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INVITED REVIEW

Active and passive involvement of claudins in the pathophysiology of intestinal inflammatory diseases

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Abstract Intestinal inflammatory diseases, four of which are discussed here, are associated with alterations of claudins. In *ulcerative colitis*, diarrhea and antigen entry into the mucosa occurs. Claudin-2 is upregulated but data on other claudins are still limited or vary (e.g., claudin-1 and -4). Apart from that, tight junction changes contribute to diarrhea via a leak flux mechanism, while protection against antigen entry disappears behind epithelial gross lesions (erosions) and apoptotic foci. *Crohn's disease* is additionally characterized by a claudin-5 and claudin-8 reduction which plays an active role in antigen uptake already before gross lesions appear. In *microscopic colitis* (MC), upregulation of claudin-2 expression is weak and a reduction in claudin-4 may be only passively involved, while sodium malabsorption represents the main diarrheal mechanism. However, claudin-5 is removed from MC tight junctions which may be an active trigger for inflammation through antigen uptake along the so-called leaky gut concept. In *celiac disease*, primary barrier defects are discussed in the context of candidate genes as PARD3 which regulate cell polarity and tight junctions. The loss of claudin-5 allows small antigens to invade, while the reductions in others like claudin-3 are rather passive events. Taken together, the specific role of single tight junction proteins for the onset and perpetuation of inflammation and the recovery from these diseases is far from being fully understood and is clearly dependent on the stage of the disease, the background of the other tight junction components, the transport activity of the mucosa, and the presence of other barrier features like gross lesions, an orchestral interplay which is discussed in this article.

Keywords Ulcerative colitis · Crohn's disease · Microscopic colitis · Celiac disease · Claudins · Barrier defect

Introduction

Chronic inflammatory diseases of the intestine are increasingly frequent within the last three decades. They can affect the small and the large intestine. The most common diseases are chronic inflammatory bowel diseases (IBD) like Crohn's disease and ulcerative colitis, as well as microscopic colitis that consists of two subtypes, lymphocytic colitis and collagenous colitis. The most common chronic inflammatory disease of the small intestine is celiac disease. A key feature of all of them is that they impair the epithelial barrier.

The epithelial cell layer as the first "line of defense" defines the epithelial barrier. It is constructed in such a way that uncontrolled passage of antigens, solutes, and water is prevented. Its barrier function consists of two parts, a transcellular and a paracellular one. The morphological correlate of the paracellular part is the tight junction (TJ), a complex meshwork of various strand-like proteins anchored in the apicolateral membrane of epithelial cells that spans across the paracellular space. Its function is to "seal" the paracellular space between adjacent epithelial cells against the outside environment to prevent the uncontrolled paracellular passage of luminal contents and to prevent the loss of solutes and water into the lumen.

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The TJ components determining the barrier function are tetraspan transmembrane proteins of two families: the large family of claudins and the smaller family of tight junction-associated Marvel proteins (TAMPs) occludin and marvelD3 as integral components of bicellular TJs [76, 90] and tricellulin, which predominantly localizes at tricellular TJs [42].

In addition, peripheral proteins belong as TJ-associated proteins to the TJ and participate as scaffold proteins in its function like zonula occludens (ZO)-1, ZO-2, and ZO-3 and cingulin. These are connected to the cytoskeleton via F-actin and myosin II [23, 32]. Among these TJ proteins, claudins are the major transmembrane components. They determine the tightness of the epithelial barrier and contribute to the polarity of epithelial cells.

In general, claudins exhibit three different functions: (i) a "gate function" through transepithelial barrier or channel formation; (ii) a "signal function" by which they affect cellular signaling, proliferation, motility, and migration as well as differentiation and receptor function; and (iii) a "fence function" that prevents intermixing of apical and basolateral membrane proteins. All three functions are regulated by stimuli from the cell interior and from the outside.

Chronic inflammatory diseases of the intestine may lead to the dysregulation of TJ proteins that in turn have two distinct effects on barrier function: (i) increased paracellular transport of solutes and water by which ions and water diffuse from the blood to the intestinal lumen and cause leak-flux diarrhea [81] and (ii) increased permeability to large molecules, as e.g., luminal pathogens that induce an immune response and maintain the inflammatory process [2, 16].

This review summarizes our current knowledge about TJ composition and function in chronic immune-mediated intestinal diseases from a clinical perspective, first by looking at the physiological expression and function of intestinal claudins and secondly by discussing their influence and behavior in chronic intestinal diseases, with an active role when the change in a claudin has functional consequences and with a passive role if it is only coincidently affected without functional consequence.

