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Chapter 8

Morphology of the Intestinal Barrier in Different Physiological and Pathological Conditions

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Additional information is available at the end of the chapter

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1. Introduction

Beside its main function of digestion and absorption, intestinal mucosa acts as an important barrier to toxic and harmful materials and protects an individual from different antigenic and inflammatory reactions. The intestinal barrier is composed of a mucin layer covering the cells, enterocytes and the apical junctional complex in between the cells [1].

The epithelium of the small intestine is characterized by villi & crypts. Villi are folds of the epithelium into the lumen with a core of lamina propria. Villi are tallest in the jejunum and shortest in ileum. The lamina propria core contains white blood cells, lacteals, a rich fenestrated capillary network, nerves; and scattered smooth muscle cells.

The villus epithelium is composed of enterocytes and goblet cells. Enterocytes are columnar absorptive cells and has an apical striated border of microvilli. Goblet cells secrete mucin to provide a protective coating. Only a few goblet cells are present in the the upper small intestine, more in the ileum.

The apical junctional complex consists of a network of tight junction proteins and the adherens junction [2]. They are anchored in the cell via the filamentous actin cytoskeleton [3]. Zonula occludens proteins (ZO-1, ZO-2 and ZO-3) are important intracellular tight junction proteins, linking the cell cytoskeleton to the transmembrane TJ proteins such as claudins, occludin and junctional adhesion molecules (JAM). Whereas occludin and JAM have a regulatory role, transmembrane protein claudins, abundantly present between adjacent healthy intestinal epithelial cells, are mainly responsible for the intestinal barrier function [4].

Crypts are folds of the epithelium that invaginate down into the lamina propria. Many of the cells in the crypt serve as precursors for enterocytes or goblet cells of the villi. Paneth cells are situated at the base of the crypts. Enteroendocrine cells are scattered through the small intestinal crypts.

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Microvilli are folds of the apical plasma membrane of each enterocyte with a core of actin cytoskeleton. A thick glycoprotein coat, the glycocalyx covers the microvilli. The glycocalyx contains hydrolytic enzymes such as enteropeptidase, dipeptidases and disaccharidases.

Lymphoid cells are found throughout the GI tract lamina propria, submucosa and even the epithelium itself and are known as gut associated lymphoid tissue or GALT. Aggregated nodules are found in the jejunum and ileum, with more prominent ones in the ileum and known as Peyer's patches.

At the site of Peyer's patches, the overlying villi are frequently absent. The epithelium covering a patch is called the follicle associated epithelium (FAE). The FAE is composed of specialized cells called "M" cells. M cells pinocytose a representative sample of intraluminal antigens and transcytose them across to intraepithelial antigen presenter cells.

Recently, disruption of the above mentioned structures during several physiological or pathological conditions has been reported, which were associated with impaired intestinal barrier function and lead to the passage of intraluminal solutes into the systemic circulation [5,6,7].

Investigations showed that the changes of intestinal barrier function were mainly due to the relaxation of the tight-junction between intestinal epithelial cells [8]. Some studies proposed that factors causing alterations in gut microbiota, hormones secreted by the enterocytes and changes of related enzymatic system caused damage of intestinal barrier, and the enteric bacteria and endotoxin reinforced the damage [9].

Histopathological data of the gastrointestinal barrier of human being is scarce. This chapter mainly focuses on the research findings of the morphological changes of the gut during intestinal barrier dysfunction in laboratory animals. Contents of the chapter will help the researchers interested in gastrointestinal morphological changes.

Discussion about the factors and type of morphological changes of the gastrointestinal barrier is provided below. For easy understanding, discussion is done under several headings.

2. Gender and age

Recently, Milićević Z and his team analyzed the effects of gender and ageing on the histoquantitative parameters of healthy jejunal and ileal mucosa. Computer-aided morphometric analysis of 24 jejunal and 25 ileal biopsy samples collected during routine endoscopy of healthy individuals with family history of intestinal malignancy was done. Jejunal mucosal thickness was significantly reduced in elderly subjects above 60 years of age ($p<0.05$), especially in elderly females compared to the adults ($p<0.05$). Jejunal villi were significantly wider in adults than in the elderly subjects ($p<0.05$), whereas ileal villi were significantly wider in elderly compared to adult subjects ($p<0.01$) and in male compared to female subjects ($p<0.05$). Other histoquantitative parameters eg. mucosa epithelium height, crypt numerical density, villous height, crypts and villous perimeter, diameter and

epithelium height of jejunal and ileal mucosa were not different significantly in their observation [10].

