We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900
Open access books available

186,000
International authors and editors

200M
Downloads

154
Countries delivered to

Our authors are among the

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS

BOOK CITATION INDEX

INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Mucosal Expression of Claudins in Celiac Disease

Dorottya Nagy-Szakál[1], Hajnalka Győrffy[2], Katalin Eszter Müller[1],
Kriszta Molnár[1], Ádám Vannay[1,3], Erna Sziksz[1,3], Beáta Szebeni[1,3],
Mária Papp[4], András Arató[1] and Gábor Veres[1]
[1]First Department of Pediatrics, Semmelweis University, Budapest,
[2]Second Department of Pathology, Semmelweis University, Budapest,
[3]Research Group for Pediatrics and Nephrology, Semmelweis University
and Hungarian Academy of Sciences, Budapest,
[4]Department of Medicine, University of Debrecen,
Hungary

1. Introduction

Celiac disease is an autoimmune gluten-sensitive enteropathy or nontropical sprue occurring in genetically susceptible individuals, triggered by dietary gluten and related prolamins, which damage small intestine and interfere with absorption of nutrients. Tight junctions play an important role in the pathomechanism of different gastrointestinal diseases. Claudins, the main tight junction proteins are found in the monolayer of the gastrointestinal epithelium (Bornholdt et al., 2011). The presence and distribution of claudin depend on the organs and the function of the tissues (Gonzales-Mariscal et al., 2003). The expression levels of various claudins correlate to the distinct physiological and pathological conditions. Claudins modulate the permeability of the epithelial barrier (Bornholdt et al., 2011). Surprisingly, there is only one study analyzing different claudins at protein level of intestinal biopsies in patients with celiac disease. At first, general information of tight junctions and the characteristics of claudins in different gastrointestinal disorders will be highlighted for a better understanding of the role of claudins in celiac disease.

2. Characteristics of tight junctions

Intercellular junctions are presented in multicellular organism as linking cells and maintaining barrier function between the two sides of cell layer (Staehelin et al., 1974). It plays a structural role in maintaining biological compartments, cell polarity, and a barrier function separating the internal and external environments (Krause et al., 2008). It also controls the paracellular transport (Balda et al., 1996). The barrier and fence function are dynamically changing and guide cell behavior. Three major types of intercellular junctions are the zonula occludens (tight junction), the zonula adherens (adherens junction) and the macula adherens (desmosome). The tight junction is an intercellular junction by interlinked rows of integral membrane proteins limiting the intercellular transport. One of the most important components of tight junction is claudin (Figure 1).
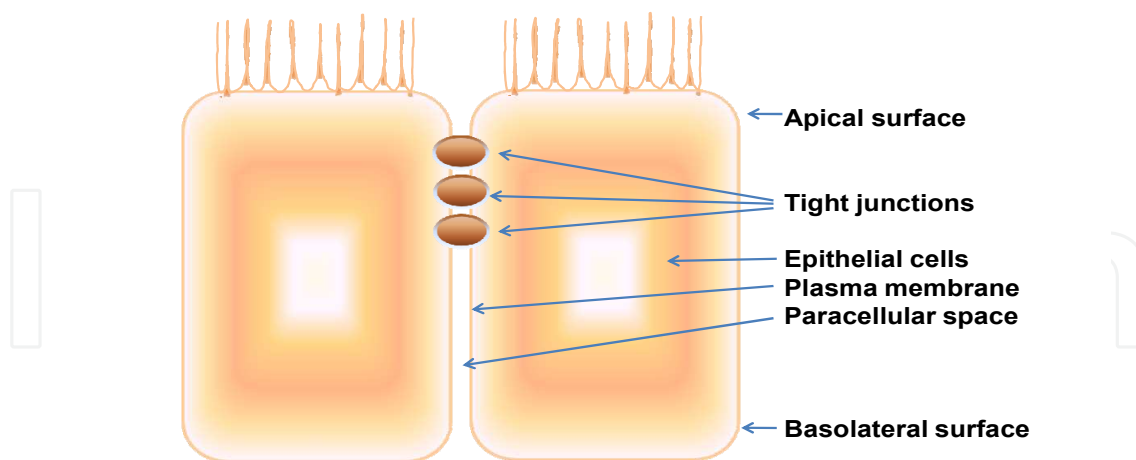
Fig. 1. Schematic structure of tight junctions.

The adherent junction links cell membranes and cytoskeletal elements connecting cells mechanically. The gap junction containing channels regulates trespassing of ions and microelements through the cell layer. Tight junction, as the most apical component of intercellular junctional complexes in basolateral spaces, constitutes the barrier between cells and has a fundamental function to separate different compartments within the organism (Farquhar et al., 1963). Tight junctions were first described in epithelia and endothelia (Stevenson et al., 1988). However, recent studies suggest that they are also found in myelinated cells. There are more than 40 different tight junction proteins in epithelia or endothelia (Gonzalez-Mariscal et al., 2003). Tight junctions have a complex structure – cortical or transmembrane protein -, and form a continuous, circumferential belt separating apical and basolateral plasma membrane domains. Tight junctions play a role not only in the maintenance of paracellular transport, but also in the cell growth and differentiation via signaling cascades. Altered tight junction structures and ratios present distinct permeability in different tissues and have a dynamic capacity responding to the altered environmental conditions. Furthermore, extracellular stimuli, such as cytokines and growth factors, also affect the distribution of tight junctions (Steed et al., 2010). Interferon-gamma, tumor necrosis factor-alpha, insulinlike growth factor-I and insulinlike growth factor -II, vascular endothelial growth factor, interleukin-1, interleukin -4, interleukin -13, and hepatocyte growth factor decrease the barrier function. Adverse effect (increased or protected barrier function) is known by transforming growth factor-beta, epidermal growth factor, interleukin-10 and interleukin-17 (Dignass et al., 1993).

