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Scanning electron microscopy of swine lymphoid organs

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Summary. The aim of this investigation was to study by scanning electron microscopy the structure of several swine lymphoid organs (lymph nodes, Peyer's patches, and tonsil). Two groups of animals were used: six-month-old pigs and six- to nine-day-old piglets. Samples were jet-washed to eliminate most free cells in order to observe the reticular framework of these organs more clearly. Peyer's patches in piglets showed two types of villi. In one of them the cellular types were absorptive cells and goblet cells. The second type of villi were shorter and wider, with M cells characterized by presenting long, thick microvilli over their surfaces. Peyer's patches of pigs did not show this second type of villi but were usually covered by absorptive villi. The soft palate tonsil was similar in both groups of animals with its surface epithelial cells full of microfolds, partially and frequently obscured by microorganisms. The appearance of the surface epithelium in the same crypt was different depending on the area. There was a large number of holes through which cells apparently passed towards the crypt lumen. The medulla in the lymph nodes was at the periphery and showed a dense reticular framework. Cortex-like lymphoid tissue was formed by lymphoid follides and diffuse lymphoid tissue with high endothelid venules and lymphatic sinuses. The serosal surface of lymphoid organs was formed either by a typical mesothelial cell layer (small intestine) or by loosely arranged connective fibers (lymph nodes).

Key words: Scanning microscopy, Swine, Lymphoid organs

Introduction

Scanning electron microscopy (SEM) allows the tridimensional study of organs, cavities, tissues and cells. As in other systems and types of cells, SEM shows the surface of lymphatic cells in situ. Furthermore, SEM techniques give us a better knowledge of the mucosal system physiology because it not only reveals the characteristics of the cut surface but also those of the luminal surface exposed to the environment. Related to this point, SEM allows the study of antigen uptake in tonsils and Peyer's patches (Owen and Bhalla, 1983). There are several ways of processing samples for SEM, depending upon the experimental objectives. The usual method is to fix the specimen by immersion or perfusion in glutaraldehyde (Owen and Bhalla, 1983). Afterwards samples are critical-point dried and metallized. To study blood or lymphatic circulation it is advisable to use the corrosion method (Ohta et al., 1977; Schmidt et al., 1983; Seki and Abe, 1985) or the freeze-fracture method (Tokunaga et al., 1974; Suzuki et al., 1977; Satodate et al., 1986). Fixation by perfusion facilitates good observation of the reticular framework, the jet-wash procedure previous to the fixation being an alternative method (Miyoshi and Fujita, 1971).

There are few works about SEM of swine lymphoid organs. The ones there are include studies of Peyer's patches (Torres-Medina, 1981; Chu and Liu, 1984), and lymph nodes (Merighi et al., 1986). Our study has been focused on the reticular framework of lymphoid organs and the relationships between their cells. Furthermore, the differences and similitudes found between the two groups of animals used in our study are described and discussed.

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Materials and methods

Two groups of healthy animals were used. One of them was formed by ten six-month-old pigs (five males and five females) and the other by ten six- to nine-day-old piglets, from the same litter. The pig group was sacrificed in a slaughter house, having been previously desensitized with an electric shock. Piglets were anaesthetized with natrium barbital (Penthotal, Abbott,

Madrid) and also sacrificed and bled. Specimens from the following lymphatic organs were collected: jejunal lymph nodes *(Lymphonodi jejunales)*, ventral superficial cervical lymph nodes *(Lymphonodi cervicales superficiales ventrales)*, ileal Peyer's patches and soft palate tonsil *(Tonsilla veli palatini)*.

Samples were obtained immediately after the death of the animals and placed in cacodylate buffer, 0.1 M, pH 7.4, at 4-8° C. The intestines were opened along the antimesenteric border and their mucosa was jet-washed with buffer. The cut surface of the other organs were also jet-washed to eliminate most of the free cells and to show the reticular framework. Samples were quickly fixed in 5% glutaraldehyde in cacodylate buffer, 0.1 M, pH 7.4. at 6° C, for 16 hours. They were then washed in this buffer and dehydrated through increasing concentrations of ethylic alcohol. Finally, they were placed in isoamyl acetate. The dehydrated samples were dried in a critical-point drying apparatus (Polaron, model E3000, Hatfield, GB) using CO_2, and mounted on aluminum stubs with a double adhesive metal tape. The specimens were coated with gold particles in a vacuum evaporator (Polaron, model E5000, Hatfield, GB) and observed in a scanning electron microscope (International Scientific Instruments, model Super III A) using an accelerating voltage of 30 kV. SEM photographs were obtained with 36 mm Ilford FP24 film (Ilford, France). Positive copies were made with Ilford Multigrade II paper (Ilford, France).

Results

Lymph nodes

SEM images of both jejunal and cervical lymph nodes were very similar, so the following description applies for both of them.