The barrier breakdown occurring in inflammatory diseases is caused for the most part by alterations of claudins and TAMPs. As this gives rise to increased uptake of antigens from the lumen, the tight junction-mediated uptake fires the inflammatory process. In turn, this inflammation releases cytokines which then alters tight junction protein expression and localization. This vicious circle has birthed the idea to focus in our review on the frequently raised question, whether the change in tight junction proteins is actively and passively involved in the chronically inflamed intestine.

Physiology of intestinal claudins—expression, localization, and function

To understand TJ alterations and their implications in chronic intestinal diseases, the physiologic function of claudins have to be elucidated. From their primary function as "gate keeper," intestinal claudins can be classified as barrier- or channel-forming. In the intestine, the latter is selective paracellular channels, which also determine water permeability. Barrier-forming claudins are subclassified as non-charge- and charge-selective [50]. Charge-selective claudins favor a privileged passage of specifically charged ions by inhibiting passage of the counter-ion. The following enumeration presents the functionally relevant intestinal claudins according to their gate function, and Table 1 summarizes claudin functions and expression patterns in the different intestinal inflammatory diseases.

Claudins with barrier function

No charge selectivity

Claudin-1 Claudin-1 and claudin-2 were the first claudins to have been isolated [28]. Claudin-1 is found in many tissues including the intestine [15, 28, 51, 110]. In human sigmoid colon, claudin-1 is detected in the TJ and the subjunctional lateral membrane of surface and crypt enterocytes [110]. The function of claudin-1 is barrier forming, since its overexpression induces an increase of the transepithelial resistance (TER) in cell culture studies [33, 43, 55]. The consequences of a loss of claudin-1 have in particular been shown for barrier function in the epidermis. Homozygous claudin-1 knockout mice die within 1 day after birth and exhibit severe transepidermal water loss and macromolecular diffusion through the epidermal TJ that permits the conclusion that claudin-1 is essential for barrier formation [30]. Other skin diseases that are associated with a downregulation of claudin-1 are atopic dermatitis and psoriasis [19, 34, 103]. Also, claudin-1 is expressed in cholangiocytes and hepatocytes and has been shown to cause neonatal sclerosing cholangitis associated with ichthyosis due to loss-of-function mutations in the claudin-1 gene [33, 35]. The physiologic regulation of claudin-1 within intestinal epithelial cells is largely unknown. Just recently, Saeedi and coworkers demonstrated that *hypoxia-inducible factor* (HIF) is a mediator that can regulate claudin-1 via prominent hypoxia response elements in the claudin-1 promoter region. Overexpression of claudin-1 in HIF1β-deficient intestinal epithelial cells (IECs) that have lost barrier properties restored exactly these properties [80]. In intestinal inflammation, proinflammatory mediators can cause a dysregulation of claudin-1. Amasheh et al. found upregulation and redistribution of claudin-1 away from the TJ in the presence of TNFα [3], whereas others demonstrated increased paracellular

Table 1 Function and expression changes of claudins in intestinal inflammatory diseases

	Function	ulcerative colitis	Crohn's disease	Collagenous colitis	Celiac disease	reference
Claudin-1	Barrier	↑, =	=	=	=	[15, 37, 51, 71, 73, 86, 104, 110]
Claudin-2	Cation channel,water channel	↑	↑	=	↑	[15, 31, 37, 86, 92, 104, 110]
Claudin-3	Barrier	↓, =	↓	=	↓	[15, 31, 71, 74, 86, 95, 110]
Claudin-4	Barrier, anion channel	↓	=	↓	=	[15, 31, 71, 86, 110]
Claudin-5	Barrier	n.a.	↓	=	↓	[15, 86, 110]
Claudin-8	Barrier	n.a.	↓	n.a.	n.a.	[110]
Claudin-15	Na^+ channel	n.a.	n.a.	n.a.	↑	[86]

↑ increased expression, ↓ decreased expression, = unchanged expression, *n.a.* not applicable due to missing peer reviewed data

permeability and downregulation of claudin-1 in conjunction with increased levels of TNFα and NFκB [24, 111]. In addition, Bruewer et al. showed that in the presence of IFNγ and/or TNFα, a substantial internalization of claudin-1 occurs after 48 h in T84 cells [14]. Diseases of the intestine that exhibit a dysregulation of claudin-1 are IBD [51, 104, 110], irritable bowel syndrome [12, 72], and HIV enteropathy [24], but not celiac disease or microscopic colitis [11, 15, 86].