3. Undernutrition

Adequate nutrition is necessary for the normal cell division and cell migration from the crypt to the villi thus in maintaining the gastrointestinal barrier. Diet with protein and vitamin restriction (75% protein and 50% vitamin restrictions) but not food restriction to weanling, Wistar/NIN male rats for 20 weeks significantly increased intestinal cell apoptosis observed by morphometry, Annexin V binding, M30 CytoDeath assay, and DNA fragmentation [11].

Providing a diet moderately deficient in protein, fat, and minerals to C57BL/6 mice from the 10 day of their life for 6 weeks resulted in decreased villous height and crypt depth in the jejunum of the undernourished weanlings, stained by haematoxylin and eosin (H&E) and observed under light microscope, increased claudin-3 expression, decreased epithelial cell proliferation measured by immunohistochemistry eg. MTS and bromodeoxyuridine assays and increased epithelial cell apoptosis as measured by annexin and 7-amino-actinomycin D staining. All these changes were associated with decreased transmucosal resistance and increased permeability to FITC-dextran indicating intestinal barrier dysfunction [12].

Cancer cachexia is also reported to induce alterations to some of the morphological parameters of the small intestine. Light microscopic observation of the H&E stained intestinal specimen of tumor-bearing mice was reported to have lower villus height and contour length than in healthy mice ($P > 0.05$). Villus width and crypt depth showed substantial evidence of atrophy ($P < 0.05$). Wasting of smooth muscle in the muscularis layer as indicated by a reduction in muscularis width was present on days 2 and 11 of tumor production ($P < 0.05$). [Villus contour length was calculated using the following equation: villus contour length = (2 × villus height) + (0.3 × villus width)] [13].

4. Psychological stress

Various kinds of psychological stresses such as chronic water avoidance stress (WAS) in rats and mice which mimics chronic depression in human being has been reported to compromise small intestinal mucosal structure and hamper intestinal barrier function. H&E and Periodic acid schiff (PAS) stain of the microtome sections of the small intestinal segments of the Sprague Dawley rats subjected to water avoidance stress for 10 days showed that villus height ($p<.005$), crypt depth ($p<0.00$), number of goblet cells in villus ($p<.0.00$) and crypt ($p<.0.03$) decreased significantly in the jejunum as compared to the control. Ileum also had atrophy but villus height and the number of goblet cells in the villi did not differ significantly. Number of polymorphonuclear neutrophil infiltration was significantly higher in stress group as compared to control ($p<0.00$) [6].

