Intercellular Spaces in the Lymph Nodule Associated Epithelium of the Rabbit Peyer’s Patch and Appendix*

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Summary. Intercellular spaces in the epithelium of the rabbit Peyer’s patch and appendix were examined by scanning and transmission electron microscopy to elucidate their three-dimensional structure and their relationship to the reticular spaces in the underlying lymph nodule.

Two types of intercellular spaces were distinguished: regularly arranged tubular channels and irregularly winding tunnels. The tubular channels were observed around normal enterocytes on the villi or internodular folds and apical portions of nodule domes. The channel spaces were lined with a successive arrangement of belt-like intercrestal surfaces of prismatic enterocytes and variously sized processes on the crests. The processes adjoining opposed crests formed a ladder-like structure or pectinate septa between neighboring channels.

The irregularly winding tunnels were formed among processes of irregularly shaped cells corresponding to FAE cells (BOCKMAN and COOPER, 1973) or M cells (OWEN and JONES, 1974) in the nodule associated epithelium. In the appendix, the tunnels were frequently organized into two-storey spaces, the adlumenal and basal spaces, which were incompletely separated by cytoplasmic processes. These tunnels continued by pores in the basement membrane to the reticular spaces in the underlying lymph nodules. Furthermore, the tunnels and the basement membrane pores constantly contained single or grouped free cells or their processes.

The findings in the present study suggest that the tubular channels are intraepithelial compartments for the absorption of nutrients and fluid, and the irregular tunnels are an intraepithelial network of spaces for the housing of lymphoid cells coming from the underlying lymph nodule.

The Peyer’s patch and appendix are important constituents of the gut associated lymphoid tissue. In both organs, the lumenal portions of the lymph nodules bulge into the intestinal lumen and closely associate with the overlying epithelium. The nodule or follicle associated epithelium (FAE) is thought to play a mediating role in conveying immunological information from the intestinal lumen to the underlying lymphatic tissue (BOCKMAN et al., 1983). For this mediation function, specialized cells have been proposed, which are morphologically distinguishable from common enterocytes. (SHI-MIZU and ANDREW, 1967; BOCKMAN and COOPER, 1973, 1975; OWEN and JONES, 1974; CHU

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et al., 1979; Bye et al., 1984). They are referred to as "follicle associated epithelial" (FAE) cells (Bockman and Cooper, 1973; Bockman, 1980) or "microfold" (M) cells (Owen and Jones, 1974).

Fine structural observations of these specialized cells have revealed the pinocytotic transport of not only experimentally administrated markers such as ferritin (Bockman and Cooper, 1973; Bye et al., 1984) and carbon particles (Joel et al., 1970; Hanaoka et al., 1971; Bockman and Cooper, 1973), but also intestinal microorganisms (Landsverk, 1981; Owen et al., 1982), from the intestinal lumen to the intercellular space of the nodule associated epithelium. Furthermore, the nodule associated epithelium is heavily infiltrated with lymphocytes from the underlying lymph nodules (PatzelT, 1936; Shimizu and Andrew, 1967; Abe and Ito, 1977; Schmedtje, 1980). Therefore, the intracellular spaces represent a contact area of lymphocytes with transported antigens. However, the three-dimensional structure of the intracellular spaces and their relationship with intercellular spaces among common enterocytes and also with reticular spaces of the underlying lymphoid tissue are little known (Bockman and Boydston, 1982; Miyoshi and Shingu, 1984).

The present study has been undertaken to directly visualize the three-dimensional extension of the intracellular spaces in the nodule associated epithelium after removal of free cells. The spatial relationship between the intracellular spaces and the reticular spaces in the underlying lymphoid tissue is discussed as it relates to the migration of lymphocytes and the immunological function of the cells.