Claudin-3 Claudin-3 is expressed in many different epithelia including the gastrointestinal tract [15, 75, 110]. In rat intestine, claudin-3 is expressed ubiquitously throughout the jejunum, ileum, and colon. Its expression is tight junctional, but in the colon, it shows also a lateral membrane localization, both in crypts and at the surface [75]. In human colon, Prasad et al., as well as Schumann et al., localized claudin-3 to the tight junction and lateral cell membrane of crypt and surface epithelial cells [74, 86]. Several studies showed that claudin-3 is barrier-forming. Overexpression of claudin-3 in MDCK II cells led to an increase in TER that was due to a significant increase in paracellular resistance as determined by two-path impedance spectroscopy and reduced permeability for ions of either charge as well as larger molecules like fluorescein (332 Da) and fluorescein isothiocyanate (FITC)-dextran (4 kDa) [60]. When stably expressed in human airway cells (IB3.1), claudin-3 reduced paracellular permeability of dextrans of different sizes (10, 70, 2000 Da) and induced a small increase in resistance across the cell layer that did not reach statistical significance [18]. Further evidence for its barrier-forming properties came from inhibition experiments. siRNA knockdown of claudin-3 in MKN28 gastric epithelial cells resulted in attenuated barrier function similar to ochratoxin A- and TRPV4-induced claudin-3 downregulation in the human colon carcinoma cell line Caco-2 and HC11 mammary cells, respectively [36, 56, 77]. Although these studies clearly showed a barrier-forming effect for claudin-3, conflicting data from alveolar epithelial cells exist in which overexpression of claudin-3 appears to be associated with decreased TER and increased permeability for paracellular markers [62] which could be due to a different TJ protein background within the respective cell model. Information about the physiologic regulation of claudin-3 is limited. Just recently, Dong et al. demonstrated intestinal claudin-3 upregulation in mice to be dependent on activation of intestinal epithelial IGF-1 receptor through glucagon-like peptide-2 (GLP-2). GLP-2 treatment increased epithelial resistance in the jejunum of mice and decreased permeability of 4-kDa FITC-dextran [22]. In addition, zinc depletion reduces TER and claudin-3 expression on a transcriptional level in Caco-2 cells [63].

Some but not all intestinal diseases alter claudin-3 expression and localization. Dysregulation occurs in IBD [31, 74, 95] and celiac disease [86], but not in microscopic colitis [15] or irritable bowel syndrome [49].

Claudin-5 Claudin-5 is expressed in various tissues [65]. It is considered primarily as a barrier-forming claudin that is associated with endothelial TJs and plays a critical role in maintaining the blood-brain barrier [45, 66, 68]. However, claudin-5 localizes to epithelial cells as well, especially to the alveolar epithelium of the lung [65, 100] and to small and large intestine in rat [75] and man [15, 110]. Here, it is strictly located in the TJ of crypts and surface epithelial cells without any gradient along the crypt-to-surface axis [110]. Several studies confirm the barrier properties of claudin-5 in epithelial and endothelial cell models. Stable transfection of a FLAG-tagged claudin-5 into Caco-2 cells increased TER [6]. In cultured rat brain capillary endothelial cells, transfection of claudin-5 reduced the permeability of ^{14}C-inulin [69] and overexpression of claudin-5 in cultured human retinal pigment epithelium cells enhanced barrier function [96]. In addition, the death

of homozygous claudin-5-knockout mice within 10 h after birth emphasizes the importance of claudin-5 for barrier integrity [68]. Experiments in endothelial cells delivered most of the information available about claudin-5 regulation. Stamatovic et al. found a lipid-raft-dependent endocytosis mechanism of internalization of claudin-5 into early and recycling endosomes that involves caveolin-1 after addition of the chemokine CCL2 [89]. Other factors that affect claudin-5 (dys)regulation and function are cyclic AMP (cAMP) and protein kinase A [44], the erythroblast transformation-specific (ETS)-related gene [109], as well as sodium caprate, β1-integrin, and bradykinin [20, 53, 70]. However, only little is known about claudin-5 regulation in intestinal epithelial cells. Recently, Watari et al. demonstrated increased TER due to increased claudin-5 expression in Caco-2 cells after checkpoint kinase 1 activation through incubation with daunorubicin and rebeccamycin [102]. Diseases of the gastrointestinal tract in which claudin-5 is dysregulated are Crohn's disease [110] and celiac disease [86] as well as *Campylobacter concisus* enteritis [67].