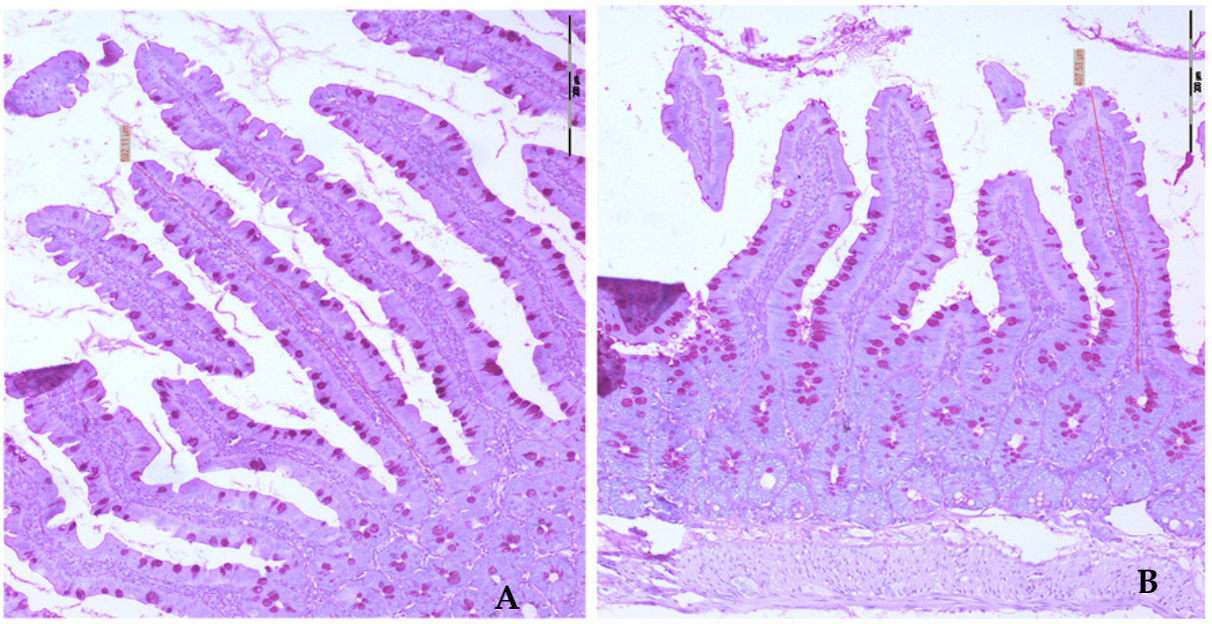


Figure 1. Light microscope findings of PAS stain of control (A) and WAS (B) rats of the mucosal layer of jejunum (× 100).

Microtome sections of the rat distal colon subjected to WAS stained with H&E showed inflammatory cell infiltration in the lamina propria of stressed rats fed with standard diet that was not observed in non-stressed rats [14].

Noise-induced stress (15 min of white noise at 90 dB daily for 3 wks) has been reported to disrupt the intestinal barrier of laboratory rats. Light microscopic observation of the ileum stained by H&E showed significantly more degranulated mast cells (mean+SE, 3.95+0.8 vs 0.35+ 0.29, respectively) and eosinophils (mean+SE 9.46+0.44 vs 4.58+0.38) per villus section adjacent to the Peyer patches in noise exposed rats than in quiet rats. The mean width of villus laminar propria was significantly greater in noise exposed rats than in quiet rats, suggesting edema. Mucosal epithelial cells of noise rats were often separated, sometimes detaching from the basement membrane, whereas those of quiet rats were intact. Recovery rats who were kept in quiet room for a further 3 weeks after the initial 3 weeks of noise exposure, showed no reduction in mast cell degranulation or mean width of villus lamina propria, but there were increased numbers of secreting goblet cells in the villi adjacent to Peyer patches and some recovery of epithelial integrity [15].

5. Nutrients

High fat diet consumption and obesity has recently been identified to be associated with compromised tight junction integrity of the enterocytes. An altered distribution such as substantial decreased staining of occludin and discontinuous signals for ZO-1, in the intestinal epithelium of leptin-deficient obese *ob*/*ob* mice was observed by immunocytochemistry of intestinal cryosections. Similar results were obtained in hyperleptinemic and functional leptin receptor deficient obese *db*/*db* mice. There was also a

shift of intestinal junctional protein from the cytoskeleton of both these obese strains, causing a decrease in paracellular sealing [9].

Medium chain triglycerides (MCTs) enhance cell proliferation of the intestinal epithelium and mucous secretion from goblet cells in the small intestine. Number of goblet cells measured by periodic acid Schiff and alcian blue stain was significantly higher in rats given MCTs as compared to rats given corn oil (5 g/kg per day) or chow for 2 weeks. Proliferating cells on the villi and the crypts in the small intestine, detected by immunohistochemistry using monoclonal mouse anti rat Ki-67 antigen and the apoptotic cells detected by polyclonal rabbit anti single stranded DNA antibody were also significantly greater in rats given MCTs than rats given corn oil or normal rats. Both proliferative and apoptotic index were significantly increased in rats receiving MCTs.

These effects of MCTs might persuade further research on the role of MCTs on the histological changes of the gastrointestinal barrier in patients suffering from inflammatory bowel disease or enterogenous infections [16].

6. Parenteral nutrition

Observation of the Ileal segments of Sprague Dawley rats stained with H&E and PAS staining after seven days of standard total parenteral nutrition (TPN) under light microscope has been associated with villous atrophy, fewer goblet cells, atrophy of Peyer's patches. Decreased luminal mucus gel was observed by cryostat sections of frozen samples in liquid nitrogen followed by celloidin stabilization and PAS stain in rats receiving TPN [17]. Seven days of Alanyl glutamine supplemented TPN was able to attenuate the changes found in standard TPN observed under light microscope [18].