**MATERIAL AND METHODS**

Peyer's patches and appendices observed in this study came from adult rabbits (weighing about 2 kg). The animals anesthetized with intraperitoneal Nembutal (25 mg/kg), and were perfused with warmed (38°C) Ringer solution via the abdominal artery for washing out the blood and plasma, and then, with a fixative composed of 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). In some cases, the organs were excessively perfused with Ringer solution for dampening the epithelium for its removal (Low and McClugage, 1984), and then followed by the perfusion of the same fixative. After perfusion, the excised Peyer's patches and appendices were kept in the same fixative for a few days.

For the scanning electron microscope (SEM) study, specimens were sliced into sections (about 100–200 μm thick). After being washed in a buffer solution, the specimens were immersed in 2% tannic acid solution for 12 hrs and then in 1% OsO₄ in 0.1 M phosphate buffer (pH 7.4) for 2 hrs. The specimens were dehydrated in a series of graded acetone, submitted by isoamyl acetate, dried by a critical point method of liquid CO₂ and coated with platinum palladium. Observation was performed with a Hitachi S-450 type of SEM.

For the transmission electron microscope (TEM) study, small blocks of the glutaraldehyde-fixed organs were cut into small pieces and post-fixed with 1.3% osmium tetroxide in the same buffer as mentioned above, dehydrated with a series of graded alcohol and embedded in epoxy resin. Thin sections of the plastic embedded specimens were doubly stained with uranium and lead, and observed in a Hitachi H-500 type of TEM.
Fig. 1. Lumenal surface of the Peyer’s patch. Domes (D) of lymph nodules are seen as swellings among columnar intestinal villi (V). ×80

Fig. 2. Vertically cut surface of the intestinal wall through the Peyer’s patch. Domes of the lymph nodules (LN) are covered with epithelium (arrowheads). Lateral and basal surfaces of the nodules are limited with lymphatic vessels (LV). V intestinal villi. ×70
OBSERVATIONS

In the survey view of the lumenal surface of the intestinal mucosa, mound-like swellings were seen among columnar intestinal villi or through the stomata of the colon mucosa (Fig. 1, 4). An appendant protuberance was also seen on the summits of some swellings (Fig. 3).

In vertically cut surfaces of the intestinal wall, lymphatic nodules were seen as oval compact masses of about 800 μm high and 500 μm thick, occupying almost the entire thickness of the intestinal mucosa (Fig. 2). Villi or internodular portions of the mucosa were seen as lamellar or fungiform folds, consisting of spongy axes and overlying epithelia (Fig. 2). The mound-like swellings mentioned above were nodule domes in conical protrusions pointing toward the lumen (Fig. 3). The domes were also covered with an epithelium of uniform thickness (about 30 μm). The lateral and basal surfaces of the nodules were encircled with slit-like spaces of the lymphatics. Many blood vessels bridged the spaces (Fig. 2).

In fractured or cut surfaces of the epithelium covering the villi or internodular portions of the mucosa, enterocytes were absorptive cells with long microvilli on the lumenal surface, and goblet cells with short microvilli and a lump of globular granules.

Fig. 3. Lumenal surface of the epithelium covering a dome (D). An appendant protuberance (Ap) is seen on the summit. The apical portion of the dome is paved mostly with polygonal surfaces of normal enterocytes, while the lower half of the dome surface is rough by mosaic arrangement of protruded enterocytes and flat-surfaced M cells. V villi. ×200

Fig. 4. Lumenal surface of the appendix. Domes of lymph nodules (arrowheads) are seen through the mucosal stomata. ×40
in the upper cytoplasm (Fig. 6, 7). The enterocytes were prismatic and, in the cross section, appeared polygonal, mostly penta- or hexagonal (Fig. 7). Crests of the prismatic enterocytes were seen as angular protrusions in cut profiles. Intercrestal surfaces were belt-like hollows extending in parallel along the long axes of the prismatic cells. Thread- or belt-like processes of varying thicknesses extended from the crests and were joined with those of neighboring cells. Faint streaks on the middle of the processes suggested the existence of a junctional structure (Fig. 6, 7). The adjoined processes between opposing crests showed a ladder-like structure or pectiniform septum in the vertical section of the epithelium (Fig. 6). A successive connection of four or five cells arranged in a circle formed tubular intercellular channels of about 3 μm in diameter, which extended along the long axes of the epithelial cells. Therefore, a set of five or six channels was arranged in a palisade fashion around each enterocyte. The neighboring channels communicated via pores between the cytoplasmic processes mentioned above (Fig. 6). The undermost pores in the septum were large, circular spaces, whose floors were lined with a thinly expanded layer of the basal cytoplasm on the basement membrane. The apical cytoplasm of the prismatic cells was adjoined laterally with that of neighboring cells by means of the structure of the terminal bar to form the roof of the intercellular channels. Free cells were sometimes seen in the intercellular channels. However, patent pores for cell migration have not yet to be observed through the underlying basement membrane.