Charge selectivity

Claudin-4 Expression of claudin-4 is not as ubiquitous as the expression of some other members of the claudin family. mRNA and protein expression of claudin-4 has been reported in lung and kidney [65], in rat gastrointestinal tract [75] including enteric neurons [46], and in human colon [15, 110]. In the human colon, claudin-4 is predominantly located in the lateral membrane with additional junctional and cytoplasmic staining in crypt and surface enterocytes [110]. Regarding its function, different effects have been observed depending on the cell model. It can act either as a regular barrier or as a sodium barrier without affecting chloride permeability and in conjunction with claudin-8 as an anion channel [41, 59, 98, 99]. In MDCK II cells, overexpression of claudin-4 increased barrier properties with an increase in TER and a decreased sodium permeability [98]. In mouse collecting duct cells, claudin-4 acts as an anion channel that facilitates NaCl-reabsorption and requires the presence of claudin-8 for correct assembly in the TJ [41]. siRNA knockdown of claudin-8 leads to a delocalization of claudin-4 to the cytoplasm with a consecutive loss of paracellular chloride reabsorption [41]. Hou et al. also demonstrated anion channel properties in mouse collecting duct cells. Knockdown of claudin-4 resulted in decreased TER due to an increase in ion permeability, while a preference change for paracellular reabsorption from anions to cations occurred [40]. In the lung, claudin-4 acts as a sodium barrier to prevent fluid loss into the alveolar space. When claudin-4 is absent, air space fluid clearance was inhibited and ventilator-induced pulmonary edema was exacerbated [107]. In contrast, increased claudin-4 expression levels resulted in better alveolar fluid clearance [78]. In the colon, claudin-4 appears to promote barrier effects based on cell culture studies. Induction of claudin-4 expression in the human colon carcinoma cell line HT-29/B6 through TGF-β-containing whey protein induced a TER increase due to increased claudin-4 promoter activity [38]. In contrast, a combination of TNFα/IFNγ induced a decrease in TER with a concomitant redistribution of claudin-4 from the TJ but no changes in expression in confluent T84 cells [74]. Claudin-4 expression is downregulated in ulcerative colitis [71], collagenous colitis [15], and irritable bowel syndrome [49]. No changes have been reported in Crohn's disease [31, 110], celiac disease [86], HIV enteropathy [24], and campylobacter enteritis [67].

Claudin-8 Claudin-8 is expressed in lung, liver, skeletal muscle, kidney, testis, and intestine [48, 52, 65, 110]. Expression in the kidney followed a heterogeneous pattern along the aldosterone-responsive parts of the renal tubule, namely, in the entire distal nephron and the late segments of the thin descending limb of Henle [52]. In mouse intestine, claudin-8 is expressed in the more distal parts of the intestine with increasing expression levels from the ileum to the colon [27]. In human intestine, segmental expression differences were not examined so far, but expression is confirmed in sigmoid colon [110]. In HT-29/B6 cells, aldosterone induced claudin-8 upregulation, increased paracellular resistance, and induced electrogenic sodium transport via the epithelial sodium channel (ENaC). The resulting tightened barrier has been proposed to prevent back-leak of sodium into the lumen [5]. This is in accordance with data from Yu et al. who demonstrated in MDCK cells that claudin-8 acts as a sodium barrier [108]. Others showed that claudin-8 recruits claudin-4 to form a TJ-based complex that seals cation passage but mediates anion transport in collecting duct cells [41]. Not much is known about claudin-8 in intestinal diseases. In only two intestinal diseases, claudin-8 was examined. Whereas in Crohn's disease claudin-8 expression was reduced [110], claudin-8 was unchanged in *C. concisus* infection [67].

Intestinal claudins forming paracellular cation and water channels

Claudins forming paracellular channels exhibit selectivities for small cations, small anions, or water. However, anion-selective claudins (e.g., claudin-10a and claudin-17) are not abundant in the intestine. The main channel formers of the gut epithelium are claudin-2 and claudin-15. Because other papers in this special issue deal with these two claudins more extensively, we provide only a short roundup here.

Claudin-2 Claudin-2 is constitutively expressed in the most leaky epithelia, e.g., the proximal nephron [48] and the small intestine [75]. In tight epithelia, it is usually not detectable, but

in human colon, it is expressed to low amounts in the crypts. Of importance, it can be upregulated in inflammatory conditions like Crohn's disease, ulcerative colitis, celiac disease, or HIV enteropathy [10]. Claudin-2 forms a paracellular channel containing a common pore for small cations and for water [4, 29, 79, 88].

Only recently, it was discussed that claudin-2 could also have a protective effect on the onset and severity of colitis, e.g., by rinsing off antigens from the apical surface due to a passive leak flux. In line with such a type of explanation, Ahmad et al. demonstrated in villin-claudin-2 transgenic mice that increased expression of claudin-2 augmented colonocyte proliferation and provided protection against colitis-induced colonocyte death in a PI-3Kinase/Bcl-2-dependent manner [1]. However, also a marked suppression of colitis-induced increase in immune activation and associated signaling was observed, suggesting in addition interference with immune tolerance [1].