7. Infections

Enteroaggregative Escherichia coli strains have been associated with persistent diarrhea in several developing countries. Electron microscope observation showed that Enteroaggregative Escherichia coli strains caused total or partial villi destruction, vacuolization of basal cytoplasm of the enterocytes, epithelium detachment, derangement of the structure and epithelial cell extrusion in ileal mucosa. Bacterial aggregates associated with mucus and cellular debris was evident in the intestinal lumen and in the intercellular spaces of the destroyed epithelium, suggesting bacterial invasion which seemed to be secondary to the destruction of the tissue [19].

8. Inflammatory bowel disease

Duodenal biopsy of Celiac disease patients stained with H&E and studied under light microscope had a raised IEL count (> 20 per 100 enterocytes) and marked villous atrophy [20].

Expression of claudin-2 was distinctly different in active Chron's disease (CD) and ulcerative colitis (UC) in comparison to its expression pattern in controls. Claudin-2

expression was upregulated along the whole length of intercellular junction (ICJ) in biopsies from patients with active CD and UC in comparison to the biopsies from control patients, where its expression was limited to the uppermost part of ICJ. There was reduced expression of ZO-1 in UC and CD patients. On transmission electron microscopic examination, the pentalaminar structure of tight junction (TJs) was destroyed in patients with CD and UC but no significant change was seen in controls. The redistribution of claudin-2 expression was in accordance with the TJ ultrastructural changes in patients with UC and CD [21].

9. Diabetes mellitus

Endoscopic biopsy of eight insulin dependent diabetes mellitus patients without concomitant celiac disease was devoid of any sign of atrophy or inflammation under light microscope, whereas observation under transmission electron microscope showed remarkable ultra-structural changes in height and thickness of microvilli, space between microvilli and thickness of tight junctions in six out of the eight patients [22].

10. Surgery

Electron microscope observation of the samples of the laboratory animals underwent intestinal surgery reported lower expression of occludin. Administration of enteral nutrition (EN) after surgery induced greater expression of occludin in the intestine than in the animals receiving total parenteral nutrition (TPN). Intestinal epithelial tight junction and microvilli were more intact in the animals receiving post surgical EN as compared to those receiving post surgical TPN [23].

Destructive changes such as intense edema of the intestinal wall, mainly in the intestinal lamina propria, as well as blood vessel dilatation and congestion were observed in the intestinal obstruction model of mice. There was also a discrete increase in the cellularity of lamina propria. In addition to that, epithelial reactive changes, superficial erosions, edema, and enlargement of the intestinal villi were observed under light microscope [24].

11. Alcohol and drugs

Ethanol (0, 1, 2.5, 5, 7.5, and 10%) produced a progressive disruption of TJ protein (ZO-1) with separation of ZO-1 proteins from the cellular junctions and formation of large gaps between the adjacent cells of Caco-2 cell line as evidenced by immunofluorescent antibody labeling of ZO-1 proteins [25].

Immunofluorescence analysis of HT-29 cells showed a fragmented and granulous ZO-1 staining, after aspirin treatment. Treating both aspirin and heat-killed Lactobacillus acidophilus strain LB (LaLB) together with the culture supernatant, resulted in fine continuous linear web at cell-cell contacts similarly to control evaluated by immunofluorescence using an anti-ZO-1 antibody [26].

Prolonged subcutaneous injection of Methamphetamine (MA) for 12 weeks decreased the villi height and increased the number of goblet cells significantly as compared to the control group of rats as evidenced by light microscope observation of the H&E and PAS stained samples of the ileum. Withdrawal of MA was able to bring the morphology back to normal [27].

Quantitative analysis of ZO-1 expression in male Wistar rats receiving oral methotraxate (MTX, 15 mg/kg) for 3-5 days showed the absence of significant differences, whereas tyrosine dephosphorylation of ZO-1 was observed. An obvious reduction of ZO-1 immunostaining along the apical membrane of intestinal villi was also observed. These findings suggest that ZO-1 alterations may contribute to the disturbance of TJ barrier in MTX-treated rats, which leads to enhanced intestinal permeability [28].

Paclitaxel, a new chemotherapeutic agent induced apoptosis in 12.5% of jejunum villus cells observed under light microscope after Terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate (dUTP)-biotin end labeling assay (TUNEL), which was reduced to 3.8% by granulocyte stimulating factor (G-CSF) treatment. Apoptosis in the control group was 0.6%. Paclitaxel treatment also resulted in villus atrophy observed under light microscope of H&E stained slides, which led to increased intestinal permeability. G-CSF treatment resulted in increased villus height and returned WBC counts to normal levels [29].