Tight junctions are integral components of cells and the disturbance of the barrier function can lead to diseases (Sawada et al., 2003). The loss of fence function (decreased cell polarity) is known in cancer cells and oncogenic papillomavirus infection (Tobioka et al., 2002; Glaunsinger et al., 2000). The defect of barrier function and consequential deficiency of paracellular transport can affect the vascular system (edema, endotoxinemia, cytokinemia, blood-borne metastasis), liver (jaundice, primary biliary cirrhosis, primary sclerosing cholangitis), respiratory tract (asthma), and hereditary diseases (hypomagnesaemia,

deafness, cystic fibrosis) (Sawada et al., 2003; Forster et al., 2008; Furuse et al., 2009). The gastrointestinal tract can be affected and the deterioration of tight junctions is responsible, at least in part, for the increased permeability in patients with bacterial gastritis, pseudomembranous or collagenous colitis, Crohn's disease, ulcerative colitis, and celiac disease (Schulzke et al., 2000, 2009).

Integral proteins, such as occludins, claudins and junctional adhesion molecules, constitute the tight junctions, and responsible together for the maintenance of barrier function. Occludin was identified as the first integral membrane protein (Furuse et al., 1993). It appears to interact with claudins and form long tight junction strands. Its overexpression increases transepithelial resistance and affects the polarization and diffusion through the membrane. Claudins are the most important components of the backbone tight junction strain (Furue et al., 1998). In this chapter, claudins and their role in different gastrointestinal diseases will be highlighted.

3. Characteristics of claudins

As an integral component of tight junctions, claudins play a central role in the regulation of cell-cell adhesion, cell polarity and transportation of paracellular ion, water, and molecules (Gonzalez-Mariscal et al., 2003). Twenty-four subgroups are known (Table 1). In general, claudin genes contain only some introns and several lack introns altogether. All claudin genes are typically small and their sequences are similar to each other. Some claudins are located close to each other in the human genome (Lal-Nag et al., 2009). For instance, claudin22 and -24 is located on chromosome 4, claudin3 and -4 on chromosome 7, claudin6 and -9 on chromosome 16, and claudin8 and -17 on chromosome 21 (Gupta IR et al., 2010). Their close proximity results simultaneous regulation and expression following different responses. The others are located on different chromosomes giving them a slightly different regulation and properties. All claudins encode 20-27 kDa proteins with four transmembrane domains and two extracellular loops where the first one is significantly longer (around 60 residues) than the second one (24 residues) (Krause et al., 2008). The first loop contains charged amino acids influencing paracellular charge selectivity. The highly conserved cysteine residues are present increased protein stability as formation of intermolecular disulfide bond. The second loop is responsible for confirmation through hydrophobic interactions. The short intracellular cytoplasmatic amino-terminal sequence (4 to 5 residues) is more conserved than the short intracellular carboxyl tail (Figure 2). The latter comprises a PZD-domain-binding motif (Guillemot et al, 2008). This part of claudins interacts directly with the tight junction-associated proteins, and determines the stability and function of proteins. Although claudins are known as the main component of the apical tight junctions, claudin can be localized in the cytoplasm as well (Acharya et al., 2004). The role of cytoplasm claudin is concluded in cell-matrix interactions and vesicle trafficking. Claudins appear to be expressed in a tissue-specific behavior. Variations in the tightness of the tight junction appear to be determined by the combination and mixing ratios of different claudins. Different tissues have altered claudin profile, and it can be also changed by abnormal conditions. Claudins have a crucial role in the regulation of the selectivity of paracellular permeability; and their lack or overexpression can influence these changes (permeability and resistance). The nephron is a representative model of illustration the different functions of claudin (Li et al., 2004). The renal epithelia contain mostly all of the

subgroups of claudins according to the function of different areas of the nephron. Although claudins are expressed in all epithelial and endothelial tissues, mutations are frequently associated with diseases of the kidney, the skin and the ear.

CLAUDINS	CHARACTERISTICS, EXPRESSION IN DIFFERENT TISSUES (INCREASED ↑ OR DECREASED ↓)
CLDN1 'tight' epithelia	Renal epithelia (collecting segment and proximal tubule), Epidermal barrier, Gallbladder, Ovarium, Inner ear, Brain capillary endothelium Breast cancer cell lines↓, Squamous cell cancer↓, Glioblastoma↓, Prostate AC↓
CLDN2 'leaky' epithelia	Renal epithelia (collecting segment and proximal tubule), Choroids plexus epithelium, Ovarium surface epithelium, Inner ear. Crohn's disease↑
CLDN3 RVP1	Capable of CPE binding Tighter segment of nephron, Gallbladder, Inner ear, Brain capillary endothelium, Liver and intestinal epithelial cells. Prostate AC↑, Ovarian CC↑, Colorectal CC↑, Breast CC↑, Glioblastoma↓, Encephalomyelitis↓
CLDN4 CPE-R	Selective CPE binding Tighter segment of nephron, Gallbladder. Pancreatic CC↑, Prostate AC↑, Ovarian CC↑, Colorectal CC↑, Breast CC↑
CLDN5	Endothelial cells (e.g. brain), Ovarium surface epithelium, Colon epithelium, Retinal pigment epithelium during development. Glioblastoma↓, Cardiofacial syndrome↓, Crohn's disease↓, Pancreatic CC↑
CLDN6	Embryonic epithelia
CLDN7	Gastrointestinal tract, Tonsillar epithelium Head and neck squamous cell carcinoma↓, Stomach CC↑
CLDN8	Tighter segment of nephron, Gastrointestinal tract Crohn's disease↓
CLDN9	Inner ear, Neonatal kidney
CLDN10	Inner ear, Most segments of nephron
CLDN11 OSP	Oligodendrocytes, Sertolli cells
CLDN12	Inner ear, Brain endothelial cells, Gastrointestinal tract
CLDN13	Gastrointestinal tract, Neonatal kidney
CLDN14	Sensory epithelium (organ of Corti), Inner ear Nonsyndromic deafness↓
CLDN15	Kidney and Gastrointestinal tract endothelial cells
CLDN16 Paracellin-1	Thick ascending limb of Henle (Mg^{2+} and Ca^{2+} resorption) Hypomagnesaemia, Hypercalciuria, nephrocalcinosis
CLDN17	Kidney, Taste receptor cells
CLDN18	Lung and stomach, Inner ear Gastric CC↓
CLDN19	Kidney, Retina, Myelinated peripheral neurons, Schwann cells
CLDN20	mRNA in skin
CLDN21	Human DNA sequence
CLDN22	mRNA in trachea
CLDN23	mRNA in colon, stomach, placenta, skin
CLDN24	Human DNA sequence