Claudin-15 Claudin-15 is widely distributed in small and large intestine in both the TJ of villus and crypt cells. Knockout of claudin-15 in mice showed that claudin-15 might act as a paracellular cation channel that facilitates sodium secretion. Loss of claudin-15 results in low intraluminal sodium concentrations that in turn hampers sodium-coupled glucose absorption via SGLT-1 [93].

Interestingly, claudin-15 knockout mice develop congenital enlargement of the small intestine [94]. The exact mechanisms leading to this megaintestine are not known. Data from zebrafish also suggest a role for claudin-15 in congenital development of the small intestine. Here, downregulation of claudin-15 in animals with a loss-of-function mutation in the transcription factor tcf2 resulted in the development of multiple intestinal lumens, a phenotype that was also seen in claudin-15 morphants supporting the central role of claudin-15 in intestine development [7].

Data on claudin-15 expression and localization in human intestinal inflammation are very limited. Only Schumann et al. examined claudin-15 in celiac disease and found an upregulation of protein expression in conjunction with a shift from the membrane into the cytoplasm [86].

Inflammatory diseases of the intestine—expression, localization, and function of intestinal claudins

Ulcerative colitis Ulcerative colitis belongs, like Crohn's disease, lymphocytic, and collagenous colitis, to the group of IBDs. Characteristic feature of ulcerative colitis is a chronic inflammation that is, apart from a rare "backwash-ileitis," restricted to the colon. The inflammation spreads out from the distal to proximal colon segments and always includes the rectum. During disease activity, patients suffer from frequent, often bloody diarrhea and abdominal pain. The disease affects usually adults in their third and fourth decade of life but a second peak occurs during the seventh and eighth life decade. Therapeutic options include various immunosuppressive agents, alone or in combination, and surgery. The therapeutic goal is to reach a long-term remission for the patient.

Many studies exist that examined the pathologic features of ulcerative colitis. Barrier function was very early assumed to be impaired. Sandle and coworkers found a decrease of mucosal resistance in ulcerative colitis to approximately 40% [82]. This finding based upon the total resistance of the tissue. Our group used one-path impedance spectroscopy to discriminate epithelial and subepithelial resistance and found an even more pronounced decrease of the epithelial resistance of approximately 85% and a concomitant increase in subepithelial resistance [83]. This was due to a reduced depth of the TJ meshwork with a reduced number of horizontally oriented TJ strands and increased thickness of the subepithelial layers due to infiltration with immune cells and edema [83]. With the discovery of claudins, the question about their role in TJ alterations in IBD arose.

The first claudin examined in ulcerative colitis was claudin-2 [37]. Heller et al. observed that claudin-2 was not detectable in the sigmoid colon of healthy subjects but was highly upregulated in the inflamed sigmoid colon of ulcerative colitis patients which may be an active event already in early disease stages causing leak flux diarrhea. They demonstrated that upregulation of claudin-2 depends on IL-13. IL-13 is a proinflammatory cytokine that is elevated in active ulcerative colitis as part of the immune response. In addition to claudin-2 upregulation, IL-13 provoked an increase in the epithelial apoptotic rate and an epithelial restitution arrest [37]. This might provide an explanation for the early occurrence of ulcer lesions in ulcerative colitis [39]. Other claudins examined in ulcerative colitis are claudin-1, claudin-3, claudin-4, and claudin-7. Data on claudin-1 are varying. Whereas Oshima et al. did not observe changes in claudin-1 expression in rectal biopsies of patients with active inflammation [71], Poritz and coworkers found increased claudin-1 expression in the inflamed colon in ulcerative colitis [73]. Claudin-1 expression appears to be dependent on the grade of inflammation. Weber et al. found claudin-1 also increased in severely inflamed but not in slightly inflamed colon of ulcerative colitis patients. Interestingly, they observed claudin-1 and claudin-2 expression not to be increased in acute, self-limited colitis but in active ulcerative colitis, in adenomas, and in IBD-associated dysplasia [104]. They speculated that increased claudin-1 and claudin-2 expressions may be involved in neoplastic developments in ulcerative colitis [104], a hypothesis that is supported by others, too [47, 57].

Data on claudin-3 in ulcerative colitis are limited. Claudin-3 expression has been shown to be unaffected [71] or reduced in active ulcerative colitis [74]. Others found increased

claudin-3 urinary concentration in active ulcerative colitis and suggested to use urinary claudin-3 concentration as a non-invasive marker of intestinal tight junction deprivation [95]. Data on the expression of claudin-4 were also divergent. In rectal biopsies, Weber et al. demonstrated elevated claudin-4 in severe active ulcerative colitis [104], whereas Oshima et al. found the opposite on protein and mRNA level in active inflammation [71]. Claudin-7, which in human sigmoid colon like claudin-4 is predominantly located in the lateral membrane and in the TJ and the cytoplasm of crypt and surface epithelial cells, was downregulated in active ulcerative colitis on mRNA and protein level [71].

