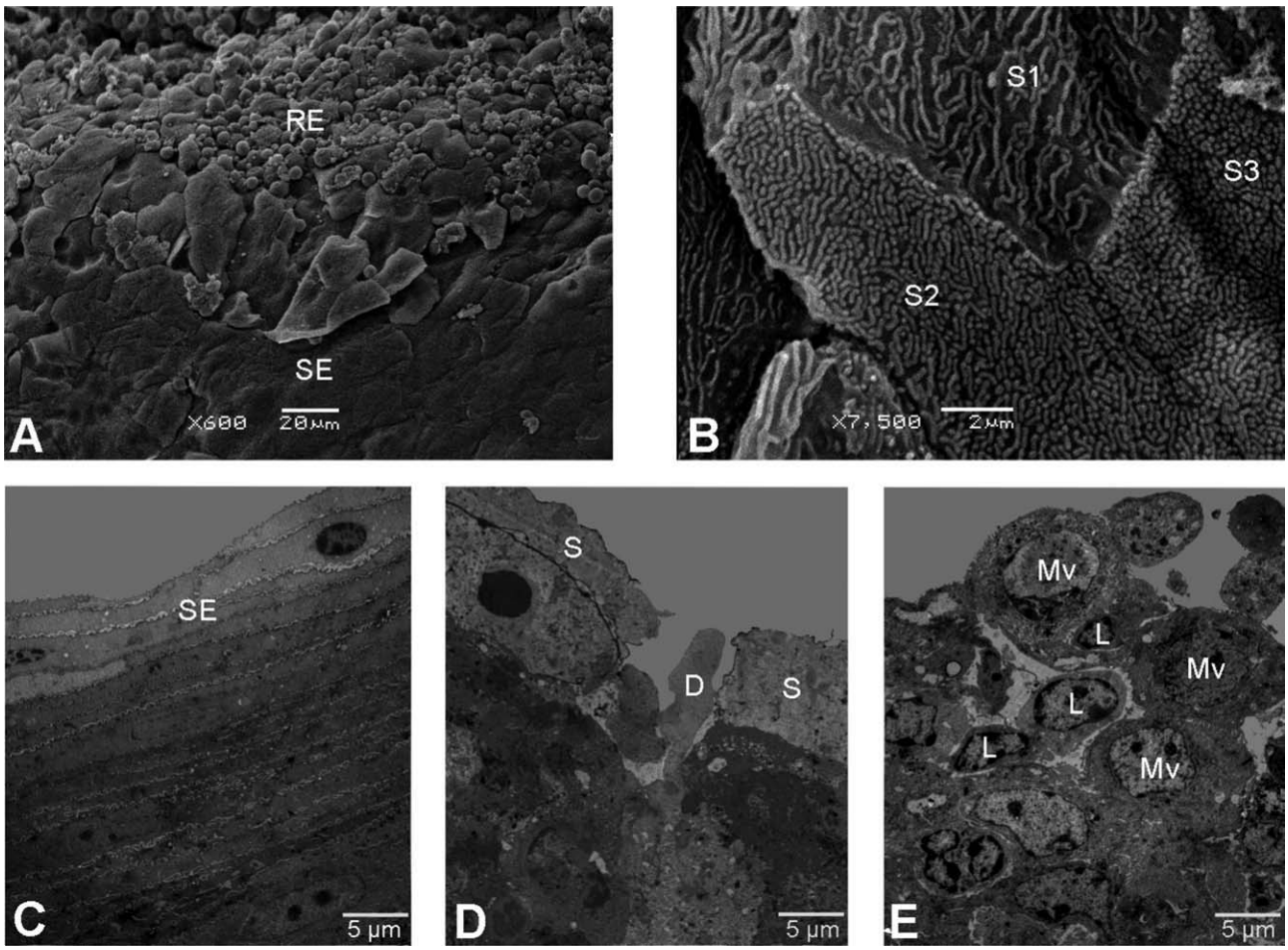

Fig. 3. Ultramicroscopic views of the ovine palatine tonsil. **A**: The crypts of the palatine tonsil are mainly covered by a squamous epithelium (SE) which is interrupted by patches of reticular epithelium (RE) containing detached round epithelial cells, lymphoid cells, and debris (SEM view). **B**: Higher magnification of the squamous epithelium showing nonkeratinized squamous cells with loosely arranged (S1) or densely packed (S2) microplicae, or short knobs (S3). **C**: TEM view of the squamous nonkeratinized epithelium (SE) consisting of many layers of epithelial cells. **D**: TEM view of a subepithelial dendritic cell process (D) which penetrates the squamous epithelium (S) to gain access to the crypt lumen. **E**: TEM view of the reticular epithelium showing detached round epithelial cells with short apical microvilli (Mv), and many lymphoid cells (L).

nodules, a reticular epithelium with detached epithelial cells and interspersed lymphoid cells could be seen.