The cut surface of pig lymph nodes displayed a capsule of 85-100 μm width, formed by a dense connective tissue with bundles of collagen fibers. The medulla was just underneath the capsule and was made up of a dense framework of reticular cells and fibers arranged parallel to the capsule (Fig. 1). Between reticular structures there were round lymphoid-like cells with either a smooth surface or some microfolds over their surface. The thickness of the medulla was variable depending on the area (50-300 μm), being maximum at the level of the efferent hilus. The cortex was usually located in the middle of the organ and was arranged in two forms: diffuse and follicular. Diffuse cortex showed a looser network of reticular cells and fibers than that of the medulla (Fig. 2). Fibers and cell prolongations were thinner and spaces between them were bigger. Scattered in this area there were 15-40 μm-diameter vessels with a prominent endothelium characteristic of postcapillary venules (PCV). Sometimes around PCV a sinus-like area partially filled by lymphoid-like cells and reticular fibers or reticular cell prolongations that completely surrounded the vessel wall could be distinguished (Fig. 3). Between lymphoid tissues there were connective tissue trabeculae limited by peritrabecular sinuses (PTCS) (Fig. 2). These sinuses had a lumen with reticular cell prolongations arranged perpendicularly to their longest axis, with variable thickness (30-70 μm). Follicular lymphoid tissue consisted of 200-500 μm diameter oval follicles. If the specimens had been jet-washed, they had an empty aspect displaying only their wall formed by reticular fibers and star-like cells. The surface that covered the lymph nodes was formed by bundles of connective tissue fibers disorderly arranged in every direction. A mesothelial cell sheet could not be seen.

Two different areas could be seen in the cut surface of piglet lymph nodes. The outer one had an appearance similar to the medulla of the pig group, but it presented numerous cavities or spaces partially filled by lymphoid-like cells, reticular cell prolongations and reticular fibers. They had thus, a sinus-like appearance. In an inner position there was an area with a larger number of free cells, considered to be the cortex-like area. This latter zone basically had two types of cells: lymphoid-like cells with smooth surfaces or with some roughness, and star-like (reticular) cells laid out between the lymphoid-like cells, approximately 7-9 x 5.5 μm in size (Fig. 4). Reticular cell prolongations were not arranged parallel to the capsule as in the medulla. Lymphoid follicles could be observed only in a few cases. Their appearance and that of the PTCS was similar to pig group counterparts. Some animals showed an afferent hilus in the middle of the organ, with blood and lymphatic vessels, surrounded by cortex-like tissue. Lymphatic vessels were characterized by presenting valves in their lumen covered by a prominent endothelium.

Peyer's patches

The mucosal surface area of Peyer's patches of the pig group presented flattened, long villi, fused to one another, giving a lattice aspect (Fig. 5). It had numerous 5-10 μm holes, some filled with mucus, probably corresponding to the openings of goblet cells. Between villi there was abundant mucus. The cut surface had many lymphoid follicles limited on their basal and lateral aspects by connective tissue cells and fibers. The follicular surface was homogeneous and made up of lymphoid-like cells (5 μm), with many microprojections, and another type of cell, with an oval or irregular form much less numerous, scattered between the first type, bigger (10 x 15 μm), and with a rough surface (reticular cells, probably). The germinal center and mantle areas could not be distinguished. The interfollicular lymphoid tissue had a similar appearance to the follicular area. The serosal surface in both groups of animals had cords of cells of 45-65 μm thickness, limited by deep furrows. The appearance of this surface was similar to that found in the spleen.

The mucosal surface of Peyer's patches in the piglet group showed two types of villi (Fig. 6). One of them was long, flattened or cylindrical, with a pointed upper end. Their surface had 5-10 μm holes. The major cell type

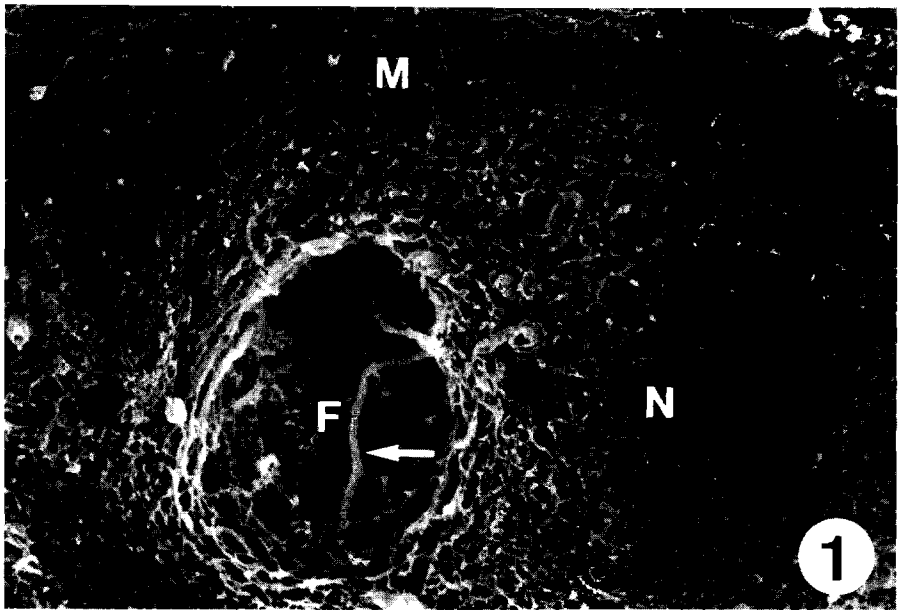

Fig. 1. Lymph node. Pig. Cut surface. Medulla (M). Non-follicular cortex (N). Lymphoid follicle (F). Capillary vessel (arrow). × 164