Just recently, claudins were examined as targets for alternative therapeutic approaches. Stio et al. observed that $1,25(OH)_2D_3$, the active form of vitamin D, reversed the increase of claudin-1 and claudin-2 and the decrease of claudin-4 and claudin-7 protein expressions in active ulcerative colitis and proposed vitamin D to be a potential therapeutic agent for the treatment of ulcerative colitis [91]. Devriese et al. reported that activity of *Butyricicoccus pullicaecorum*, a butyrate-producing bacterium reduced in the stool of patients with active ulcerative colitis, correlates inversely with claudin-1 mRNA-expression and proposed it as a pharmacobiotic in ulcerative colitis [21].

Crohn's disease Crohn's disease differs from ulcerative colitis through dissemination across the entire intestinal tract, although terminal ileum and/or the colon are more abundantly affected. Inflammation is segmentally distributed and can affect more than one area of the gastrointestinal tract at the same time. Often, it does not affect the rectum. Patients suffer from abdominal pain in stenotic disease and mostly from non-bloody diarrhea. The nature of pain and diarrhea depends on the gastrointestinal segments involved. Thus, symptoms in Crohn's disease are more diverse than in ulcerative colitis. Crohn's disease tends to start in young people but can occur at any age. The immune-suppressive therapeutic approach is often similar to that for ulcerative colitis.

Zeissig et al. performed the most comprehensive study on claudin expression and localization in Crohn's disease [110]. The epithelial resistance in mild to moderately inflamed sigmoid colon of Crohn's disease patients was approximately 60% of that of controls according to one-path impedance spectroscopy analysis suggesting TJ alterations. In fact, tight junction morphology as determined by freeze-fracture electron microscopy showed severe disturbances with reduced and discontinuous TJ strands, suggesting altered claudin expression and/or subcellular localization. They examined claudin-1, claudin-2, claudin-3, claudin-4, claudin-5, claudin-7, claudin-8, claudin-11, claudin-12, claudin-14, claudin-15, and claudin-16 and occludin and found claudin-5 and claudin-8 downregulated and redistributed off the TJ as well as upregulation of claudin-2 in the inflamed colon. Especially, claudin-5 deficiency has to be assumed to play an active role in antigen entry into the mucosa.

In Fig. 1, redistribution of claudins off the TJ is exemplarily shown for claudin-8. The upregulation of claudin-2 is triggered by TNFα but was not as strong as in ulcerative colitis, where it was threefold higher. Claudin-2 upregulation was located in the crypt base and was even higher in patients with lower claudin-8 expression, which is consistent with results from cell culture experiments demonstrating that claudin-2 expression is inversely related to claudin-8 expression and that it can be replaced by claudin-8 [108]. In this manner, claudin-2 and claudin-8 have to be assumed to play a role for the diarrhea (leak flux mechanism). Claudin-1, claudin-4, and claudin-7 were unchanged regarding protein expression and localization within the TJ, whereas protein expression of claudin-11, claudin-12, claudin-14, claudin-15, and claudin-16 was not detectable in sigmoid colon [110]. Others found similar results. Claudin-1 expression for example was unchanged in inflamed duodenum and colon [51, 73] and was proposed as a surrogate marker to differentiate between Crohn's disease and ulcerative colitis [73].

A recent study by Goswami et al. performed in Crohn's disease with active duodenal inflammation confirmed that claudin-2 is elevated, whereas claudin-3 and claudin-4 are downregulated and correlated these observations with simultaneous alterations in the TJ ultrastructure [31]. In contrast, treated Crohn patients and patients in remission exhibited no significant changes in epithelial resistance and claudin expression indicating the importance of claudins for the normal function of the epithelial barrier [31, 110]. Proinflammatory cytokines can disturb the integrity of the epithelial barrier. In contrast to ulcerative colitis, a predominant T_h17 immune response is observed in Crohn's disease. High TNFα and IFNγ serum levels are evident and possess the ability to decrease TER and to increase claudin-2 expression as demonstrated in human epithelial HT-29/B6 and Caco-2 cells [26, 110].