Table 1. The characteristics and altered expression of claudins in different human tissues and cancers. Claudins were mostly investigated in the renal epithelium where the claudin pattern and the subsequent changes of permeability are easily followed by. (Abbreviations: CLDN: Claudin, AC: Adenocarcinoma, CC: Carcinoma, RVP: Rat Ventral Prostate, CPE: Clostridium Perfringens Enterotoxin, CPE-R: Clostridium Perfringens Enterotoxin Receptor, OSP: Oligodendrocyte Specific Protein)
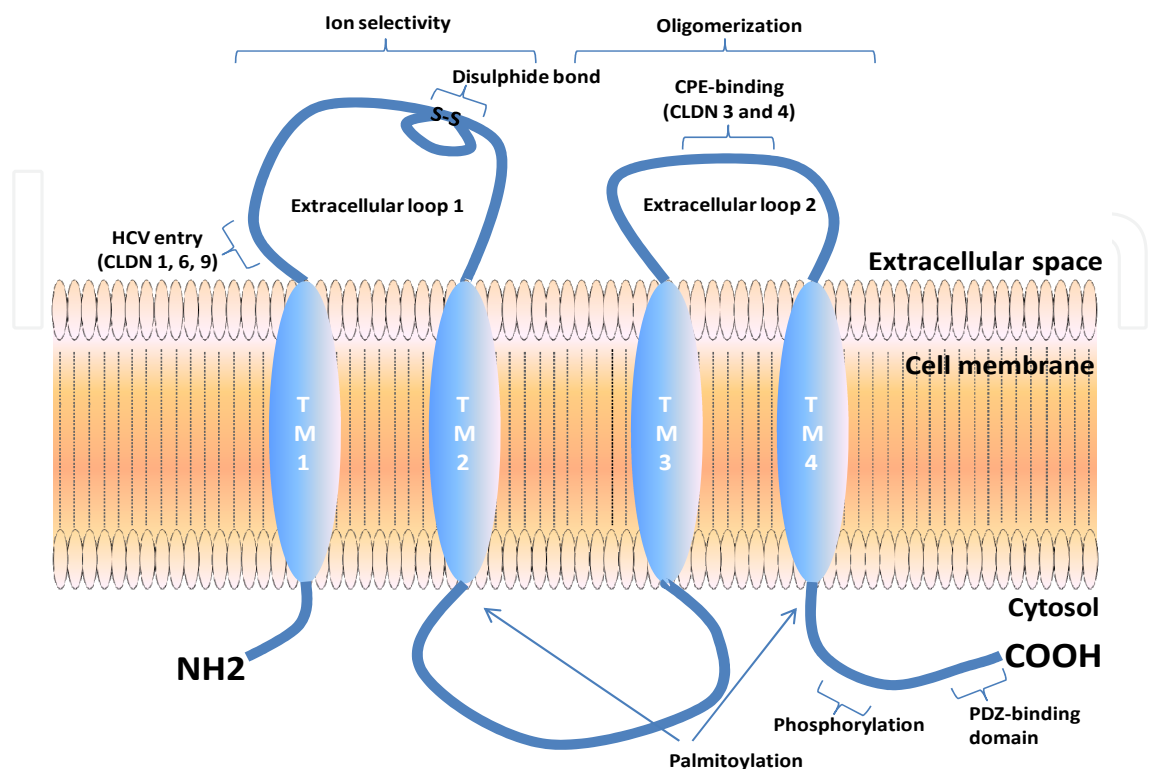
Fig. 2. Schematic structure of claudin.

3.1 Claudins and tumour of the gastrointestinal tract

Altered claudin expression is associated with different disorders of the intestine (Table 2).

Gastrointestinal tract	Disorders and altered CLDN pattern (increased↑ or decreased↓ expression)
Esophagus	Barrett's epithelia: CLDN2 and -3 ↑ Adenocarcinoma: CLDN2 and -3 ↑
Stomach	Gastric metaplasia CLDN2,-3 and -4 ↑
Duodenum, Ileum, Jejunum	Adenocarcinoma: CLDN2 ↑ GIST: CLDN2,-3, -4, -5 and -7 ↑ Angiosarcoma: CLDN2 and -5 ↑ Hemangioma: CLDN2 ↑ Leiomyoma: CLDN2 ↑ Leiomyosarcoma: CLDN1, -2, -3, -4, -5 and 7 ↑ Celiac disease: CLDN2 and -3 ↑ Gluten-intolerance: CLDN4 ↑
Colon	Adenocarcinoma: CLDN2 ↑ IBD: CLDN2 ↑ and CLDN3, -4, -5 and -8 ↓

Table 2. Claudin expression in the gastrointestinal tract in different disorders.
(Abbreviations: CLDN: Claudin, GIST: gastrointestinal stromal tumour, IBD: inflammatory bowel disease)

