Mechanical components of rat intestinal villi as revealed by ultrastructural analysis with special reference to the axial smooth muscle cells in the villi

Yasue Hosoyamada¹ and Tatsuo Sakai²

¹Department of Nutrition, Chiba College of Health Science, Chiba; and ²Department of Anatomy, School of Medicine, Juntendo University, Tokyo, Japan

Summary. The ultrastructure of the rat intestinal interstitium with regard to the mechanical components was analyzed from a functional viewpoint utilizing serial horizontal as well as longitudinal sections through the lamina propria mucosae, including both villi and crypts. The axial smooth muscle cells in the villi (villus-axial SMs) exhibited different configurations at various levels of the wall. They were separated from the voluminous fluid-filled spaces by sheet-like processes of fibroblasts in the upper part of the intravillous interstitium, formed a sheet around the central lymphatics, and were covered by the sheet-like processes of fibroblasts in the lower part of the intravillous interstitium. These villus-axial SMs were poorly developed and associated with the lymphatic walls in the upper part of the pericryptal interstitium; they were tapered and connected to microtendons composed of fascicles of longitudinal collagen fibrils in the lower part of pericryptal interstitium. At the apical termination, the villus-axial SMs were connected to myofibroblasts, which sent off many processes into the subepithelial meshwork layer of fine cell processes and extracellular matrices. The villus-axial SMs possibly develop longitudinal tension against the intravillous hydraulic pressure developing from the transepithelial absorption through the intestinal epithelium.

Introduction

The interstitium plays substantial roles in the function of organs and manifests specific structural arrangements in different organs. In the intestinal wall, the interstitium is differentiated into the lamina propria mucosae (LPM) both within the intestinal villi and among the crypts and tela submucosa between the muscularis mucosae and smooth muscle layer. Ultrastructural studies of the intestinal interstitium have revealed unique structural features, including a subepithelial meshwork layer established by both cytoplasmic processes of myofibroblasts (Marsh and Trier, 1974; Ohtani et al., 1988) as well as a subepithelial meshwork layer of extracellular matrices (Komuro, 1985; Komuro and Hashimoto, 1990), the differentiation of myofibroblasts between the villous and cryptal regions of the interstitium (Daimon and Okura, 2004), and vascular organization in the villi (Ohtani, 1987).

Recently Hosoyamada and Sakai (2005) revealed that the density of collagen fibrils in the interstitium as well as the thickness of individual collagen fibrils are differentiated among portions of the intestine (duodenum, jejunum, ileum and large intestine) in addition to the regions within the wall. The study pointed out that the structure of the intestinal villi was sustained by the dynamic balance between the outward expanding force from the interstitial pressure and the inward circumferential tension from the subepithelial meshwork layer.

The smooth muscle cells in the axial region of the villi (villus-axial SMs) are well recognized by previous ultrastructural studies (Güldner et al., 1972). They are thought to develop longitudinal tension against the...
interstitial pressure and possibly to produce rhythmical contraction of the villi (Womack et al., 1988). However, precise findings are still lacking concerning the mechanical relationship of the villus-axial SMs to the other mechanical components of the intestinal wall such as the subepithelial meshwork layer, including myofibroblasts as well as the collagen fibrils and extracellular matrices in the interstitium.

The present study was undertaken to provide a better understanding of the ultrastructural details of the mechanical components of the intestinal villi with special reference to the attachments of the villus-axial SMs at the apical and basal terminations.

Fig. 1. Light micrograph of the rat duodenal wall showing a longitudinal section of the intestinal villi. The ascending blood vessel in the middle (a) reaches the apex of villus to diverge into the capillary network beneath the intestinal epithelium. The descending blood vessel (d) is found in association with the ascending one. The central lymphatics (asterisks) are distended in the lower part of the intravillous LPM. In the pericryptal LPM and tela submucosa, cross or oblique sections of arteries (A) and veins (V) running along the muscularis mucosae are found. Several profiles of the villu-axial SMs are found in the intravillous LPM. Scale bar = 500 μm