The nodule-associated epithelium consisted of two types of cells (Fig. 5, 10, 11, 14). They were prismatic absorptive cells, as mentioned above, and irregularly shaped cells with low ridges on the luminal surface. Globular or rod-like microorganisms were

Fig. 5. Closer-view of the epithelium covering a lower portion of the dome slope in the Peyer’s patch. Two types of cells are distinguished: the absorptive cells (A) with gathered long microvilli and the M cells (M) with short microvilli. ×3,000
Fig. 6 and 7. Legends on the opposite page.
Intercellular Spaces in the Nodule Associated Epithelium

frequently noticed among those ridges (Bockman and Boydston, 1982) (Fig. 13, 14). This peculiar surface structure indicated that the irregularly shaped cells were the FAE cell of Bockman and Cooper (1973) or M cell of Owen and Jones (1974). Prismatic cells were dominant in the apical portion of the nodular dome (Fig. 3, 8), while the irregularly shaped cells were much numerous in the middle or lower portions of the dome slope (Fig. 3, 5, 10). Goblet cells were scarcely seen in the nodule-associated epithelium.

When not washed, the cut surface of the nodule-associated epithelium showed single or grouped free cells among the processes of the irregularly shaped cells (Fig. 9), while vacant tubular channels were also seen around the prismatic cells (Fig. 9a). The tubular channels in the nodule-associated epithelium were essentially the same as those

Fig. 6. Vertically cut surface of the epithelium covering the intestinal villi. Elongated intercellular spaces are interposed between prismatic epithelial cells with crests (arrows) issuing various-sized processes. The processes are adjoined with those on opposing crests of neighboring cells. The intercellular spaces are sealed apically with the terminal bars and basally with cytoplasmic expansions on the basement membrane (BM). F free cells in the intraepithelial space, G goblet cell, L intestinal lumen, V blood vessel. ×2,000. Inset. High magnification view of the cytoplasmic processes on the crests. Arrowheads indicate fine streaks for cell adjoinings. ×4,300

Fig. 7. Cross-cut surface of the epithelium of the intestinal villi. Epithelial cells are polygonal with five or six of angles with crests. Thin cytoplasmic processes on the crests extend to adjoin (arrowheads) with those of neighboring cells. G goblet cells with granules in the apical cytoplasm, F free cells. ×2,900
in the epithelium covering villi or internodular folds. Free cells were mostly spherical and about 5 μm in diameter. Their surfaces were equipped with some finger-like, or blebbed processes (Fig. 9). After removal of the free cells (Fig. 8, 10, 12), intercellular spaces under the irregularly shaped cells appeared as winding and branching tunnels. The surface lining the tunnels was quite smooth. The tunnel spaces also communicated with tubular channels through pores in the pectiniform septa (Fig. 10).

The intercellular spaces of the epithelium covering the appendix nodules were a two-storey structure in most parts (Fig. 9b, 12). The spaces of the upper floor were usually distended two or three times greater than those of the lower floor. The spaces of both floors were roughly separated by a cytoplasmic sheet formed by the successive adjoining of middle portions of the epithelial cells. However, there were many pores or tunnels in the sheet.