12. Radiation injury

Radiation exposure in cancer patients damages the intestinal epithelium and thus can hamper intestinal barrier function [30].

Disruption of the integrity of the intestinal barrier was observed in rat ileum following abdominal X-irradiation, depending on the post irradiation time and the delivered dose. The loss of barrier integrity was characterized by a disorganization of proteins of tight and adherent junctions as evidenced by immunohistochemical analyses of junctional proteins (ZO-1 and beta-catenin) observed by confocal microscope. A disorganization of the localization for ZO-1 and beta-catenin was also observed [31].

Epithelial cell damage was observed in the duodenum, jejunum, ileum and distal colon in paraffin and frozen section at 1, 6, 24, 96h, 1.5 and 3 months after a single dose of 25Gy administered percutaneously to the liver of rats. However, prolonged denudation of the villi together with destruction of the crypt lining was only observed in the ileum, resulting in deficient regeneration. In the colon, changes were minor. Radiation mucositis with granulocyte (MP0+) infiltration was seen from 1 to 24h in the duodenum and jejunum, when ED1+ macrophages, CD3+ T-lymphocytes, and CD34+ hematopoietic precursor cells were recruited, accompanied by an increase in the chemokines MCP-1, MIP-1α, MIP3α and Il-8. In the ileum, early granulocyte infiltration was delayed but continuous. Recruitment of macrophages and lymphocytes was deficient and induction of chemokines such as the adhesion molecules PECAM-1, ICAM-1 was lacking [32].

13. Others

Thirty percent total body surface area (TBSA) burn resulted in a significant increase in intestinal permeability. Burn injury resulted in a marked decrease in the levels of tight junction proteins occludin and ZO-1 at 6 and 24 h following burn analyzed by immunoblotting and immunohistochemistry and seen under confocal microscope [33].

Gut barrier dysfunction was evident in patients with multiple organ dysfunction (MODS). Breakdown and reorganization of occludin and ZO-1 away from tight junctions was found in all MODS patients analyzed by immunoblotting and immunofluorescence staining [34].

In animal model of cirrhosis of liver, ileal structure was altered by the presence of villous atrophy, lymphangiectasias and submucosal oedema as seen in H&E stained samples under light microscope [35].

Condition	Methods used	Findings	Author
Gender and age	Endoscopic biopsy, H&E stain, Computer-aided morphometric analysis.	Jejunal mucosal thickness was significantly reduced in elderly subjects above 60 years of age ($p<0.05$), especially in elderly females compared to the adults ($p<0.05$). ileal villi were significantly wider in elderly compared to adult subjects ($p<0.01$) and in male compared to female subjects ($p<0.05$).	Milićević Z
Under nutrition Diet with 75% protein and 50% vitamin restrictions to weanling, Wistar/NIN male rats for 20 weeks	Apoptosis observed by morphometry, Annexin V binding.	Significantly increased intestinal cell apoptosis.	Bodiga VL 2005
Diet moderately deficient in protein, fat, and minerals to C57BL/6 mice	H&E, immunohistochemistry eg. MTS and bromodeoxyuridine assays, annexin and 7-amino-actinomycin D staining.	Decreased villous height and crypt depth in the jejunum. Increased claudin-3 expression, decreased epithelial cell proliferation, increased epithelial cell apoptosis.	Ueno PM 2011
Cancer cachexia	Light microscopic observation of the H&E stained intestinal specimen of tumor-bearing mice.	Decreased villus height and contour length than in healthy mice ($P > 0.05$). Villus width and crypt depth showed substantial evidence of atrophy ($P < 0.05$).	Susan E 2000