Fig. 2. Electron micrographs of horizontal sections of rat duodenal villi at the various levels of villi and crypts, including a) the upper part of villi (200 μm from the apex), b) the lower part of villi (450 μm from the apex), c) the upper part of crypts (600 μm from the apex), and d) the lower part of crypts (800 μm from the apex). a: The columns of villus-axial SMs (SM) are separated from the highly developed fluid-filled spaces (*) by sheet-like processes of fibroblasts (F). C: blood capillaries. b: The lymphatics (L) are surrounded by a layer of villus-axial SMs which appear in these sections as cross sectional profiles. The fibroblasts partly separate the villus-axial SMs and subepithelial meshwork layer from the moderately developed fluid-filled spaces. c: The wall of lymphatics (L) is associated with the discontinuous layer of villus-axial SMs which appears in these sections as islands of cross sectional profiles. The fluid-filled spaces are represented as narrow intervals between the fibroblasts surrounding the villus-axial SMs and lymphatics on one hand and the epithelium (E) and subepithelial meshwork layer on the other. MF: myofibroblasts. d: Sheet-like processes of fibroblasts (F) and villus-axial SMs (SM) are found between neighboring crypts. The villus-axial SMs are surrounded by longitudinal microtendons which appear as aggregations of cross sectional profiles of collagen fibrils. Scale bar = 2 μm
Fig. 2. Legend on the opposite page.
Materials and Methods

The present study used six male Wistar rats weighing 120~140 g (Charles River Japan). All were perfused with a fixative containing 2% paraformaldehyde in a 0.1 M cacodylate buffer, pH 7.4, at 4°C via a cannula inserted into the abdominal aorta for about 5 min. After fixation, the duodenum was dissected out and immersed in 2.5% glutaraldehyde in the same fixative overnight.

The specimens were cut into slices of 250 μm thickness by a vibrating microtome (DTK-1000, Dosaka EM Co. Ltd., Osaka) and processed further by the cold dehydration technique described elsewhere (Sakai and Kriz, 1987). Briefly, the specimens were immersed in a 1.2% extract of oolong tea (OTE, Suntory Co., Tokyo) in an acetone buffer solution (0.05 M maleate buffer pH 6.0 containing 10% acetone), followed by 1% uranyl acetate in the same acetone buffer solution, and then dehydrated with a graded series of acetone at 0°C to -30°C before embedding in Epon 812. Semithin sections were obtained with a diamond knife, stained with toluidine blue, and observed and photographed under a Nikon Optiphot microscope. Ultrathin sections were processed with a diamond knife, stained doubly with uranyl acetate and lead citrate, and observed in a Hitachi H7100 electron microscope.

For observation of the interstitium at different levels in the region of villi and crypts, a continuous series of horizontal semithin sections was processed at a thickness of 0.5 μm for light microscopy along with ultrathin sections in specific levels for electron microscopy. The levels of horizontal sections were estimated by the numbers of sections counted from the top of villi to a depth of 875 μm at the junction between the tela submucosa and smooth muscle layer. The ultrathin sections were obtained at depths of 200 μm, 450 μm, 600 μm, and 800 μm.

Results

Villus-axial SMs and myofibroblasts at different levels of villi and crypts

The interstitial tissue of the lamina propria mucosae (LPM) was found within the villi as well as around crypts in the rat small intestine. At first sight, the interstitial tissue of LPM within the villi (intravillous LPM) was relatively loose in appearance compared with that around the crypts (pericryptal LPM). Careful observation of semithin sections of the duodenum in longitudinal as well as transverse planes revealed that both the intravillous and pericryptal LPM were subdivided into upper and lower parts, respectively (Fig. 1).

In the upper part of the intravillous LPM, solitary columns of SMs comprised groups of villus-axial SMs enclosed within a compartment established by sheet-like processes of fibroblasts (Fig. 2a). Within the compartment, the villus-axial SMs and cell processes of fibroblasts were connected by a small amount of delicate fibrillar substances. The anchoring fibers with or without groups of unmyelinated nerve fibers were surrounded by sheet-like processes of fibroblasts. Beneath the intestinal epithelium, cytoplasmic processes of myofibroblasts and fine meshworks of collagen fibrils constituted a subepithelial meshwork layer mechanically supporting the basement membrane and epithelial cells.