TEM observation of the nodule-associated epithelium in thin sections revealed grouped or isolated free cells in the labyrinthine intercellular tunnels under the irregularly shaped cells (Fig. 11, 13). The free cells showed nuclei with lumped chromatin enveloped by ample cytoplasm being usually electron lucent, except for grouped or dispersed small mitochondria and fragmental endoplasmic reticulum. Free cells at the basal level of the epithelium were relatively small, but contained stacks of well developed granular endoplasmic reticulum.

Fig. 9. Cut surfaces of the nodule-associated epithelia in the Peyer’s patch (a) and appendix (b), without being washed after sectioning. The irregularly shaped spaces under M cells (M) with short microvilli are fully occupied by free cells (F). BM basement membrane, L intestinal lumen, V blood vessel. a: ×2,500, b: ×2,000

Fig. 10. A cut surface of the epithelium of the lower dome slope in the Peyer’s patch. Intercellular spaces after removal of free cells are irregular and communicate with each other, forming labyrinthine tunnels. Underlying lymphoid tissue is composed of reticular cells (R), macrophages (*) and spherical lymphocytes. M M cells, BM basement membrane. ×2,100

Fig. 11. TEM-image of M cells (M) in the Peyer’s patch. Microvilli of the M cells are short, but thick when compared with those of absorptive cells (A). The cytoplasm of the M cells is electron lucent and embraces grouped free cells (F). L intestinal lumen. ×8,100
Fig. 10 and 11. Legends on the opposite page.
The framework of subepithelial lymphoid tissue was a spongy texture of reticular cells. The reticular cells showed polygonal perikaryon and some thin cytoplasmic processes extending radially (Fig. 8, 10). These processes were anastomosed with those of neighboring cells. Expanded cytoplasm of reticular cells was applied to the reverse side of the basement membrane of the epithelium, and also attached to the outer surface of blood vessels in the lymphatic tissue (Fig. 8, 10, 11). Labyrinthine spaces contained many round cells with finger-like processes, suggesting their lymphocytic nature. Macrophages with numerous globular processes were also seen (Fig. 10).

The intercellular tunnels in the nodule-associated epithelium were continuous through pores in the basement membrane to the reticular spaces of the underlying lymphoid tissue, as seen in fractured surfaces (Fig. 12, 15) and in sections (Fig. 16). Free cells were seen in those pores (Fig. 15, 16). SEM observation of the basement membrane after removal of the epithelial cells (Fig. 17) showed the basement membrane as a mesh-sheet with many round or oval pores of varying diameters ranging from 5 μm to 20 μm. The surface of the sheet was quite smooth without any detectable structure under the SEM. Blood vessels under the basement membrane extended along the thread-like extension of the rims of pores.
Intercellular Spaces in the Nodule Associated Epithelium

**DISCUSSION**

To slice fixed Peyer's patches and appendices into thin sections was more effective for the removal of free cells from the labyrinthine tissue spaces than was washing and squeezing off free cells from the cut surfaces of fresh organs (MIYOSHI and SHINGU, 1984). Furthermore, a more appropriate thickness of the sections was useful for the visualization of the three-dimensional extensions of delicately branched cells.

Two types of intercellular spaces, the tubular channels and the irregularly shaped tunnels, observed in the epithelium of the rabbit Peyer's patch and appendix apparently differed from each other in their structures and presumed functions. In the intestine (TOMASINI and DOBBINS, 1970), gallbladder (KAYE et al., 1966; OGAWA and YAMAMOTO, 1976) or urinary tubule (SCHMIDT-NIELSEN and DAVIS, 1968) actively absorbing fluid, the intercellular spaces in the epithelium are known to be greatly distended. DiBONA et al. (1974) have shown in the rabbit jejunum that acute volume loading of isotonic saline in the circulation causes the distension of the intercellular spaces in the epithelium. Therefore, the expansion of the intercellular spaces in the epithelium covering the villi and internodular folds may be due to the local effect of prolonged perfusion of the