Lingual Tonsil

The lingual tonsil was confined to a few lymphoid cells that were located in the connective tissue cores of the vallate papillae which were surrounded by deep narrow grooves (Fig. 5). SEM showed that the papillae were covered by a keratinized epithelium composed of squamous cells with loosely arranged microplicae (S1 cells). No patches of reticular epithelium were observed. TEM additionally demonstrated that the epithelium was built up of ~5–15 layers. The upper layers were well keratinized and contained small apical protrusions.

Tonsil of the Soft Palate

The epithelial cell populations lining the tonsil of the soft palate were very heterogeneous (Fig. 6). The epithelial lining of the rostral half of the tonsil was quite similar to that of the pharyngeal and tubal tonsils. SEM showed that the epithelium was composed of flattened polygonal cells with short (Mv1 cells), medium-sized (Mv2 cells), or long (±1 µm) microvilli (Mv3 cells), and cells with both microvilli and cilia. Mv3, membranous and microfold cells were low in number. The characteristics of these cells on TEM were similar to those of the cells present on the pharyngeal and tubal tonsils. TEM further revealed that it was obvious that the latter cells had close contact with the numerous lymphoid cells which infiltrated the epithelium. Some protruding goblet and squamous cells with small densely packed microplicae were also present in this region. Additionally, the epithelium was sometimes disrupted allowing lymphoid cells to be exposed to luminal antigens.

The caudal part of the tonsil resembled the palatine tonsil, since it consisted of many layers of squamous cells with loosely arranged or densely packed microplicae or short knobs (S1, S2, and S3 cells). A summary of the various epithelial cell types present on each ovine tonsil is presented in Table 1.