Glucocorticoids can improve intestinal barrier function, an effect which can also be studied in cell models. Although the TNFα- and IFNγ-induced TER decline in Caco-2 cells was similar, TER was still higher in glucocorticoid-treated than in non-treated cells because treatment with dexamethasone before TNFα and IFNγ exposure increased TER. This effect was accompanied by decreased claudin-2 and increased claudin-4 protein expression in the glucocorticoid-treated Caco-2 cells and was mediated through activation of MAPK phosphatase-1 [26]. A similar effect is seen with the anti-TNFα agent adalimumab. In the presence of TNFα and IFNγ, adalimumab prevented TER decrease and downregulation/redistribution of claudin-1, claudin-2, and claudin-4 as well as occludin in T84 cells and inhibited the formation of irregular membrane structures [25]. Thus, both glucocorticoids and anti-TNFα agents augment and protect

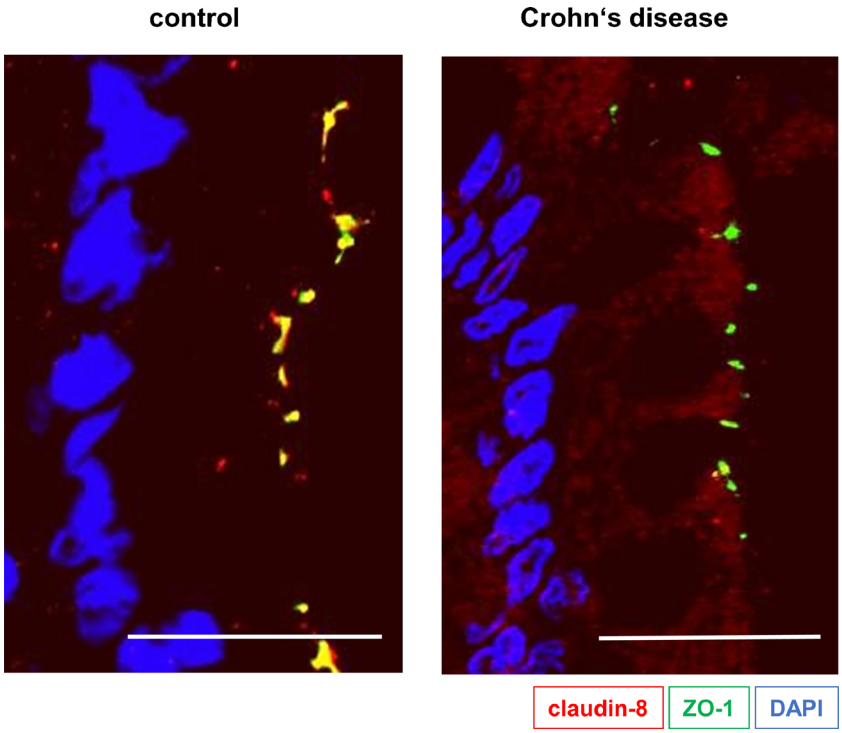

Fig. 1 Distribution and localization of claudin-8 in the sigmoid colon of patients with Crohn's disease. Claudin-8 shifted from an exclusive TJ localization to the apical cytoplasm in Crohn's disease, where it stained weakly, primarily not co-localizing with ZO-1. Bar = 20 μm (reprinted and adapted from [110] with permission from BMJ Publishing Group Ltd. and British Society of Gastroenterology)

mucosal barrier integrity and function and are involved in the rearrangement of the TJ, inducing "mucosal healing."

Microscopic colitis Microscopic colitis consists of two subtypes, lymphocytic and collagenous colitis. The endoscopic appearance is usually normal and histological analysis of endoscopically obtained biopsies is required to establish the diagnosis. Increased numbers of intraepithelial lymphocytes (more than 20 per 100 enterocytes) without mucosal gross lesions are a characteristic feature of both subtypes. However, in contrast to lymphocytic colitis, the histological picture in collagenous colitis offers additional thickening of the apical subepithelial collagenous layer of more than 10-μm thickness due to irregular collagen deposition. Both subtypes usually affect older people. The main symptom is watery, non-bloody diarrhea that also often occurs during the night. Other symptoms include abdominal pain, weight loss, nausea, bloating, and fatigue. Treatment options include antidiarrheal drugs and 5-ASA agents, but most effective are (topic) steroids. Finally, anti-TNFα agents promise relief in a subset of patients with a severe disease course.