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**Fig. 13.** TEM-view of the nodule-associated epithelium of the appendix. Free cells in the adlumenal intraepithelial spaces (F2) are larger and more electron lucent than those in the lower space (F1). A channel (large arrow) is formed between intraepithelial space and reticular space. Microorganisms (arrowheads) are seen on the luminal surface of M cell. R reticulum, L intestinal lumen. ×1,800

**Fig. 14.** Lumenal surface of the nodule-associated epithelium in the appendix. Microorganisms (arrowheads) are seen on M cells (M) with irregular microfolds. A absorptive cells. ×4,200
Fig. 15. A free cell (F) in a pore in the basement membrane (BM) of the epithelium covering the appendix dome. E epithelial cell, IS intercellular space, R reticular cell. × 6,100

Fig. 16. TEM-image of the basal portion of the appendix dome epithelium (E). Free cells (F) are seen in a pore of the basement membrane (arrowheads). × 2,900

Fig. 17. Epithelial face of the basement membrane (BM) after removal of epithelial cells covering the dome in the appendix. The basement membrane is a porous sheet. Blood capillaries (arrowheads) extend beneath the basement membrane. × 720
saline and fixatives performed in the present study. Although the tubular channels were extensively dilated, the regularity of their arrangement around enterocytes, their constant lining structures consisting of belt-like intercrestal cell surfaces and pectinate septa, as well as their uniform dimensions support the actual existence of the tubular channel system in the intestinal absorptive epithelium. The space system may possibly play a role in absorption or exchange of nutrients as intraepithelial compartments for the volume regulation of absorbed fluid or supplied plasma (Trier and Madara, 1981).

Instead of the simultaneous fixation, the perfusion of fixatives did not cause dilatation of the irregular, tunnel-like spaces around the irregularly shaped cells, the spaces being filled with compact masses of free cells. The direct visualization of those spaces under the SEM, therefore, was only possible after removal of the free cells. The irregularly shaped cells apparently correspond to the specifically differentiated epithelial cells, called FAE cells (Bockman and Cooper, 1973) or M cells (Owen and Jones, 1974). The finding in the present study that irregularly shaped cells were abundant at the lower portion of the dome slope, but scarce in its upper region coincides well with the distribution of M cells in the mouse Peyer’s patch (Bye et al., 1984), and also with the concentration of intraepithelial lymphocytes in the nodule associated epithelium of the Peyer’s patch in the rabbit (Schmedtje, 1980) and mouse (Abe and Ito, 1977). However, only Smith and Peacock (1980) have proposed a much denser distribution of M cells at the upper part of the dome of the mouse Peyer’s patch. A reverse opinion on the M cell distribution has been presented by Bye et al. (1984) and these discrepancies remained unsettled in the present study.

The basement membrane pores of the nodule-associated epithelium are apparently pathways for the free cell traffic between the intraepithelial spaces and the reticular spaces. However, the patency of the pores is considered to be kept by the passage of free cells, because our TEM observation of the basement membrane supported the concept of a constant existence of free cells or their processes in the pore lumina. Furthermore, the return of cells from the intraepithelial spaces into the reticular spaces has been proposed by some investigators (Marsch, 1975; Ferguson, 1977; Schmedtje, 1980). The free cells under the irregularly shaped cells observed in the present study were mostly lymphoid cells, such as medium-sized lymphocytes with ample cytoplasm, small lymphocytes and typical plasma cells. Their location in the epithelium is characteristic of each cell type. The medium-sized lymphocytes were in the adlumenal space, while the small lymphocytes and plasma cells were in the basal spaces or the pores of the basement membrane. Although the location of the free cells does not prove cell returning, it clearly indicates the functional importance of undifferentiated cells such as medium-sized lymphocytes (Hall et al., 1967; Matter et al., 1972) being close to the entrance of antigens which should be taken up by M cells from the intestinal lumen (Bockman and Cooper, 1973; Owen and Nemanic, 1978; Bockman et al., 1983) and of differentiated cells of small lymphocytes and plasma cells close to the subepithelial space.
REFERENCES