Condition	Methods used	Findings	Author
Total parenteral nutrition	Ileal segments of rats stained with H&E and PAS staining and observed under light microscope.	Villous atrophy, fewer goblet cells, atrophy of Peyer's patches. Decreased luminal mucus gel.	Khan J 1999 Iiboshi Y 1994
High fat diet	Immunocytochemistry of intestinal cryosections of mice	Altered distribution such as substantial decreased staining of occludin and discontinuous signals for ZO-1	Suzuki T 2010
Medium chain triglycerides (MCTs)	Periodic acid Schiff and alcian blue stain. Immunohistochemistry using monoclonal mouse anti rat Ki-67 antigen, polyclonal rabbit anti single stranded DNA antibody.	Number of goblet cells measured was significantly higher in rats given MCTs as compared to rats given corn oil (5 g/kg per day) or chow for 2 weeks. Proliferating cells on the villi and the crypts in the small intestine, significantly greater in rats given MCTs than rats given corn oil or normal rats.	Iishi K 2009
Psychological stress	H&E and periodic acid Schiff stain of the microtome sections of the small intestinal segments of rats under light microscope. Microtome sections of the rat distal colon stained by H&E. Light microscopic observation of the ileum stained by H&E.	Villus height ($p<.005$), crypt depth ($p<0.00$), number of goblet cells in villus ($p<.0.00$) and crypt ($p<.0.03$) decreased significantly in jejunum as compared to control. Number of polymorphonuclear neutrophil was significantly higher in stress group as compared to control ($p<0.00$). Inflammatory cell infiltration in the lamina propria of stressed rats. Significantly more degranulated mast cells and eosinophils per villus section adjacent to the Peyer patches in noise exposed rats than in quiet rats. The mean width of villus laminar propria was significantly greater in noise rats than in quiet rats. Mucosal epithelial cells of noise rats were often separated, sometimes detaching from the basement membrane.	Saudi WSW 2009 Larauche M 2012 Baldwin AL 2006

Condition	Methods used	Findings	Author
Diabetes mellitus	Transmission electron microscope.	Remarkable ultra-structural changes in height and thickness of microvilli, space between microvilli and thickness of tight junctions.	Secondulfo M 2004
Surgery	Electron microscope.	Samples of the laboratory animals underwent intestinal surgery reported lower expression of occludin.	Zhang MM 2009
Infection	Electron microscope.	Enteroaggregative Escherichia coli strains caused total or partial villi destruction, vacuolization of basal cytoplasm of the enterocytes, epithelium detachment, derangement of the structure and epithelial cell extrusion in ileal mucosa of laboratory animals.	Andrade JA 2010
Inflammatory bowel disease	Hematoxylin-eosin and studied under light microscope. Immunohistochemistry. Electron microscope	Raised IEL count (> 20 per 100 enterocytes) and marked villous atrophy of duodenal biopsy. Reduced expression of ZO-1 in UC and CD patients. Pentalaminar structure of tight junction (TJs) was destroyed in patients with CD and UC	Dewar DH 2012 Das P 2012
Alcohol	Immunofluorescent antibody labeling of ZO-1 proteins.	Ethanol (0, 1, 2.5, 5, 7.5, and 10%) produced a progressive disruption of TJ protein (ZO-1) with separation of ZO-1 proteins from the cellular junctions and formation of large gaps between the adjacent cells of Caco-2 cell line.	Ma TY 1999
Drugs	Immunofluorescence analysis. H&E and PAS staining of ileum, observed under light microscope. Immunostaining.	HT-29 cell line showed a fragmented and granulous ZO-1 staining, after aspirin treatment. Prolonged Methamphatamine abuse in rats decreased the villi height and increased the number of goblet cells significantly as compared to the control group of rats. Reduction of ZO-1 along the apical membrane of intestinal villi was observed in methotraxate treatment in rats.	Montalto M 2004 Khan J 2008 Hamada K 2010

Condition	Methods used	Findings	Author
	Light microscope observation after Terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate (dUTP)-biotin end labeling assay (TUNEL) and hematoxylin and eosin (H&E) staining.	Paclitaxel, a new chemotherapeutic agent induced apoptosis in 12.5% of jejunum villus cells. Apoptosis in the control group was 0.6%. also resulted in villus atrophy which led to increased intestinal permeability. Granulocyte colony-stimulating factor (G-CSF) treatment resulted in increased villus height and returned WBC counts to normal levels.	Zhang C 2011
Radiation injury	Immunohistochemical analyses of junctional proteins (ZO-1 and beta-catenin) and confocal microscopy. Light microscope observation.	Disorganization of the proteins of tight and adherent junctions. A disorganization of the localization for ZO-1 and beta-catenin was also observed. Prolonged denudation of the villi together with destruction of the crypt lining observed in the ileum. In the colon, changes were minor. Radiation mucositis with granulocyte (MP0+) infiltration was seen from 1 to 24h in the duodenum and jejunum, when ED1+ macrophages, CD3+ T-lymphocytes, and CD34+ hematopoietic precursor cells were recruited.	Dublineau I 2004 Cameron S 2011
Burn	Immunohistochemistry.	Marked decrease in the levels of tight junction proteins occludin and ZO-1 at 6 and 24 h.	Costantini TW 2009
Multiple organ dysfunction	Immunoblotting and immunofluorescence staining.	Breakdown and reorganization of occludin and ZO-1 away from tight junctions was found in all MODS patients.	Zhang JB 2010
Cirrhosis of liver	Light microscope.	In animal model of cirrhosis of liver, ileal structure was altered by the presence of villous atrophy, lymphangiectasias and submucosal oedema.	V Lorenzo Zúñiga 2006