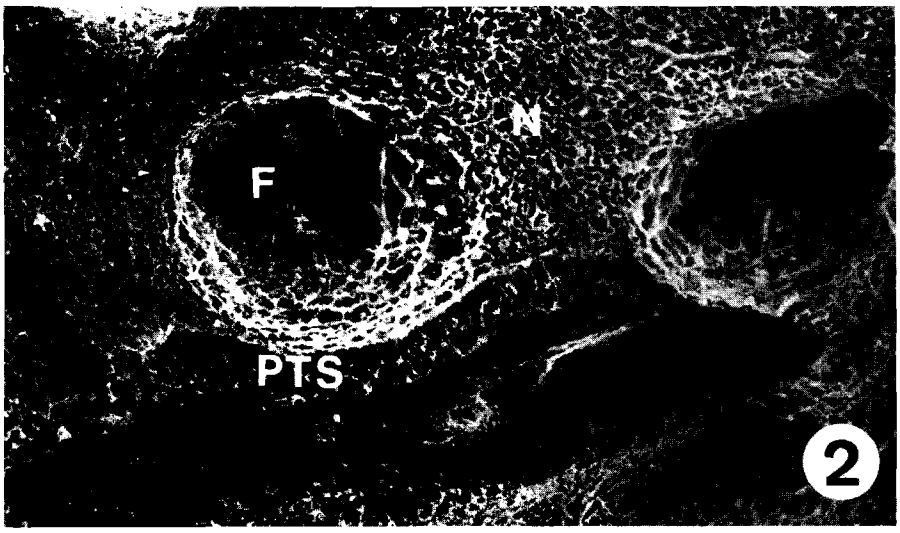

Fig. 2. Lymph node. Pig. Cut surface. Non-follicular cortex (N). Lymphoid follicle (F). Peritrabecular sinus (PTS). × 120

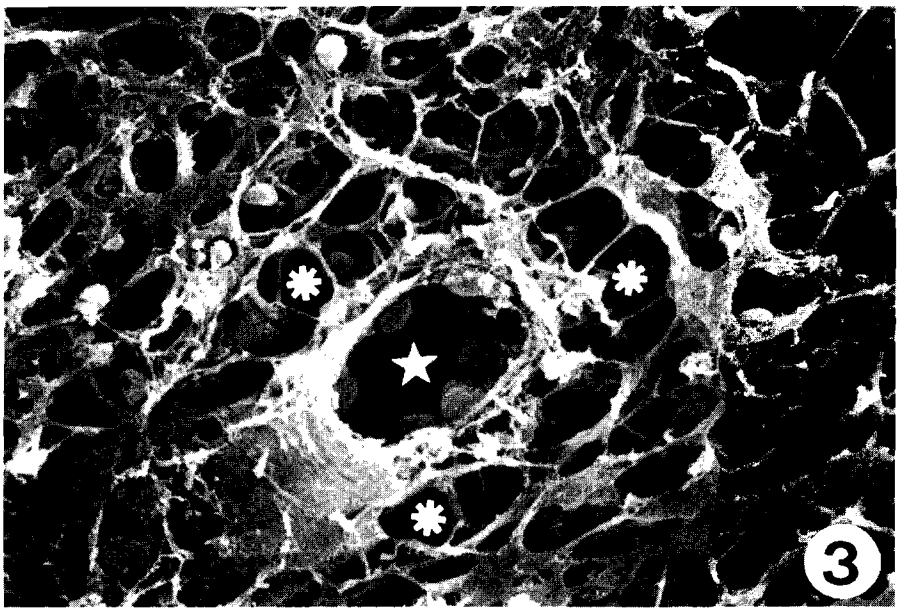

Fig. 3. Lymph node. Pig. Postcapillary venule (star) and sinus-like space (asterisk) surrounding it. × 1,100

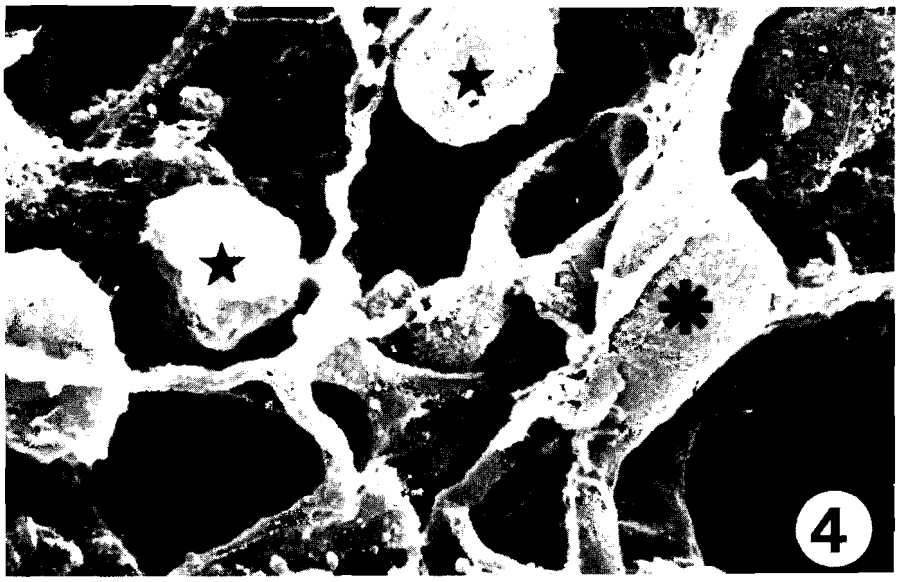

Fig. 4. Lymph node. Piglet. Reticular cells (asterisk) and lymphocyte (star). × 5,200