Since the damage of the cell-cell adhesion is an important role in the carcinogenesis, several papers have studied changes of claudins during tumor development and progression. All claudins were found in gastrointestinal carcinomas, and their expression was tumour-specific. The Barrett's metaplasia of the esophagus requests attention for its precancerous behaviour (Thomson et al., 1983). Claudin2 and -3 expressions in Barrett's esophagus were higher compared to the normal foveolar epithelium. The esophageal adenocarcinoma showed higher claudin2 and -3 expression compared with normal and Barrett's epithelia. The similar claudin expression profile of Barrett's esophagus and adenocarcinoma supports their sequential development (Győrffy et al., 2005). The low expression of the claudin4 is associated with the poor prognosis in the most common tumour of the esophagus, squamous cell carcinoma (Sung et al., 2011). Gastric intestinal metaplasia showed higher expression of claudin2, -3 and -4 as compared with normal antral foveolar mucosa (Győrffy, 2009). Gastric adenocarcinoma expresses various claudin. Lower expression of claudin1 is common in the intestinal type of gastric adenocarcinoma according to Lauren classification (Jung et al., 2011). Claudin3 and -4 overexpression prevents the lymphatic invasion (Jung et al., 2011), but the overexpression of the claudin6, -7 and -9 increases the invasiveness of tumour cells in experimental model (Zavala-Zendejas, 2011). Claudin4 is a good general prognostic marker in the gastric adenocarcinoma (Jung et al., 2011). Autoantibodies against claudin18 prevent the development of the lung metastasis (Klamp et al., 2011). Tumours of small and large bowels exhibited higher claudin2 expression compared to normal epithelia (Győrffy, 2009). Decreased claudin4 expression correlates with the invasiveness and metastasis (Ueda et al., 2007). In addition, claudin18 overexpression is associated with poor prognosis of the colorectal cancer (Matsuda et al., 2010). However, colorectal adenoma and adenocarcinoma could not be differentiated according to their claudin profile (Győrffy, 2009).

3.2 Claudins and inflammatory bowel disease

Beside the neoplastic or precancerous lesions, some of the inflammatory processes show alteration of the tight junctions. In inflammatory bowel diseases, including Crohn's disease and ulcerative colitis, the intestinal barrier function is impaired due to deterioration in the structure of the epithelial tight junction. Claudin, as a key component of tight junction, might play an important role in the pathogenesis of inflammatory bowel diseases. In addition, tumour necrosis factor in inflammatory bowel diseases is upregulated, which induces barrier defects and is associated with the induction of claudin2 expression. Increased expression of claudin2 is detected along the inflamed crypt epithelium, whilst absent or barely detectable in normal colon (Weber et al., 2010). This higher expression of channel-forming claudin2 can cause reduced epithelial barrier in inflammatory bowel diseases (Suzuki et al., 2011). In the inflamed colonic mucosa of patients with ulcerative colitis, the protein expression of claudin1 was increased compared to non-inflamed ulcerative colitis colon and normal colon (Poritz et al., 2011). In addition, the higher expressions of claudin1 and -2 correlated positively with inflammatory activity of inflammatory bowel diseases and this increased expression may be involved at early stages of transformation in inflammatory bowel diseases -associated neoplasia (Weber et al., 2008). In experimental model of colitis in rats, significant decrease of claudin2, -12, -15 levels were detected in the colonic mucosa after dextrane-sodium sulphate induces colitis (Arimura et al., 2011). In contrast, some members of the claudin family such as claudin3 and -4 were

present throughout normal colonic epithelium and were reduced or redistributed in the inflamed surface epithelium (Prasad et al., 2005). Food components can strengthen the epithelial barrier as for example the flavonoid quercetin. Quercetin has been shown to upregulate claudin4 within the epithelial tight junction. This might be a therapeutic option in inflammatory bowel diseases patients to rebuild the tight junction complex (Hering et al., 2009).

3.3 Claudins and intestinal infections

Claudins may serve as cell surface receptors for epithelial pathogens. Intestinal pathogens such as Vibrio cholerae, Salmonella, E. coli, Shigella, Giardia lamblia, and Rotavirus were found to directly alter tight junction permeability. Claudin3 and -4 have been shown to act as a receptor for C. perfringens enterotoxin (Katahira et al., 1997). Rotavirus infection of Caco-2 intestinal cells altered distribution of claudin1 and other tight junction proteins (Dickmann et al., 2000). In the pathogenesis of Helicobacter pylori infection, disruption of the tight junction implicated host cell signaling pathways including the dysregulation of claudin4 and -5 was observed (Fedwick et al., 2005). Moreover, claudin1, -6, and -9 are coreceptors for cellular entry of hepatitis C virus (Angelow et al., 2008). The importance of intestinal barrier function in the pathogenesis of necrotizing enterocolitis has been suggested in a rat model, where necrotizing enterocolitis was associated with increased claudin3 mRNA levels in both jejunum and ileum (Clark et al., 2006).

3.4 Claudins in food allergy, obstructive jaundice and obesity

In food allergy, mast cells are classically associated with allergen-induced immunoglobulin E mediated responses. Concerning our topic, mast cell deficient mice-model demonstrated dysregulation of claudin3 expression (Gorschwitz et al., 2009). Furthermore, claudin1 expression was elevated in the small intestine in patients with food allergy (Pizzuti et al., 2011). Experimental and clinical studies have shown that there is an increased intestinal permeability permitting the escape of endotoxin from gut lumen in patients with obstructive jaundice. In these subjects, claudin1 and -7 were significantly decreased whereas claudin4 expression was increased. This pattern may be a key factor contributing to the disintegration of mucosal barrier (Assimakopoulos et al., 2011). Recently, obesity and diabetes have been characterized by low-grade chronic systemic inflammation. According to a novel hypothesis, this systemic inflammation is closely linked to the plasma endotoxemia due to increased intestinal permeability in obese animals (Cani et al., 2008). It is of interest, that excessive dietary fat increased small intestinal permeability resulting from the suppression of tight junction protein expression. Claudin1 and -3 were influenced by diet.