Table 1. Summary of the morphological findings in different conditions affecting gastrointestinal barrier.

Although still at conceptual level, evidences are persuasive that use of the certain compounds, such as zinc, glutamine, probiotics etc has the potential to attenuate morphological changes by the above factors and might represent a simple device to prevent the occurrence or aggravation of chronic pathologies caused by intestinal barrier dysfunction.

14. Glutamine

Glutamine is an amino acid important for the growth of enterocytes. Electron microscopy of the intestine in a GLN-deprived infant rat model demonstrated intestinal intercellular junction breakdown [36].

Deprivation of GLN decreased claudin-1, occludin, and ZO-1 protein expression and caused a disappearance of perijunctional claudin-1 and a reduction of occludin but had no effect on ZO-1. Transmission electron microscopy revealed that methionine sulfoximine-treated cells in the absence of GLN formed irregular junctional complexes between the apical lateral margins of adjoining cells. These findings indicate that TJ protein expression and cellular localization in Caco-2 cell monolayers rely on GLN [37].

15. Zinc

Trace elements such as zinc may assist in the maintenance of intestinal barrier integrity. Caco-2 cells grown in zinc-deficient media had reduced TEER and altered expression of ZO-1 and occludin ie. localized away from the cell boundaries and less homogenicity as compared with the Caco-2 cells grown in zinc-replete media. This findings were accompanied by disorganization of F-actin filaments [38].

Electron microscopic studies showed that percentage of the disrupted (opened) tight junctions in experimental colitis were reduced by 50% with zinc supplementation [39].

16. Vitamin A

Retinoic acid (RA), the active form of vitamin A had significant trophic effects in resected and sham-resected rats. Exogenous RA stimulated the adaptive response of the intestine in 70% small bowel resection by 2 weeks, as manifested by a significant increase in crypt depth, villus height, and intestinal surface area of rats. The enlarged crypts and villi were due to adaptive hyperplasia and not to cellular hypertrophy. RA was also trophic in the intestine of control rats that were only subjected to transection and reanastomosis. Villus heights and crypt depths were measured in 20–50 well-oriented hematoxylin-eosin-stained crypt-villus units with the aid of a slide micrometer and Scion Image software. Apoptotic cells were identified by standard morphological changes, including nuclear condensation, perinuclear clearing, and cell shrinkage, and by staining for activated caspase-3 [40].

17. Arginine

Arginine is a dibasic amino acid with various metabolic and immunologic effects. Animal models of intestinal obstruction, treated with arginine presented preservation of the tissue structure. The villous epithelium was preserved and only discrete edema and enlargement were present at lamina propria [24].

18. Probiotic

Light microscope observation of Giemsa staining samples revealed that there was close interaction between luminal bacteria and the apical aspect of surface ileal enterocytes in rats subjected to WAS. Bacterial interactions with ileal enterocytes were not observed in sham stressed animals. Pretreatment with probiotics prevented the bacteria epithelial cell contacts induced by WAS.

TEM confirmed the findings demonstrated with light microscopy. While there were no bacteria adhering to the apical surface of enterocytes in sham stressed rats, multiple bacteria were observed closely adhering and internalised into ileal enterocytes in stressed rats. Electron dense condensation around the internalised bacteria consistent with polymerised actin was observed indicating that enterocytes underwent cytoskeletal rearrangements. Pretreatment with probiotics prevented WAS induced bacteria epithelial cell interactions [41].

19. Prebiotic

Prebiotic treated mice exhibited a decreased hepatic expression of inflammatory and oxidative stress markers. This decreased inflammatory tone was associated with a lower intestinal permeability and improved tight-junction ZO-1 and occludin integrity evidenced by qPCR and immunohistochemistry, compared to controls [9] .