Fig. 5. Small intestine. Peyer's patch. Pig. Mucosal surface. Villi (V) bridge each other. Mucus (star) is between villi. × 69

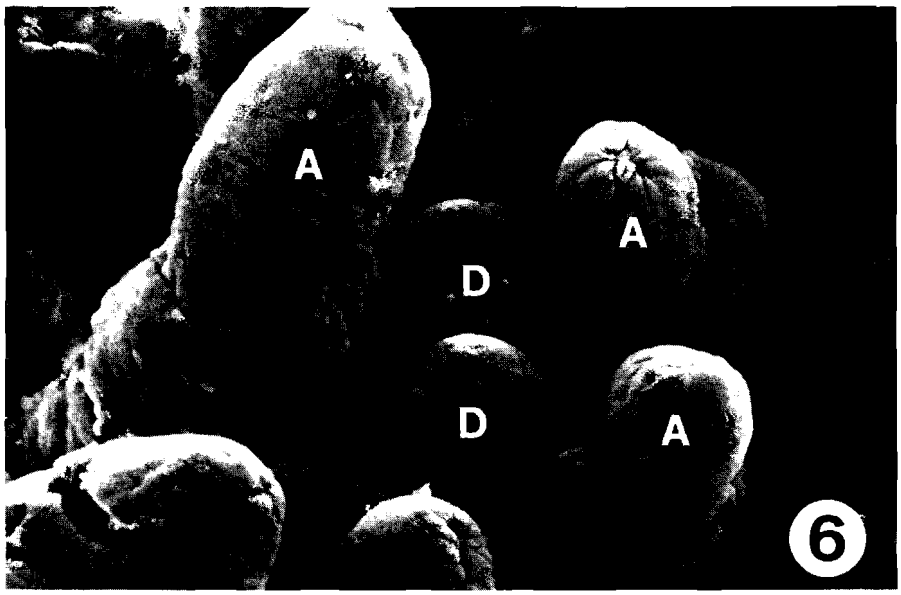

Fig. 6. Small intestine. Peyer's patch. Piglet. Mucosal surface. Absorptive villi (A) and villi with dome (D). × 1,200

Fig. 7. Small intestine. Peyer's patch. Piglet. Mucosal surface. M cells (arrow) scatterd between absorptive epithelial cells (star). × 1,780

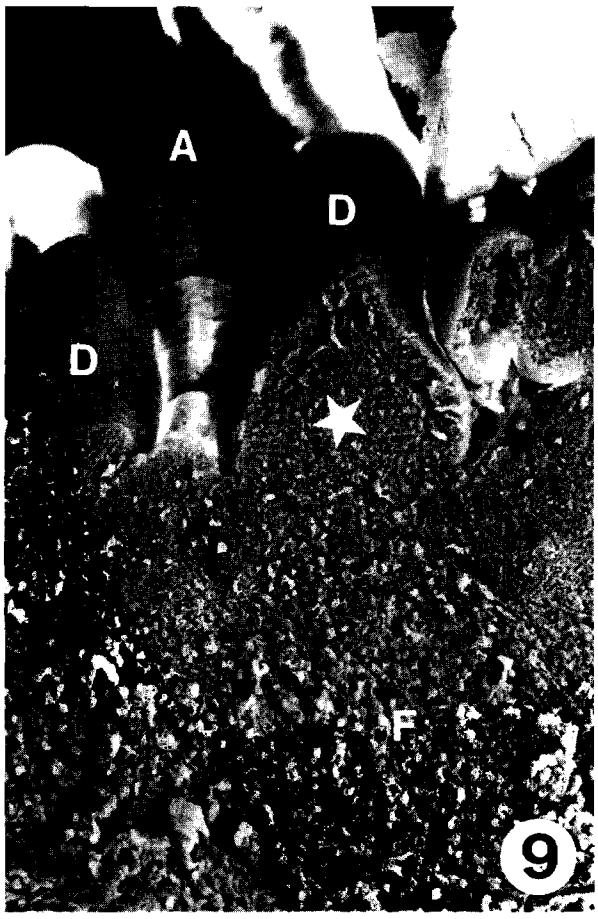

Fig. 9. Small intestine. Peyer's patch. Piglet. Cut surface. Absorptive villi (A) and villi with dome (D). Dome area (star) and lymphoid follicle (F) × 240