4. Tight junctions and its effect on intestine in celiac disease

4.1 Celiac disease and intestinal barrier function

Deterioration of intestinal barrier function is one of the most important steps in the pathomechanism of celiac disease (Sapone et al, 2011). According to functional, structural and molecular analyses, intercellular junctions between epithelial cells are abnormal in the gut of patients with celiac disease (Madara et al, 1987; Poritz et al, 2011). Decreased intestinal barrier function leads to a continuous abnormal passage of antigens through the

epithelial layer. The main antigen of gluten in wheat, the gliadin can regulate cell activation, especially inhibits cell development and induces apoptosis. Gliadin almost immediately can change the barrier function of the intestinal mucosa inducing a reorganization of actin filaments and altered expression of different tight junction proteins (Drago et al, 2006; Fasano et al., 2000). In a human Caco-2 intestinal epithelial cell-model, gliadin altered barrier function almost immediately by decreasing transepithelial resistance and increasing permeability to small molecules (4 kDa). In addition, gliadin induced a reorganisation of actin filaments and altered expression of the tight junction proteins occludin, claudin3 and -4, the tight junction-associated protein zonula occludens-1 and the adherens junction protein E-cadherin (Sander et al., 2005). The activation of T helper 1 and T helper 17 cells results tissue damage and disrupts barrier function. Namely, expression of interleukin-17A, interferon-gamma and interleukin-6 is enhanced and leads to increased immune reaction and promotes differentiation. On the other hand, reduced function of adaptive immunity is also detected. Decreased regulatory T cells in the epithelial mucosa are related to disturbed adaptive capacity. In addition, upregulation of regulatory T cell markers (like FoxP3 and tranforming growth factor-beta) was reported which phenomenon may be explained as a secondary compensatory response to injury.

4.2. Claudins and the gut microbiota

The intestinal epithelium is one of the most immunologically active surfaces in the body. Beside the barrier function, immunological reactions against food antigens and toxins are involved in the maintenance of healthy gut status. However, inappropriate increase of the immune response can lead to decreasing tolerance and intestinal immune disorders (e.g. celiac disease). The commensal bacteria and their dynamic interaction of the host gut play an essential role in the preservation of gut homeostasis. Intestinal flora is involved in the regulation of gut intestinal epithelial cells, maintenance of barrier function, and also in the restitution and reformation (stabilization) of tight junctions (Yu et al., 2012).

Highlighting the importance of claudins, recent studies suggested that invasive bacterial pathogens (e.g. *Streptococcus pneumonia* and *Haemophilus influenza*) decrease the CLDN7 and -10 expression via TLR-dependent pathway leading decreased integrity of the epithelial cells (Clarke et al., 2011). This mechanism due to epithelial opening and bacterial translocation through the epithelial layer leads increased permeability and bacterial invasion.

Recent studies suggested that the altered intestinal microbiota plays a role in the development of different disorders such as celiac disease, inflammatory bowel diseases and irritable bowel syndrome. In celiac disease, rodent studies suggest that gut microbiota influences mucosal integrity and plays a role in the early pathogenesis of CD (Cinova et al., 2011). In human celiac disease, intestinal flora may be a key component switching oral tolerance to immune response against gliadin (Ray et al., 2012). In celiac disease, intestinal dysbiosis, along with increased Gram-negative bacteria and decreased Bifidobacteria was determined (Sanz et al., 2011). Infants who developed CD later in life had an unstable and immature microbiome with decreased abundance of the phylum Bacteriodetes along with high amount of Firmicutes compared to healthy individuals (Sellitto et al., 2012). The metabolomic analysis reveals increased lactate in CD which may be a predicting factor of CD.

5. Claudins and celiac disease

5.1. Claudin-protein level in patients with celiac disease

To the best of our knowledge, there is only one study to address the question to determine the expression of different claudin proteins in patients with celiac disease. The aim of this prospective study was to compare claudin2, -3 and -4 expressions in proximal and distal part of duodenum in children with celiac disease and in controls (Szakal et al., 2010). Biopsy samples from the proximal and distal part of duodenum were taken from newly diagnosed children with celiac disease. The villous/crypt ratio and the percentage of lymphocytes in the intraepithelial region using monoclonal CD3 antibodies were determined (Marsh scoring). The expression pattern of claudins in the duodenal mucosa was investigated by immunohistochemistry. The monoclonal anti-claudin2 and -4 and the polyclonal anti-claudin3 antibodies were purified from rabbit antiserum. For visualization, biotinilated goat anti-rabbit secondary antibody and standard avidin-biotin peroxidase technique with diaminobenzidine were used as chromogen. The number of positive cells was calculated in the surface epithelium (with 100 enterocytes) on the top of the villi. Increased expression of claudin2 and -3 was detected in distal part of duodenal mucosa in pediatric patients with celiac disease compared to the proximal region and controls. It should be emphasized that claudin4 expression was comparable between the different groups studied (see later).

Moreover, there was an association between expression of claudin and the grade of atrophy. Changes in the composition and the overturn of the different types of claudin may lead to structural alteration of tight junctions. This phenomenon may be responsible for the increased permeability and the modified cell-to-cell adhesion in the pathomechanism of celiac disease. In addition, comparative substudy showed that both proximal and distal parts of duodenum are also reliable for taking biopsy sample to prove villous atrophy. However, using sensitive methods, the distal part of duodenum depicted earlier signs of mucosal deterioration. Histological scoring grade (Marsh classification), the percentage of CD3 positive T cells and the expression of different claudin showed slightly more severity in the distal part of duodenum compared to the bulbus duodeni.

5.2 Claudin-mRNA expression in celiac disease and gluten-sensitive disease

As described previously, celiac disease is an autoimmune enteropathy triggered by the ingestion of gluten. Gluten sensitive individuals cannot tolerate gluten and may develop gastrointestinal symptoms similar to those in celiac disease. However, in contrast to celiac disease, the overall clinical picture is generally less severe and is not accompanied by the elevation of tissue transglutaminase autoantibodies or autoimmune comorbidities (Sapone, 2011). In this study, innate and adaptive immunity in celiac disease were compared with gluten sensitivity. Intestinal permeability was evaluated using a lactulose and mannitol probe, and mucosal biopsy specimens were collected to study the expression of genes involved in barrier function and immunity. In contrast to celiac disease, gluten sensitivity was not associated with increased intestinal permeability. In fact, this was significantly reduced in gluten sensitive individuals compared to controls paralleled by significantly increased mRNA expression of claudin4. In comparison to controls, adaptive immunity markers interleukin-6 and interleukin-21 were significantly increased in celiac disease but not in gluten sensitivity, while expression of the innate immunity marker Toll-like receptor 2 was increased in gluten

sensitive individuals but not in celiac disease. In addition, expression of the T-regulatory cell marker FOXP3 was significantly reduced in gluten sensitive individuals relative to controls and celiac disease patients. Authors concluded, that the two gluten-associated disorders, celiac disease and gluten sensitivity, are different clinical entities, and it contributes to the characterization of gluten sensitivity as a condition associated with prevalent gluten-induced activation of innate, rather than adaptive immune responses. In addition, previous study conducted by Szakal et al showed no elevation of claudin4 in the intestinal mucosa of patients with celiac disease (Szakal, 2010). This finding strengthen the hypothesis of Sapone et al that claudin4 could be an important marker to differentiate between celiac disease and gluten sensitivity (Sapone, 2011). Further studies are necessary to characterize gluten sensitivity as a well-defined entity in the family of celiac-related disorders.