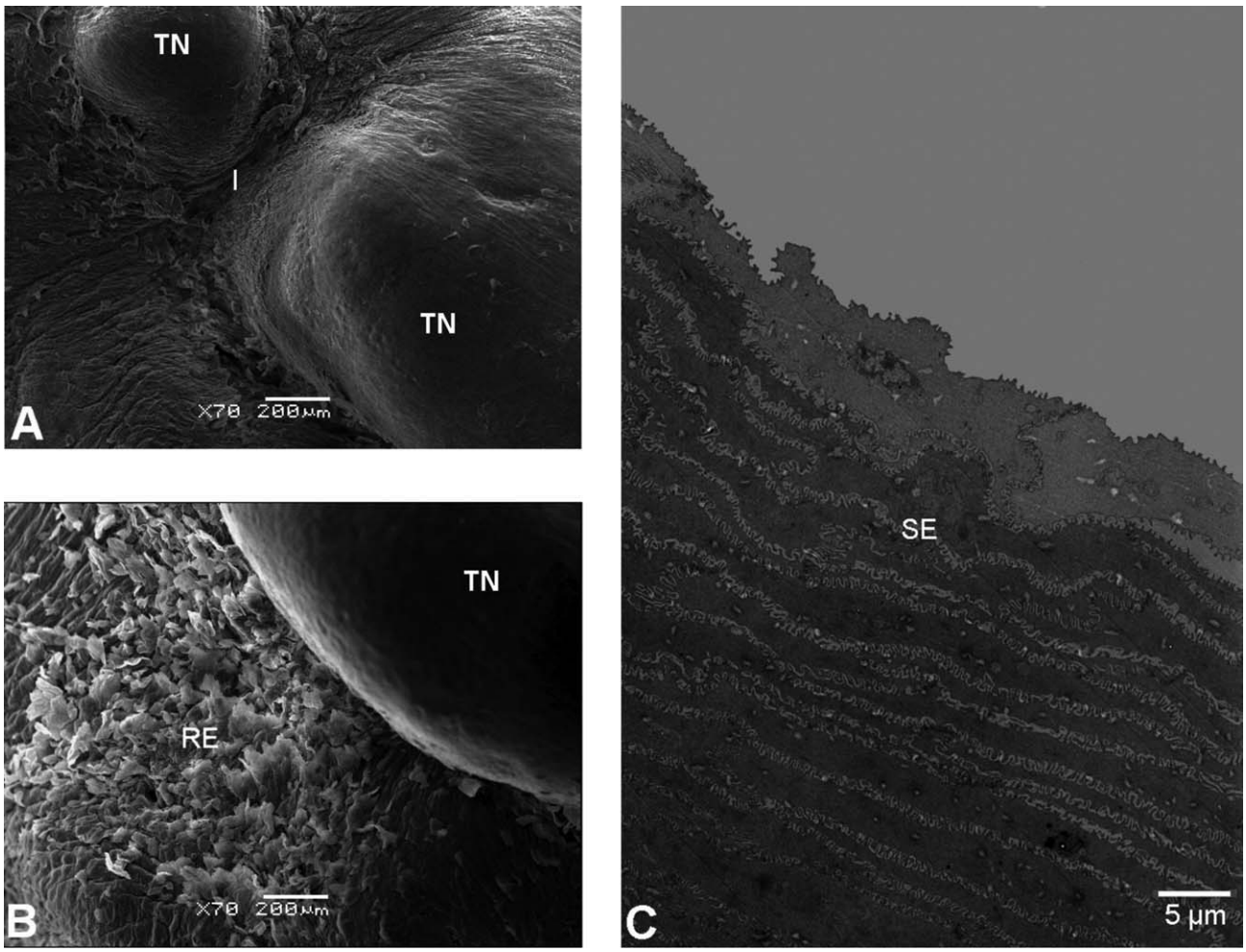

Fig. 4. Ultramicroscopic views of the ovine paraepiglottic tonsil. **A**: The paraepiglottic tonsil consists of a few tonsillar nodules (TN) that are separated by invaginations (I) (SEM view). **B**: Patches of reticular epithelium (RE) consisting of loosely arranged squamous epithelial cells are often present in the invaginations near the tonsillar nodules (TN) that are lined by a nonkeratinized squamous epithelium. **C**: TEM view of the squamous nonkeratinized epithelium (SE), consisting of several layers of epithelial cells, overlying the tonsillar nodules.

DISCUSSION

This electron microscopic study demonstrates that a large variety of cell types is present on the surfaces of the pharyngeal and tubal tonsils and the rostral part of the tonsil of the soft palate. In contrast to these nasopharyngeal tonsils, the palatine, paraepiglottic, and lingual tonsils are mainly covered by squamous cells with apical microplicae or small knobs. As M cells and dendritic cells exert a sampling function in the epithelia overlying lymphoid tissues (Debard et al., 1999), the present study aimed to identify these cell types on the ovine tonsils using electron microscopy.

The ultrastructure of the epithelial lining of the ovine pharyngeal tonsil has already been investigated by Chen et al. (1990, 1991). The results obtained by these authors are largely similar to those generated in the present study. Our classification of the various types of microvillous cells (Microvillous cell type 1 and 2) that are present on the pharyngeal tonsil was, however, based on the work of Kumar and Timoney (2001). According to these authors, who thoroughly investigated the equine tonsils, both cell types can be considered as different maturation stages of M cells. The membranous cells with reduced numbers of very small microvilli appear to be fully differentiated M cells (Belz and Heath, 1995; Kumar et al., 2001). These cells were, however, scarce. Intermediate cells with both reduced numbers of smaller cilia and interspersed microvilli could be precursors of M cells, maturing by their transformation from ciliated cells to microvillous cells (Spit et al., 1989). Our TEM observations support these views since the observed intermediate cells were often surrounded by intraepithelial lymphoid cells, and their apical cytoplasm contained many electron-lucent vesicles and mitochondria. Moreover, all microvillous cells of the nasopharyngeal follicle associated epithelia of the rat and sheep have been classified as M cells by Spit et al. (1989) and Chen et al. (1991), respectively. The presence of patches of reticular epithelium indicate, however, that M cells are not solely necessary for immune induction. Our results further demonstrate that the epithelial lining of the ovine