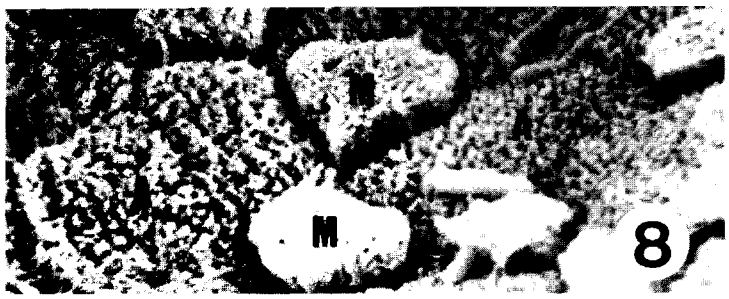

Fig. 8. Small intestine. Peyer's patch. Piglet. A closer view of Fig. 7. M cells (M) and absorptive cells (A). × 6,600

in this villus, had a polygonal shape and net borders close to those of adjacent cells. Its surface was covered by tightly packed microvilli. Scattered on this surface were the openings of goblet cells. The second type of villi was shorter and thicker with a round upper end that apparently did not have goblet cells. There were fewer villi of this type than the first one and it presented two types of cells (Fig. 7). The most predominantly was similar to the cell described for the first type of villi. The second type of cells were scattered between those. They

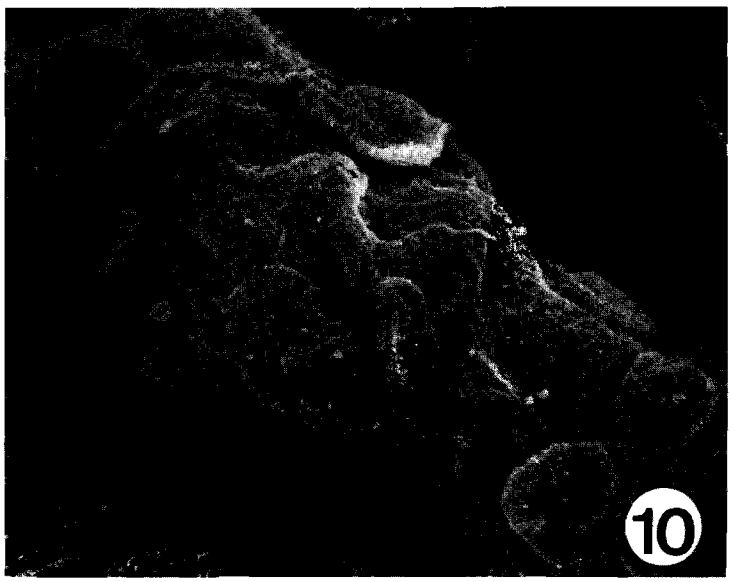

Fig. 10. Small intestine. Pig. Serosal surface. Mesothelial cells (star) partially covering the surface. Underlying this, a loose connective tissue layer (asterisk). × 1,200

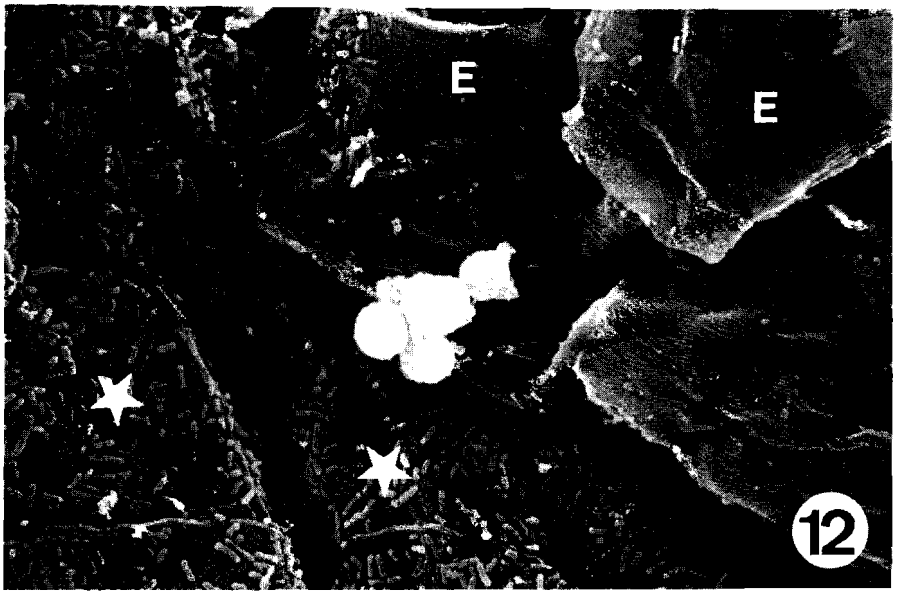

Fig. 11. Tonsil. Pig. Epithelial surface. Cords of epithelial cells limited by deep furrows. × 220

Fig. 12. Tonsil. Piglet. Epithelial surface (E) covered by bacteria (star) × 2,060

Fig. 13. Tonsil. Pig. Cut surface. Crypt opening (star) and squamous stratified epithelium (asterisk). × 152

Fig. 14. Tonsil. Pig. Epithelial cells of a crypt with many microfolds. Upper third of a crypt. × 5,300