6. Conclusion and future remarks

Gluten-induced changes in the tight junction and the ratio of claudins influence immune processes and barrier mechanism underlying celiac disease pathogenesis. As sensitive methods, detection of claudins in the upper gastrointestinal tract may help to detect abnormalities in an early stage and provide information to determine the prognosis of celiac disease (Szakal et al., 2010; Prasad et al, 2005; Visser et al, 2009). Nevertheless, modification of claudins and tight junction might be therapeutic approach in the future. Furthermore, influence of tight junctions' regulation may be a novel approach of treatment in several diseases due to the fact that celiac disease may serve as a model for other autoimmune disorders. The advantage in celiac disease is that the causative agent (gluten) is well known compared to other autoimmune disorders such as in inflammatory bowel diseases. Development of agents making tight junctions close might be used as anti-inflammatory, anti-metastatic and anti-diarrhea drugs. In contrast, drugs opening tight junctions are new applications of treating tumors and help reaching closed compartment of the body (e.g. brain-blood barrier).

7. Acknowledgments

8. References

Acharya, P., Beckel, J., Ruiz, WG., Wang, E., Rojas, R., Birder, L., Apodaca, G. (2004). Distribution of the tight junction proteins ZO-1, occludin, and claudin-4, -8, and -12 in bladder epithelium. Am J Physiol Renal Physiol. 287(2):F305-18.

Angelow, S., Ahlstrom, R., & Yu, AS. (2008). Biology of claudins. *Am J Physiol Renal Physio,* 295 : F867-F876.

Arimura Y, Nagaishi K, & Hosokawa M. (2011). Dynamics of claudins expression in colitis and colitis-associated cancer in rat. *Methods Mol Biol.* 762:409-25.

Assimakopoulos, SF., Tsamandas, AC., Louvris, E., Vagianos, CE., Nikolopoulou, VN., Thomopoulos, KC., Charonis, A., & Scopa, CD. (2011). Intestinal epithelial cell proliferation, apoptosis and expression of tight junction proteins in patients with obstructive jaundice. *Eur J Clin Invest.* 41:117-125.

Balda, MS., Whitney, JA., Flores, C., Gonzalez, S., Cereijido, M., & Matter, K. (1996). Functional dissociation of paracellular permeability and transepithelial electrical resistance and disruption of the apical-basolateral intramembrane diffusion barrier by expression of a mutant tight junction membrane protein. *J Cell Biol*. 134(4):1031-49.

Bornholdt, J., Friis, S., Godiksen, S., Poulsen, SS., Santoni-Rugiu, E., Bisgaard, HC., Lothe, IM., Ikdahl, T., Tveit, KM., Johnson, E., Kure, EH., & Vogel LK. (2011). The level of claudin-7 is reduced as an early event in colorectal carcinogenesis. *BMC Cancer*. 11:65.

Cani, PD., Bibiloni, R., Knauf, C., Waget, A., Neyrinck, AM., Delzenne, NM., & Burcelin R. (2008). Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high fat diet induced obesity and diabetes in mice. *Diabetes*. 57:170-181.

Cinova, J., De Palma, G., Stepankova, R., Kofronova, O., Kverka, M., Sanz, Y., Tuckova, L. (2011). Role of intestinal bacteria in gliadin-induced changes in intestinal mucosa: study in germ-free rats. *PLoS One*. 6(1):e16169.

Clark, JA., Doelle, SM., Halpern, MD., Suanders, TA., Holubec, H., Dvorak, K., Boitano, SA., & Dvorak B. (2006). Intestinal barrier failure during experimental necrotizing enterocolitis: protective effect of EGF treatment. *Am J Physiol Gastrointest Liver Physiol*. 291;G938-G949.

Clarke ,TB., Francella, N., Huegel, A., Weiser, JN. (2011). Invasive bacterial pathogens exploit TLR-mediated downregulation of tight junction components to facilitate translocation across the epithelium. *Cell Host Microbe*. 9(5):404-14.

Dickmann, KG., Hempson, SJ., Anderson, J., Lippe, S., Zhao, L., Burakoff, R., & Shaw, RD. (2000). Rotavirus alters paracellular permeability and energy metabolism in Caco-2 cells. *Am J Physiol Gastrointest Liver Physiol*. 279:G757-G766.

Dignass, AU., & Podolsky DK. (1993). Cytokine modulation of intestinal epithelial cell restitution: central role of transforming growth factor beta. *Gastroenterology*. 105(5):1323-32.

Drago, S., El Asmar, R., Di Pierro, M., Grazia Clemente, M., Tripathi, A., Sapone, A., Thakar, M., Iacono, G., Carroccio, A., D'Agate, C., Not, T., Zampini, L., Catassi C., Fasano, A. (2006). Gliadin, zonulin and gut permeability: Effects on celiac and non-celiac intestinal mucosa and intestinal cell lines. *Scand J Gastroenterol*. 41(4):408-19.

Farquhar, MG., & Palade, GE. (1963). Junctional complexes in various epithelia. *J Cell Biol*. 17:375-412.

Fasano, A., Not, T., Wang, W., Uzzau, S., Berti, I., Tommasini, A., Goldblum, SE. (2000). Zonulin, a newly discovered modulator of intestinal permeability, and its expression in celiac disease. *Lancet*. 355(9214):1518-9.