Both fibre sources, wheat bran (rich in cellulose and hemicellulose) and pollen from Chinese Masson pine (Pinus massoniana) (rich in lignin) increased villus height of mucosa in jejunum (+10% on average) and ileum (+16% on average) in animal model of 48 weaned piglets [42].

Diet rich in arachidonic and docosahexaenoic acids, galacto- and fructo-oligosaccharides and Lactobacillus paracasei NCC2461 resulted in increased villus length in the small intestine to restore impaired intestinal barrier function and growth after neonatal stress in rats [43].

20. Flavonoid

Quercetin is the most common flavonoid in nature. High amounts of quercetin are found in onions, kale, and apples [44].

Flavonoids, quercetin and myricetin, enhanced barrier function in human intestinal Caco-2 cells. Suzuki and Hara recently reported that a 48-h exposure of quercetin enhanceds the

intestinal barrier function through increasing claudin-4 expression in human intestinal Caco-2 monolayers. Quercetin promoted the assembly of TJ proteins, ZO-2, occludin, and claudin-1 and the expression of claudin-4 by inhibiting the PKCδ isoform [45].

Kaempferol, a natural flavonoid present in fruits, vegetables, and teas, provides beneficial effects for human health. Confocal microscopy showed kaempferol-induced assembly of occludin and claudin-3 occurred at the TJ of Caco -2 cells at 6 h postadministration [46].

Condition	Methods used	Findings	Author
Deprivation of Glutamine	Electron microscope observation	Decreased claudin-1, occludin, and ZO-1 protein expression and caused a disappearance of perijunctional claudin-1 and a reduction of occludin	Potsic B 2000
Zinc supplementation	Electron microscope observation	Percentage of the disrupted (opened) tight junctions in experimental colitis were reduced by 50% with zinc.	Sturniolo GC, 2002
Zinc-deficient media		Caco-2 cells have reduced TEER and altered expression of ZO-1 and occludin ie. localized away from the cell boundaries and less homogenous compared with Caco-2 cells grown in zinc-replete media	Finamore A 2008
Vitamin A	Light microscope, H&E and staining for activated caspase-3	Significant increase in crypt depth, villus height, and intestinal surface area of 70% bowel resection in rats.	Wang L 2007
Arginine	Light microscope	Animal models of intestinal obstruction treated with arginine presented preservation of the tissue structure. The villous epithelium was preserved and only	Viana ML 2010

Condition	Methods used	Findings	Author
		discrete edema and enlargement were present at lamina propria.	
Probiotics	Light microscope observation of Giemsa stained sample	Prevented WAS induced close interaction between luminal bacteria and the apical aspect of surface ileal enterocytes in rats.	M Zareie 2007
Prebiotics	qPCR and immunohistochemistry	Improved tight-junction ZO-1 and occludin	Cani PD 2009
Wheat bran and pollen from Chinese Masson pine	Light microscope observation of H&E stained samples	Increased villus height of mucosa in jejunum (+10% on average) and ileum (+16% on average) in animal model of 48 weaned piglets	Schedle K 2008
Diet containing arachidonic and docosahexaenoic acids, galacto- and fructo-oligosaccharides and Lactobacillus paracasei NCC2461	Light microscope observation	Increased villus length in small intestine after neonatal stress in rats.	García-Ródenas CL 2006
Quercetin	Confocal microscope observation	Promotes the assembly of TJ proteins, ZO-2, occludin, and claudin-1 and the expression of claudin-4	Suzuki T 2009
Kaempferol	Confocal microscope observation	Assembly of occludin and claudin-3 at the TJ of Caco -2 cells	Suzuki T 2011

Table 2. Summary of the histological findings of different modulating agents improving gastrointestinal barrier.

21. Conclusion

Emerging experimental evidences from animal models suggest that altered barrier function is a potential pathway for intestinal and extra intestinal inflammation. Although thousands of research findings are available dealing with gut barrier function during different physiological and pathological conditions, few articles focused on the histolological changes. In this chapter discussion was made in an attempt to provide a generalized idea of

morphological changes during several conditions. Future researches are suggested to deal with the effect of different modulating agents on the histolological parameters of intestinal barrier. Therapeutic restoration of barrier function could improve pathophysiology and clinical outcomes of different diseases.

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