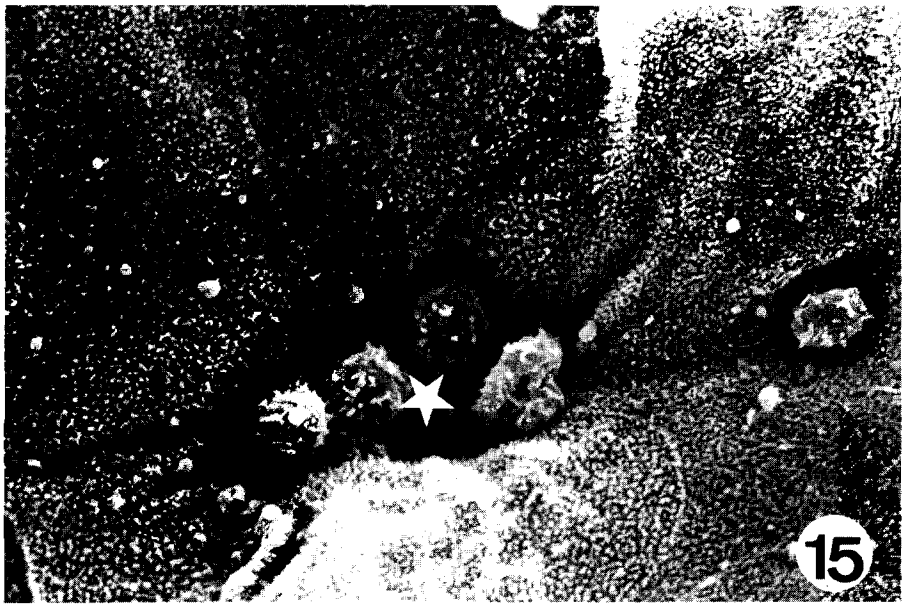

Fig. 15. Tonsil. Pig. Epithelial cells of the lower third of a crypt with microprojections. Leukocytes (star) between several epithelial cells. × 3,300

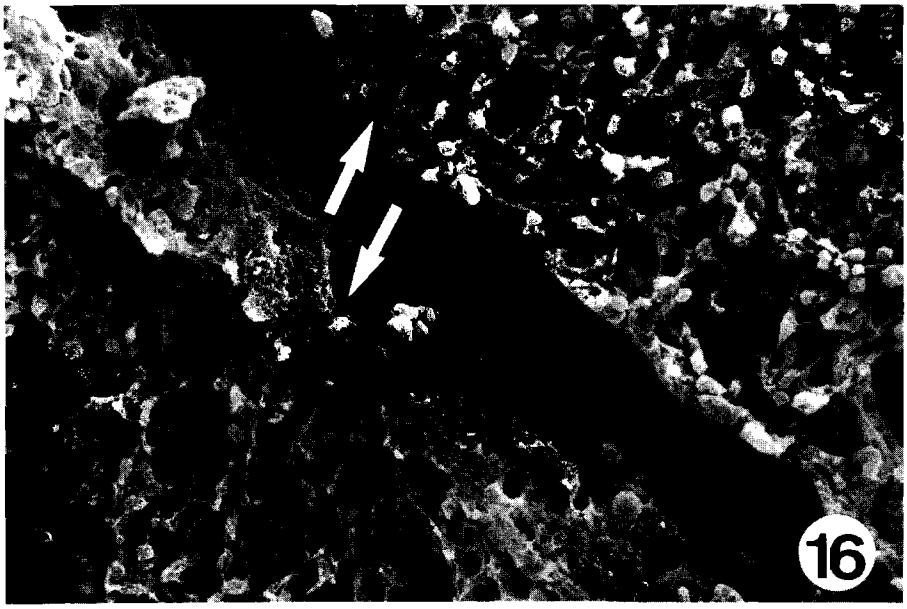

Fig. 16. Tonsil. Pig. Lumen of a crypt with openings (arrows) of channel-like structures. × 540

were of a similar size (6.5-7 μm) or a little smaller, with prominent cell borders limited from the other cells by a deep furrow. Their surface was covered with long microvilli arranged more irregularly than in the other type of cell. The number of these cells in this type of villi was never higher than 10% of the total number of cells. They were considered to be M cells from their appearance (Fig. 8). Occasionally there were bacteria scattered heterogeneously over the mucosal surface. The cut surface of this second type of villi was formed by a dense aggregate of lymphoid-like cells with a smooth surface (Fig. 9). Below this area there were lymphoid follicles with an appearance similar to those of the pig group. Due to their mucosal and cut surface characteristics this second type of villi was considered a typical villi with dome. Serosa was formed by cords of flattened cells with microvilli and well defined boundaries (Fig. 10). Underlying them, there was a loose connective tissue layer.