Fedwick, JP., Lapointe, TK., Meddings, JB., Sherman, PM., & Buret, AG. (2005). Helicobacter pylori activates myosin light chain kinase to disrupt claudin-4 and claudin-5 and increase epithelial permeability. *Infection and immunity*. 7844-7852.

Forster, C. (2008). Tight junctions and the modulation of barrier function in disease. *Histochem Cell Biol*. 130(1):55-70.

Furuse, M., Fujita, K., Hiiragi, T., Fujimoto, K., & Tsukita S. (1998). Claudin-1 and -2: novel integral membrane proteins localizing at tight junctions with no sequence similarity to occludin. *J Cell Biol*. 141(7):1539-50.

Furuse, M., Hirase, T., Itoh, M., Nagafuchi, A., Yonemura, S., & Tsukita, S. (1993). Occludin: a novel integral membrane protein localizing at tight junctions. *J Cell Biol.* 123(6 Pt 2):1777-88.

Furuse, M. (2009). Knockout animals and natural mutations as experimental and diagnostic tool for studying tight junction functions in vivo. *Biochim Biophys Acta.* 1788(4):813-9.

Glaunsinger, BA., Lee, SS., Thomas, M., Banks, L., & Javier, R. (2000). Interactions of the PDZ-protein MAGI-1 with adenovirus E4-ORF1 and high-risk papillomavirus E6 oncoproteins. *Oncogene.* 19(46):5270-80.

Gonzalez-Mariscal, L., Betanzos, A., Nava, P., & Jaramillo, BE. (2003). Tight junction proteins. *Prog Biophys Mol Biol.* 81(1):1-44.

Gonzalez-Mariscal, L., Lechuga, S., & Garay, E. (2007). Role of tight junctions in cell proliferation and cancer. *Prog Histochem Cytochem.* 42:1-57

Gorschwitz, KR., Ahrens, R., Osterfeld, H., Gurish, MF., Han, X., Abrink, M., Finkelman, FD., Pejler, G., & Hogan, SP. (2009). Mast cells regulate homeostatic intestinal epithelial migration and barrier function by a chymase/Mcpt4-dependent mechanism. *PNAS.* 106:22381-22386.

Guillemot, L., Paschoud, S., Pulimeno, P., Foglia, A., & Citi, S. (2008). The cytoplasmic plaque of tight junctions: a scaffolding and signalling center. *Biochim Biophys Acta.* 1778(3):601-13.

Gupta, IR., & Ryan, AK. (2010). Claudins: unlocking the code to tight junction function during embryogenesis and in disease. *Clin Genet.* 77(4):314-25.

Győrffy, H., Holczbauer, Á., Nagy, P., Szabó, Zs., Kupcsulik, P., Páska, Cs., Papp, J., Schaff, Zs., & Kiss, A. (2005). Claudin expression in Barrett's esophagus and adenocarcinoma. *Virchows Arch.* 447: 961–8

Győrffy H. (2009). Study of claudins and prognostic factors in some gastrointestinal diseases. *Magy Onkol.* 53:377-83.

Hering, NA., & Schulzke, JD. (2009). Therapeutic options to modulate barrier defects in inflammatory bowel disease. *Dig Dis.* 27:450-4.

Jung, H., Jun, KH., Jung, JH., Chin, HM., & Park, WB. (2011). The expression of claudin-1, claudin-2, claudin-3, and claudin-4 in gastric cancer tissue. *J Surg Res.* 167:e185-91.

Katahira, J., Sugiyama, H., Inoue, N., Horiguchi, Y., Matsuda, M., & Sugimoto, N. (1997). Clostridium perfringens enterotoxin utilizes two structurally related membrane proteins as functional receptors in vivo. *J Biol Chem.* 272:26652-26658.

Klamp, T., Schumacher, J., Huber, G., Kühne, C., Meissner, U., Selmi, A., Hiller, T., Kreiter, S., Markl, J., Türeci, Ö., & Sahin, U. (2011). Highly specific auto-antibodies against claudin-18 isoform 2 induced by a chimeric HBcAg virus-like particle vaccine kill tumor cells and inhibit the growth of lung metastases. *Cancer Res.* 71:516-27.

Krause, G., Winkler, L., Mueller, SL., Haseloff, RF., Piontek, J., & Blasig, IE. (2008). Structure and function of claudins. *Biochim Biophys Acta.* 1778(3):631-45.

Lal-Nag, M., & Morin, PJ. (2009). The claudins. *Genome Biol.* 10(8):235.

Li, WY., Huey, CL., & Yu, AS. (2004). Expression of claudin-7 and -8 along the mouse nephron. *Am J Physiol Renal Physiol.* 286(6):F1063-71.

Madara, JL., & Pappenheimer, JR. (1987). Structural basis for physiological regulation of paracellular pathways in intestinal epithelia. *J Membr Biol.* 100(2):149-64.

Matsuda, M., Sentani, K., Noguchi, T., Hinoi, T., Okajima, M., Matsusaki, K., Sakamoto, N., Anami, K., Naito, Y., Oue, N., & Yasui W. (2010). Immunohistochemical analysis of colorectal cancer with gastric phenotype: claudin-18 is associated with poor prognosis. *Pathol Int.* 60:673-80.

Pizzuti, D., Senzolo, M., Buda, A., Chiarelli, S., Giacomelli, L., Mazzon, E., Curioni, A., Faggian, D., & De Lazzari, F. (2011). In vitro model for IgE mediated food allergy. *Scand J Gastroenterol.* 46:177-87.

Poritz, LS., Harris, LR., Kelly, AA., & Koltun WA. (2011). Increase in the Tight Junction Protein Claudin-1 in Intestinal Inflammation. *Dig Dis Sci.* 2011 Jul 12. (Epub ahead of print)

Prasad, S., Mingrino, R., Kaukinen, K., Hayes, KL., Powell, RM., MacDonald, TT, & Collins, JE. (2005). Inflammatory processes have differential effects on claudins 2, 3 and 4 in colonic epithelial cells. *Lab Invest.* 85:1139-62.