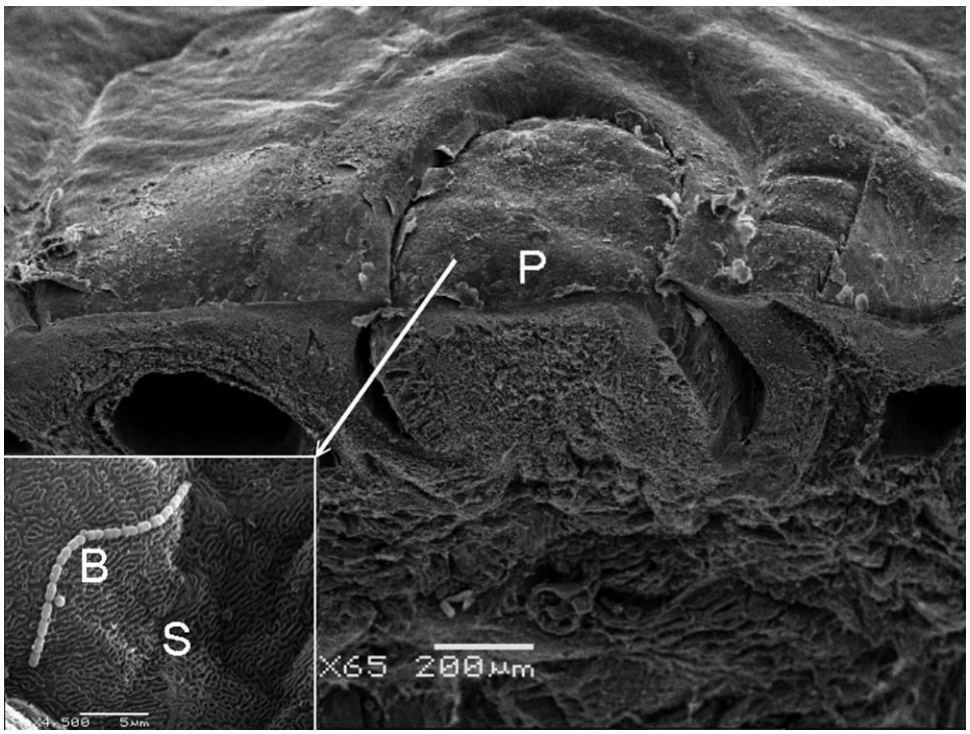

Fig. 5. SEM view of the ovine lingual tonsil which consists of lymphoid cells within the connective tissue cores of the vallate papillae. Each vallate papilla (P) is surrounded by deep grooves. Insert: Higher magnification (×4500) of the epithelial lining of the lingual tonsil consisting of keratinized squamous epithelial cells with microplicae (S). Notice the bacteria (B) on the tongue surface.

pharyngeal tonsil resembles that of the horse, cattle, and goat (Schuh and Oliphant, 1992; Kahwa and Balemba, 1998; Kumar et al., 2001; Kumar and Timoney, 2001; Kumar et al., 2006a).

The ovine tubal tonsil is very similar to the pharyngeal tonsil which could be explained by their closely adjacent position in the nasopharynx. The ultrastructure of its epithelium has already been investigated previously by Stanley et al. (2001) rendering results that are largely similar to ours. Epithelial channels that were also observed by Chen et al. (1991) in the pharyngeal tonsil of sheep seem to form another route for luminal antigens to gain access to the tonsillar lymphoid tissue. Kumar and Timoney (2005b) also found Mv1 and Mv2 cells which were both designated as M cells.

This study demonstrated that the crypts of the ovine palatine tonsil were lined by nonkeratinized squamous cells with loosely arranged or densely packed microplicae, or short knobs. Patches of reticular epithelium with complete loss of the surface cells, leaving the underlying nonepithelial cells exposed, were also seen. As these detached epithelial cells are round and have apical microvilli they could be M cells (Kumar and Timoney, 2005c). The ovine palatine tonsils seem to resemble the human, equine, bovine and caprine palatine tonsils (Perry, 1994; Kumar and Timoney, 2005c; Kumar et al., 2006b; Palmer et al., 2009). In contrast, M cells with long microvilli (0.5–4.8 μm) projecting from irregular and widely spaced membranous folds have been observed in the canine palatine tonsils (Belz and Heath, 1996), while fungiform cells with well developed microvilli of different lengths are described in the rabbit palatine tonsil (Oláh and Everett, 1975). Further functional studies are needed to elucidate whether any of the presumed squamous cells with apical microplicae in the present study exhibit M cell-like functions (Andrews, 1976; Cleaton-Jones, 1976; Gebert and Pabst, 1999). However, a small number of M cells restricted to the uppermost layers of a squamous epithelium would not greatly contribute to the immunological function (Koshi et al., 2001). In contrast, together with the dendritic cells, the reticular epithelium containing microvillous cells and exposed lymphoid cells, could be the major routes through which luminal antigens get in contact with the lymphoid tissue.