Tonsil

The surface of tonsil in the pig group was formed by cords of cells, parallel with each other, and limited by deep furrows (Fig. 11). Scattered over the surface the oval openings of the crypts could be seen. They were usually over 300-400 μm in size. Cells covering the tonsillar surface were of epithelial type, polygonal with many microfolds and with net borders. Bacteria, cell debris and detached epithelial cells could be seen (Fig. 12). The cut surface showed three well-defined layers. The outer was formed by a stratified squamous epithelium of 120-240 μm thick (Fig. 13). Cellular borders had small filaments that contacted with those of adjacent cells. Immediately below there was a 60-90 μm thick dense connective tissue layer. The third layer was the lymphoid area which occupied more than two-thirds of the total cut surface area. Non-follicular areas were formed by a reticular cell and fiber network with PCV of similar appearance to those of lymph nodes. Specimens not having been jet-washed showed many free lymphoid-like round cells with a rough surface in this area. Follicular lymphoid tissue was formed by lymphoid follicles scattered between diffuse lymphoid tissue but with a tendency to be gathered around crypts. The appearance of their cells was similar to those of non follicular areas but their surface was usually smooth or displayed a few microprojections. Between these cells blood vessels of 4.5-6 μm diameter could be seen. There was a thin layer of reticular cells and fibers incompletely covering the follicles. Crypts were situated between lymphoid tissue and were perpendicular to the organ surface. Their lowest end reached the basement of the tonsil. The cellular type that coated the luminal surface was different

depending on the zone. The upper part of the crypts (Fig. 14) had an epithelium similar to that of the surface, with many microfolds. On the other hand, the middle and the lowest parts of the crypts (Fig. 15) presented well-defined epithelial cells with short microprojections over their surface. There were many 3.5 to 7.5 μm holes in the epithelial cell cytoplasm or between two cells which allowed the passage of leukocytes and macrophage-like cells with microfolds or microprojections over their surface to the lumen. The cut surface of the crypts showed a stratified squamous epithelium thinner than that of the tonsillar surface. Round cells with smooth or rough surface inside channel-like structures were seen between the epithelial cells (Fig. 16). Sometimes there were so many cells crossing the epithelium that it was difficult to ascertain the limit between the crypt epithelium and the adjacent lymphoid tissue.

Piglet soft palate tonsillar surface had a similar appearance to that of the pig but it showed two types of polygonal epithelial cells. One, the most, with microfolds and the other scattered between them and with very few microfolds or microprojections. The cut surface exhibited the same layers as the pig but only in a few specimens could lymphoid follicles be seen. Crypts were also similar to those of the pig.

Discussion

With the methodology used in this work we have been able to observe the tridimensional architecture of swine lymphoid organs, viz. its reticular framework and the relationships between several cell types. Both groups of animals displayed lymph node sinus structure (subcapsular and PTCS) similar to that described in other species (Fujita et al., 1972; Fujita, 1978; Fujita and Kashimura, 1981; Heath and Spalding, 1987). PTCS were conspicuous and their lumen had reticular cell prolongations or fibers. One of the characteristics of the lymphoid organs studied was the presence of PCV. In the lymph nodes these vessels were found mostly in the diffuse lymphoid tissue of the cortex. The morphology was similar to that described in the literature (Fujita and Kashimura, 1981; Irino et al., 1981; He, 1985), with a prominent endothelium. A channel that surrounded the PCV was observed with reticular cell prolongations and round free cells in its lumen. Due to the fact that it was very narrow it could only be seen after having washed off most of the free cells. We have found no reference to this structure in the lymph nodes, but it has been described in thymic PCV (Kato and Schoefl, 1987; Ushiki, 1986). These authors described this perivascular space, which had many lymphocytes and with an outer limit of a discontinuous layer of reticular cells, only around PCV. Kato and Schoefl (1987) think that this perivascular channel and the respective PCV may function in the thymus as a pathway of lymphocytes into or out of the blood circulation. With SEM we could clearly distinguish the medulla and the cortex. The cortex of the pig had two intermingled areas. The lymphoid follicles, after washing off free cells, showed a wall made up of reticular and cell fibers. The other area was formed by diffuse lymphoid tissue with many lymphoid cells tightly packed and arranged inside a loose network of reticular fibers and cells. Our findings are similar to those of Merighi et al. (1986), but they show that follicles are coated by a dense fibrous capsule while our observations revealed that the wall had many holes which apparently allowed direct contact between the cells of the two cortical areas. As seen with optical microscope OM, piglet lymph nodes had few lymph follicles. However, since in this group of animals we did not use jet-washing we cannot rule out the possibility that there were small lymph nodes or follicles in early stages of development that without free cells in them could have been seen much more easily. The reticular network of the medulla was more dense than that of the cortex and its cells and fibers were arranged parallel to the organ capsule. These findings are different from those described in other species (Fujita et al., 1972; Fujita, 1978; Fujita and Kashimura, 1981; He, 1985; Heath and Spalding, 1987) but are similar to those of Merighi et al. (1986) and Hoshi et al. (1986, 1988) in the pig.