The villus-axial SMs in the lower intravillous LPM were found either as solitary columns or as a perilymphatic layer of SMs. Both populations of villus-axial SMs were surrounded by sheet-like processes of fibroblasts (Fig. 2b). In this way the fibroblasts divided the interstitium of lower intravillous LPM into two compartments: one containing villus-axial SMs, nerve fibers and extracellular matrices, and the other consisting of fluid filled spaces with circulating cells. In the lower

Fig. 3. Electron micrographs of longitudinal sections of the rat duodenal villi and crypts. a: Overview showing the entire length of the villi and crypts. The areas indicated by vertical lines and numbers are shown in higher magnification in b–d and Figure 4. b–d: Higher magnification of the regions indicated in a, respectively. b: In the upper part of villi (*2 in a), the villus-axial SMs from isolated columns which are surrounded by sheet-like processes of fibroblasts (F). The fluid-filled spaces (*) are conspicuous at this level of LPM. c: At the junction between villi and crypts (*3 in a), the villus-axial SMs are associated with the wall of central lymphatics (L). d: In the lower part of crypts (*4 in a), the villus-axial SMs have tapered and connected with longitudinal bundles of collagen fibrils forming the microtendon. C: capillary, *E: epithelial intercellular space, IC: intestinal crypt, MF: myofibroblast. Scale bars = 50 μm (a), 10 μm (b–d)
Fig. 4. Electron micrograph of longitudinal sections of the apex of rat duodenal villi of the regions indicated as *1 in Figure 3a. The villus-axial SMs (SM) are connected with myofibroblasts (MF) which send off cell processes to establish the subepithelial meshwork layer. *E: epithelial intercellular space, *I: interstitial fluid-filled space, F: fibroblast. Scale bar = 5 μm
intravillous LPM, the fluid-filled spaces were decreased and the lymphatics were increased compared with the upper intravillous LPM. The solitary columns of SMs in the upper intravillous LPM were a continuation of the two populations of SMs in the lower intravillous LPM. In the lower intravillous LPM of the rat duodenum, the subepithelial meshwork layer was conspicuously thick, contained an abundance of densely packed collagen fibrils, and was much more well-developed compared with that in the upper intravillous LPM.

The villus-axial SMs in the upper pericryptal LPM were occasionally found around the lymphatics, but were obviously decreased in number compared with the perilymphatic SMs in the lower intravillous LPM (Fig. 2c). The subepithelial mesh work layer with myofibroblasts and collagen fibrils in the pericryptal LPM was less well-developed compared with that in the lower intravillous LPM.

The villus-axial SMs in the lower pericryptal LPM were found between neighboring crypts, were surrounded by loose bundles of collagen fibrils running in longitudinal directions, and did not make contact with lymphatics. The collagen fibrils appeared to serve as microtendons which transmit the mechanical force of villus-axial SMs to the lower structures such as the muscularis mucosae (Fig. 2d).

Attachments of villus-axial SMs

The villus-axial SMs arose from the lower pericryptal LPM and attached themselves to the mechanical components in the upper intravillous LPM. Ultrastructural observations of horizontal and longitudinal sections of duodenal mucosa revealed different arrangements of the villus-axial SMs at several levels of the LPM (Fig. 2, 3).

The lower formation of the villus-axial SMs was composed of slender projections of SMs surrounding collagen fibrils running in longitudinal directions (Fig. 3d). The longitudinal collagen fibrils obviously served as microtendons to transmit the mechanical forces of villus-axial SMs to the moderately dense bundles of collagen fibrils in the lowermost part of the pericryptal LPM just above the muscularis mucosae. The lower ends of villus-axial SMs with collagen fibrils were found mostly in the middle position between adjacent crypts.

The lower belly portion of villus-axial SMs was associated with the lymphatic endothelium (Fig. 3b, c). The villus-axial SMs were occasionally found near the endothelium of meandering wide lymphatics in the upper pericryptal LPM; those in the lower intravillous LPM were much better developed, constituting a thick covering layer around individual longitudinal lymphatics. In the upper pericryptal LPM, the villus-axial SMs and lymphatics

Fig. 5. Electron micrograph of longitudinal sections of the apex of rat duodenal villi, showing attachments of villus-axial SMs (SM) via myofibroblasts (MF) to the subepithelial meshwork layer of intestinal epithelium (E). Scale bar = 2 μm
Fig. 6. Schematic diagram of the structural organization of the interstitium of the intestinal wall. **a:** In the villi on the left side, the ascending (red) and descending blood vessels (blue), blood capillaries in the subepithelial meshwork layer (purple), and lymphatics in the center of villi (green) are illustrated. In the villi on the right side, the villus-axial SMs (orange) and myofibroblasts (green) in addition to SMs in the smooth muscle cell layer (pink) are shown. **b:** At the apex of villi, the villus-axial SMs are connected to the epithelial basement membranes via the myofibroblasts which send off numerous processes into the subepithelial meshwork layer. **c:** At the lower part of the pericryptal LPM, the villus-axial SMs are tapered and connected to microtendons of collagen fibrils, which are anchored to the relatively dense connective tissue in the lowest part of the LPM.

were directly surrounded by a loose interstitium with fibroblasts and collagen fibrils. In the lower intravillous LPM, the villus-axial SMs and lymphatics were substantially covered by sheet-like processes of fibroblasts and separated from the voluminous fluid-filled spaces which were characteristically developed in the intravillous LPM.