The ultrastructure of the epithelial lining of the paraepiglottic tonsil had not yet been demonstrated in any species. This study reveals that its epithelium largely resembles that of the palatine tonsil, which is likely since both tonsils are located close to each other. The reticular epithelium which is often present in the invaginations between tonsillar nodules is probably most important for the exposure of the lymphoid tissue to luminal antigens.

The small lingual tonsil is the only ovine tonsil that is entirely lined by a stratified keratinized epithelium composed of squamous cells with densely packed microplicae. Just like in horses, this tonsil did not present cells with characteristics of M cells (Kumar and Timoney, 2005d). In horses, however, the epithelium of the lingual tonsil was reticulated at the level of the crypts (Kumar and Timoney, 2005d). Because of its small size

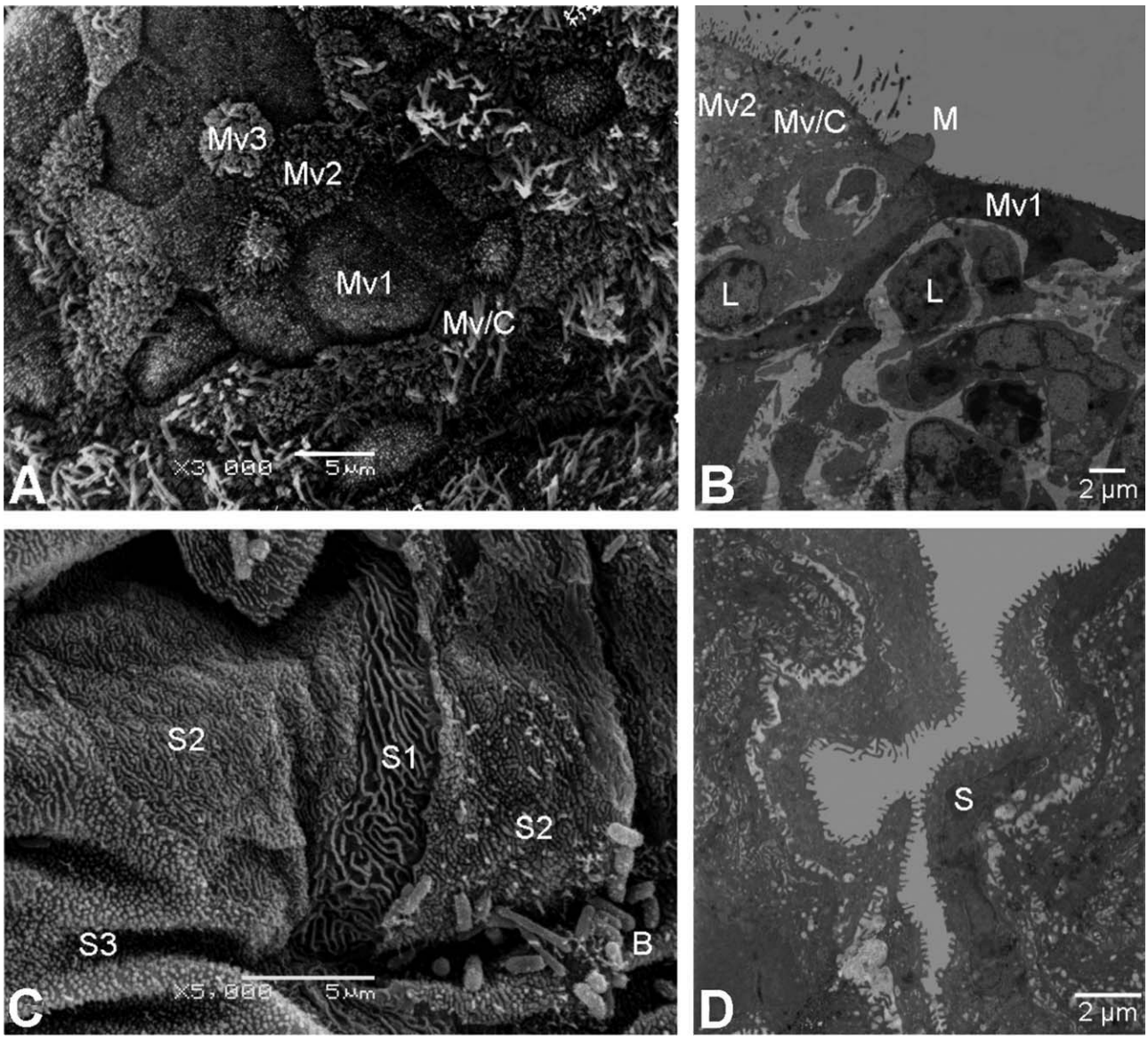

Fig. 6. Ultramicroscopic views of the ovine tonsil of the soft palate. **A**: The epithelium of the rostral part of the tonsil contains many intermediate cells with both microvilli and cilia (Mv/C), and cells with short (Mv1), medium-sized (Mv2) or long (Mv3) microvilli (SEM view). **B**: TEM view of the epithelium of the rostral part of the tonsil showing intermediate cells (Mv/C), microvillous type 1 and 2 cells, a small and round membranous cell with apical membrane ruffles (M), and infiltrating lymphoid cells (L). **C**: The epithelium of the caudal part of the tonsil consists of squamous cells with loosely arranged (S1) or densely packed (S2) microplicae, or densely packed small knobs (S3). Notice the bacteria (B) on the surface. **D**: TEM view of the nonkeratinized squamous epithelium of the caudal part of the tonsil.

TABLE 1. Summary of the various epithelial cell types present on each ovine tonsil

Tonsil	C	Mv1	Mv2	Mv3	Mv/C	Mf	M	S	D	Reticular epithelium
T. pharyngea	+++	++	++	−	+	±	±	±	−	+
T. tubaria	+++	++	++	−	+	±	±	±	−	+
T. palatina	−	−	−	−	−	−	−	+++	±	++ with Mv
T. paraepiglottica	−	−	−	−	−	−	−	+++	−	++ with Mv
T. lingualis	−	−	−	−	−	−	−	+++	−	−
T. veli palatini	+	+	+	+	+	±	±	+	−	+

The presence of patches of reticular epithelium, possibly containing rounded epithelial cells with microvilli (Mv), is additionally indicated where appropriate.
C = ciliated cells, Mv1 = microvillous cells type 1, Mv2 = microvillous cells type 2, Mv3 = microvillous cells type 3, Mv/C = intermediate cells, Mf = microfold cells, M = membranous cells, S = squamous cells, D = dendritic cells.