The mucosal surface of Peyer's patches (PP) of the pig did not apparently show M cells, but there were many goblet cells. This image could be the result of the covering of dome areas by longer and thinner absorptive villi. This fact has already been described by Chu et al. (1979) and by our observations with the OM. In the last case we observed that absorptive villi bridged dome areas. On the other hand, the mucosa of piglet PP showed the two types of villi described in the literature. One of them was longer and with the attributes of the absorptive villi (Landsverk, 1979; Torres-Medina, 1981). The other type was shorter and thicker with two types of cells; enterocytes with homogeneous and tightly packed microvilli and another cell type, of reduced number with thicker and longer microvilli, the so-called M cells. We have not found any author that describes the structure of PP of normal pigs under SEM. Only Torres-Medina (1981) studied gnotobiotic pig PP, with results similar to ours. Concerning the differences found with other species such as rodents or primates (Owen and Jones, 1974a,b) these authors found that the microvilli of M cells were shorter than those of absorptive cells. We occasionally observed bacteria over the mucosal surface but they were not apparently as numerous over a specific area or cell type as has been described by others (Chu et al., 1982; Chu and Liu, 1984; Buller and Moxley, 1988) who observed microorganisms mostly over M cells. Perhaps the washing of our specimens eliminated the majority of bacteria on the mucosa. Dome areas and lymphoid follicles showed a similar appearance, with tightly packed lymphoid-like cells generally with a smooth surface, but we were unable to find differences between germinal center and mantle areas of the follicles. This fact was also seen in the lymphoid follicles of other lymphoid organs (tonsil, lymph nodes) in spite of the fact that these compartments could be seen with OM. Fujita et al. (1972) did not seen different areas in secondary lymph nodes.

As mentioned by Owen and Bhalla (1983) we used

two methods of specimen preparation to study the tonsils, the most important difference between them being the application of a jet-wash previous to the fixation. The jet-wash was used to observe the reticular framework. When we wanted to see the epithelial surface, we did not wash the specimen. The outer layer of epithelial cells of the tonsillar surface had many microfolds in all the animals. Furthermore, piglets displayed another cell type with few or no microfolds. The emergence of microfolds in the epithelial cells occurs during the ontogenic development of the organ, cilia and microvilli appearing first which then fuse to form microfolds (Saito and Takagi, 1976). Nevertheless, the presence in our study of this second type of cell does not seem to be attributable to this fact. Also it cannot be argued that these cells were only detached cells because they also had many microfolds. We did not distinguish the fungiform cells seen in human tonsils (Howie, 1980) and rabbit tonsils (Olah and Everett, 1975). This type of cell has a similar function to the M cell of Peyer's patches (Howie, 1980). The presence of this cell type could be related to the type of tonsil or to the animal species. One characteristic of the crypt epithelium, not described by other authors, was the different appearance of its cells depending on the level in the crypt. In the upper third of the crypt, the epithelium was similar to the surface epithelium but in the rest of the crypt the cells had microprojections and microfolds. A possible explanation is the presence of cell receptors in this membrane which interact with antigens and eventually are driven to lymphoid areas of the tonsils. Anyway, we think this specialization of the cell surface would not apparently be necessary due to the existence of holes between or in the cells which would allow the migration of antigenic material through them. Howie (1980) also found holes in the crypt epithelium but they were thought to be artefacts caused by the detaching of fungiform cells during the processing of the specimen. In our samples some of these holes were partially or completely filled with lymphoid cells or another type of leukocytes, probably indicating migration of these cells to the crypt lumen, thus explaining the important number of leukocytes in the crypt lumen when tonsils are observed by OM. The cut surface of the crypts showed channels that crossed all the thickness of the stratified squamous epithelium. These channels sometimes had free cells and usually a tortuous course. Howie (1980) has also described similar channels in human palatine tonsils but a direct communication between these channels and the crypt lumen was not seen. As stated by this author, we think that these structures could have been caused by the migration of leukocytes across the epithelium.

There are few publications about the serosal surface of organs and cavities of animals and humans under SEM (Andrews and Porter, 1973; Furubayashi et al., 1984; Fentie et al., 1986; Pfeiffer et al., 1987; Faroon et al., 1989). Serosal membranes, which coat the majority of cavities and organs, are formed by a loose connective tissue covered by a single layer of mesothelial cells (Andrews and Porter, 1973). With SEM these cells displayed microvilli of variable length and number, depending on the organ. In our observations we found two types of covering of lymphoid organs. One of them (Peyer's patches) was formed by mesothelial-like cells with microprojections and microvilli that established connections with those of adjacent cells. The second type (lymph node) did not apparently show the mesothelial layer but had many interlaced connective tissue fibers. Pfeiffer et al. (1987) described pig intestine serosa as cords of cells with microvilli and well defined boundaries which corroborates our results. The length of microvilli in the intestine was higher than other organs, at least in the cow (Furubayashi et al., 1984) and the number of microvilli was also higher than that of other organs (reticulum, omasum, urinary bladder, pleura) or similar to others (rumen, pericardium, liver). We could observe in the intestinal serosa discontinuities in the mesothelial covering. According to Fentie et al. (1986) this could be due more to artefact during the processing of the specimens than to an anatomical characteristic of this serosa. The function of microvilli in serosal surfaces is not well defined but they probably have a protective function from frictional damage produced by continuous movement of the viscera (Andrews and Porter, 1973). This hypothesis was confirmed by the findings of Furubayashi et al. (1984) who observed a higher density of microvilli in the largest organs or in the most active ones. As in our case, caprine superficial lymph nodes have a capsule consisting of dense connective tissue (Faroon et al., 1989) which does not show mesothelial cells but muscle cells. The function of this type of cell could be the regulation of the lymph flow from the node. We did not distinguish smooth muscle cells in our samples.

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