The upper belly of villus-axial SMs formed solitary columns independent of lymphatics (Fig. 4). The solitary columns were surrounded by sheet-like processes of fibroblasts and were separated from the voluminous fluid-filled spaces.

The upper termination of the villus-axial SMs was represented by an association with myofibroblasts which
were connected to the subepithelial meshwork layer with cytoplasmic processes of myofibroblasts and networks of collagen fibrils (Fig. 5). Thus, the mechanical force of villus-axial SMs was obviously transmitted to the villous epithelium through the myofibroblasts.

Discussion

Myofibroblasts in the intestinal villi

The interstitium of intestinal villi contained at least three types of interstitial cells — i.e. the fibroblasts, myofibroblasts, and villus-axial SMs, each showing a characteristic localization and morphology serving different roles in the functions of the intestinal villi.

The fibroblasts possessed sheet-like cytoplasmic processes and substantially separated the fluid-filled spaces from the formed elements of the interstitium, including the villus-axial SMs, lymphatics, and subepithelial meshwork layer of extracellular matrices and myofibroblasts. The morphology of the fibroblasts has been previously reported in part by Güldner et al. (1972), who showed that the solitary columns of villus-axial SMs were encapsulated by sheet-like processes of myofibroblasts.

The myofibroblasts play a central role in the mechanical support of the intestinal villi. They make contact with the villus-axial SMs on one hand, and project numerous processes beneath the intestinal epithelium to make a subepithelial meshwork layer together with delicate collagen fibrils on the other (Takahashi-Iwanaga and Fujita, 1985; Ohtani et al., 1988; Komuro, 1989; Daimon and Okuma, 2004). This subepithelial meshwork layer serves as a tensile sheath to develop tension against the expanding force exerted by interstitial hydrostatic pressure (Hosoyamada and Sakai, 2005). Furthermore, the myofibroblasts connect the subepithelial meshwork layer with the villus-axial SMs, depressing the intestinal epithelium via the subepithelial meshwork layer downward against the upward expanding force from the interstitial hydrostatic pressure. Thus, the myofibroblasts counteract the interstitial hydrostatic pressure both in a circumferential direction as a part of the subepithelial meshwork layer and in a longitudinal direction as the mechanical mediator of villus-axial SMs.

Villus axial SMs

The villus-axial SMs have been frequently observed by light and electron microscopy since the first report by Trautman (1909). Güldner et al. (1972) reported that the bundles of villus-axial SMs in the intestinal villi were surrounded by sheet-like processes of fibroblasts. Ushiki (1990) observed that the bundles of villus-axial SMs accompanied the central lymphatics in the villi. However, the ultrastructural details of the villus-axial SMs remain obscure, especially regarding their overall configuration as well as mechanical attachments at the apical and basal terminations. The present study has revealed the structural details of the villus-axial SMs for the first time (Fig. 6).

The villus-axial SMs develop longitudinal tension which is mediated via myofibroblasts and subepithelial meshwork layer to the intestinal epithelium, as proposed by Lee (1986) based on physiological experiments. The most well-developed villus-axial SMs are found around the lymphatics in the lower part of the villi and become progressively thinner at the level of crypts to connect with the thin bundles of collagen fibrils that serve as microtendons of the villus-axial SMs. This arrangement indicates that villus-axial SMs develop tension most effectively in the villi and not so well at the level of crypts. Thus, the villus-axial SMs can serve to develop longitudinal tension in the villi against the hydrostatic pressure produced by transepithelial transport by the intestinal epithelial cells, and do not appear to pull the whole villi toward the lamina muscularis mucosae or the submucosal connective tissue. This idea can be confirmed by observations of the rhythmical contractions of the rat intestinal villi, a phenomenon which has been frequently reported since the first notification by Carleton and Florey (1927).

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References