(Casteleyn et al., 2007) and tight epithelium it is not likely that the ovine lingual tonsil is an important inductive site for mucosal immunity (Hathaway and Kraehenbuhl, 2000).

The epithelium covering the rostral part of the ovine tonsil of the soft palate resembles that of the other nasopharyngeal tonsils, while the epithelium overlying the caudal half is similar to the epithelium of the palatine and paraepiglottic tonsils. A third microvillous cell type with long microvilli was present in the rostral part of the ovine tonsil of the soft palate and was not observed in any other tonsil. According to Bye et al. (1984) and Kumar et al. (2001) such cells with long microvilli could be precursors of mature M cells. The nasopharyngeal location of this tonsil in sheep, which is unique amongst domestic mammals, hampers the comparison with other species, such as the horse and pig in which the tonsillar tissue is located at the ventral side of the soft palate (Belz and Heath, 1996; Cocquyt et al., 2005; Kumar and Timoney, 2006; Casteleyn et al., 2007).

This study shows that the epithelia of the ovine nasopharyngeal tonsils contain cells that resemble M cells ultramicroscopically. The palatine and paraepiglottic tonsillar epithelia contain not only typical squamous cells with apical microplicae, but they also enclose cells with densely packed small knobs and short microvilli. Further studies are, however, needed to further characterize these putative M cells. Lectin histochemistry and immunohistochemistry are often applied to specifically identify M cells, not only at the light microscopical level, but also at the ultrastructural level using immunogold labeling (Verbrugghe et al., 2006, 2008). Specific markers for ovine M cells do, however, not yet exist. In functional studies, tonsils could be instilled with fluorescent latex microspheres of which the adherence to and distribution on the epithelium can be investigated using confocal laser scanning microscopy and SEM, respectively (Jepson et al., 1993, 1996). Additionally, internalized microspheres can be visualized on frozen sections (Jepson et al., 1996). TEM applied on tonsils that have been exposed to colloidal gold could identify the true antigen sampling cells and then allow their morphological description (Frey et al., 1996). Understanding the exact nature of ovine M cells could not only lead to control strategies for diseases such as scrapie, based on the prevention of pathogen invasion, but might also be most interesting in the development of vaccine and drug vehicles that selectively bind to M cell surfaces (Jepson and Clark, 1998; Kraehenbuhl and Neutra, 2000).

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