Ray, K. (2012) Microbiota: Tolerating gluten-a role for gut microbiota in celiac disease? *Nat Rev Gastroenterol Hepatol.* 9(5):242.

Sander, GR., Cummins, AG., Henshall, T., & Powell, BC. (2005). Rapid disruption of intestinal barrier function by gliadin involves altered expression of apical junctional proteins. *FEBS Lett.* 579(21):4851-5.

Sanz, Y., De Pama,, G., Laparra, M. (2011). Unraveling the ties between celiac disease and intestinal microbiota. *Int Rev Immunol.* 30(4):207-18.

Sapone, A., Lammers, KM., Casolaro, V., Cammarota, M., Giuliano, MT., De Rosa, M., Stefanile, R., Mazzarella, G., Tolone, C., Russo, MI., Esposito, P., Ferraraccio, F., Cartenì, M., Riegler, G., de Magistris, L., & Fasano A. (2011). Divergence of gut permeability and mucosal immune gene expression in two gluten-associated conditions: celiac disease and gluten sensitivity. *BMC Med.* 9:23.

Sawada, N., Murata, M., Kikuchi, K., Osanai, M., Tobioka, H., & Kojima, T. (2003). Tight junctions and human diseases. *Med Electron Microsc.* 36:147-56.

Schulzke, JD., Ploeger, S., Amasheh, M., Fromm, A., Zeissig, S., & Troeger, H. (2009). Epithelial tight junctions in intestinal inflammation. *Ann N Y Acad Sci.* 1165:294-300.

Schulzke, JD. (2000). Epithelial transport and barrier function: pathomechanisms in gastrointestinal disorders. Proceedings of a conference. March 26-27, 1999. Berlin, Germany. *Ann N Y Acad Sci.* 915:1-375.

Sellitto, M., Bai, G., Serena, G., Fricke, WF., Sturgeon, C., Gajer, P., White, JR., Koenig, SS., Sakamoto, J., Boothe, D., Gicquelais, R., Kryszak, D., Puppa, E., Catassi, C., Ravel, J., Fasano, A. (2012) Proof of concept of microbiome-metabolome analysis and delayed gluten exposure on celiac disease autoimmunity in genetically at-risk infants. *PLoS One.* 7(3):e33387.

Smedley, JG., Saputo, J., Parker, JC., Fernandez-Miyakawa, ME., Robertson, SL., McClane, BA., & Uzal, FA. (2008). Noncytotoxic Clostridium perfringens enterotoxin variants localize CPE intestinal binding and demonstrate a relationship between CPE-induced cytotoxicity and enterotoxicity. *Infection and immunity.* 76:3793-3800.

Staehelin, LA. (1974). Structure and function of intercellular junctions. *Int Rev Cytol.* 39:191-283.

Steed, E., Balda, MS., & Matter, K. (2010). Dynamics and functions of tight junctions. *Trends Cell Biol.* 20:142-9.

Stevenson, BR., Anderson, JM., & Bullivant, S. (1988). The epithelial tight junction: structure, function and preliminary biochemical characterization. *Mol Cell Biochem*. 83:129-45.

Sung, CO., Han, SY., & Kim, SH. (2011). Low expression of claudin-4 is associated with poor prognosis in esophageal squamous cell carcinoma. *Ann Surg Oncol*. 18:273-81.

Szakal, DN., Gyorffy, H., Arato, A., Cseh, A., Molnár, K., Papp, M., Dezsofi, A., & Veres, G. (2010). Mucosal expression of claudins 2, 3 and 4 in proximal and distal part of duodenum in children with coeliac disease. *Virchows Arch*. 456:245-50.

Thijs, WJ., van Baarlen, J., & Kleibeuker, JH. (2004). Duodenal versus jejunal biopsies in suspected celiac disease. *Endoscopy*. 36:993-6.

Thompson, JJ., Zinsser, KR., & Enterline, HT. (1983). Barrett's metaplasia and adenocarcinoma of the esophagus and gastroesophageal junction. *Hum Pathol*. 14:42-61

Tobioka, H., Isomura, H., & Kokai, Y. (2002). Polarized distribution of carcinoembryonic antigen is associated with a tight junction molecule in human colorectal adenocarcinoma. *J Pathol*. 198:207-12.

Ueda, J., Semba, S., Chiba, H., Sawada, N., Seo, Y., Kasuga, M., & Yokozaki, H. (2007). Heterogeneous expression of claudin-4 in human colorectal cancer: decreased claudin-4 expression at the invasive front correlates cancer invasion and metastasis. *Pathobiology*. 74:32-41.

Visser, J., Rozing, J., & Sapone, A. (2009). Tight junctions, intestinal permeability, and autoimmunity: celiac disease and type 1 diabetes paradigms. *Ann N Y Acad Sci*. 1165:195-205.

Weber, CR., Nalle, SC., Tretiakova, M., Rubin, DT., & Turner, JR. (2008). Claudin-1 and claudin-2 expression is elevated in inflammatory bowel disease and may contribute to early neoplastic transformation. *Lab Invest*. 88:1110-20.

Weber, CR., Raleigh, DR., Su, L., Shen, L., Sullivan, EA., Wang, Y., & Turner, JR. (2010). Epithelial myosin light chain kinase activation induces mucosal interleukin-13 expression to alter tight junction ion selectivity. *J Biol Chem*. 285:12037-46.

Yu, LC., Wang, JT., Wei, SC., Ni, YH. (2012). Host-microbial interactions and regulation of intestinal epithelial barrier function: From physiology to pathology. *World J Gastrointest Pathophysiol*. 3(1):27-43.

Zavala-Zendejas, VE., Torres-Martinez, AC., Salas-Morales, B., Fortoul, TI., Montaño, LF., & Rendon-Huerta, EP. (2011). Claudin-6, 7, or 9 overexpression in the human gastric adenocarcinoma cell line AGS increases its invasiveness, migration, and proliferation rate. *Cancer Invest*. 29:1-11.