The main symptom, watery diarrhea, suggests a mucosal barrier disturbance, but only a few studies exist that examined the epithelial barrier. A study that elucidated mechanisms of diarrhea in collagenous colitis was performed by Bürgel et al. [15]. They found two mechanistic principles that cause diarrhea in collagenous colitis: (i) malabsorption through reduced activity of electroneutral NaCl absorption and (ii) a leak-flux component due to an epithelial barrier dysfunction which could be attributed to TJ alterations. They examined claudin-1, claudin-2, claudin-3, claudin-4, and claudin-5 and occludin and found claudin-4 and occludin protein expression to be downregulated. In parallel, one-path impedance spectroscopy revealed that epithelial resistance is diminished in collagenous colitis by approximately 44%. They suggested that this decrease was attributed to the changes in TJ composition and concluded that a leak-flux mechanism contributes to the watery diarrhea in collagenous colitis.

For lymphocytic colitis, data exist only in abstract form so far. Barmeyer et al. found claudin-4, claudin-5, and claudin-8 downregulation of protein expression and internalization of claudin-5 and claudin-8 from the TJ into the cytoplasm and concluded that in lymphocytic colitis, a leak-flux mechanism contributes to the diarrhea [11]. The importance of this contribution remains unclear. In a small subgroup of patients that were free of symptoms after 6 weeks of oral treatment with budesonide, restauration of electrogenic sodium transport, which is disturbed in lymphocytic and collagenous colitis [8, 9], occurred but epithelial resistance was still reduced indicating an ongoing barrier disturbance [8]. However, this subset of patients was too small for a definite statement. On the other hand, in the absence of apoptotic leaks and gross lesions, the reduction of claudin-5 and claudin-8 in the epithelial tight junction of the colon has to be attributed to play an active role for antigen uptake and perpetuation of inflammation in lymphocytic colitis.

Celiac disease Celiac disease is an autoimmune disorder that affects the small bowel of genetically susceptible individuals. It appears as enteritis secondary to gluten sensitivity and exhibits one or more of the following pathologic features: villous atrophy, increased intraepithelial lymphocyte count (>30 / 100 enterocytes), chronic inflammatory infiltrate in the lamina propria, and crypt hyperplasia. Duodenal biopsies in combination with serum celiac disease antibodies are relevant for the diagnosis. Affected are all age categories. Symptoms are diverse and include primarily gastrointestinal symptoms, but also atypical symptoms manifesting at the skin, inner organs, joints, or psychiatric and neurological symptoms as well as infertility, recurrent miscarriage, or missed menstrual periods are possible and have to be taken into account. The only therapy is lifelong gluten-free diet, under which the epithelium completely restitutes.

Disease susceptibility genes involved in TJ formation like MYO9B, PARD3, and MAGI2 suggest a primary leaky barrier that promotes disease progression [64, 101]. This hypothesis is supported by the observation of increased intestinal permeability in healthy relatives of celiac disease patients [97] that also exhibit TJ morphology changes despite normal villous architecture and negativity for anti-TTG-IgA [61]. Despite this coherence, the role of disease susceptibility genes in regulating paracellular permeability and gliadin transport is not resolved so far [54, 58].

In active celiac disease, paracellular permeability is increased and TJ morphology is substantially altered [84, 85]. Freeze-fracture electron microscopy demonstrated a thin and tattered TJ meshwork with reduced depth, reduced number of TJ strands, and increased numbers of aberrant strands and strand discontinuities [84]. This is accompanied by molecular changes in the TJ protein composition. Schumann et al. demonstrated in duodenal biopsies of patients with active celiac disease an upregulation of the channel-formers claudin-2 and claudin-15 and downregulation of the barrier-formers claudin-3, claudin-5, and claudin-7 and occludin, whereas claudin-1 and claudin-4 expression was unchanged [86]. Claudin-2 upregulation predominantly localizes to the TJ of crypts and appeared to be posttranscriptional (Fig. 2; [86]). Szakal et al. made similar observations in the duodenum of children with severe celiac disease [92]. But, the role of TJ changes for the diarrhea may be less important than that of the villus atrophy-related malabsorptive mechanism. Claudin-3 did not colocalize with ZO-1 in celiac patients when compared with controls, whereas localization of claudin-1 and claudin-4 and occludin was not altered [86]. In contrast, intense intracellular staining of claudin-5 (Fig. 2) and claudin-15 indicates a relevant protein shift from the TJ into the cytoplasm. While claudin-5 can be linked to barrier function and may have an active role along the leaky gut concept, the redistribution of claudin-15 off the TJ may be more relevant for intestinal glucose malabsorption than for barrier function [93].

The key cytokines in celiac disease are IL-21 and IFNγ [13, 17]. Whereas the role of IL-21 on the TJ in celiac disease has not been evaluated yet, the effect of IFNγ on claudin-2 remains unclear. On the one hand, uptake of the α2-gliadin-33mer was increased in the presence of high IFNγ levels [87] indicating its importance in celiac disease, but on the other hand, IFNγ inhibited the expression of claudin-2 [105, 106].

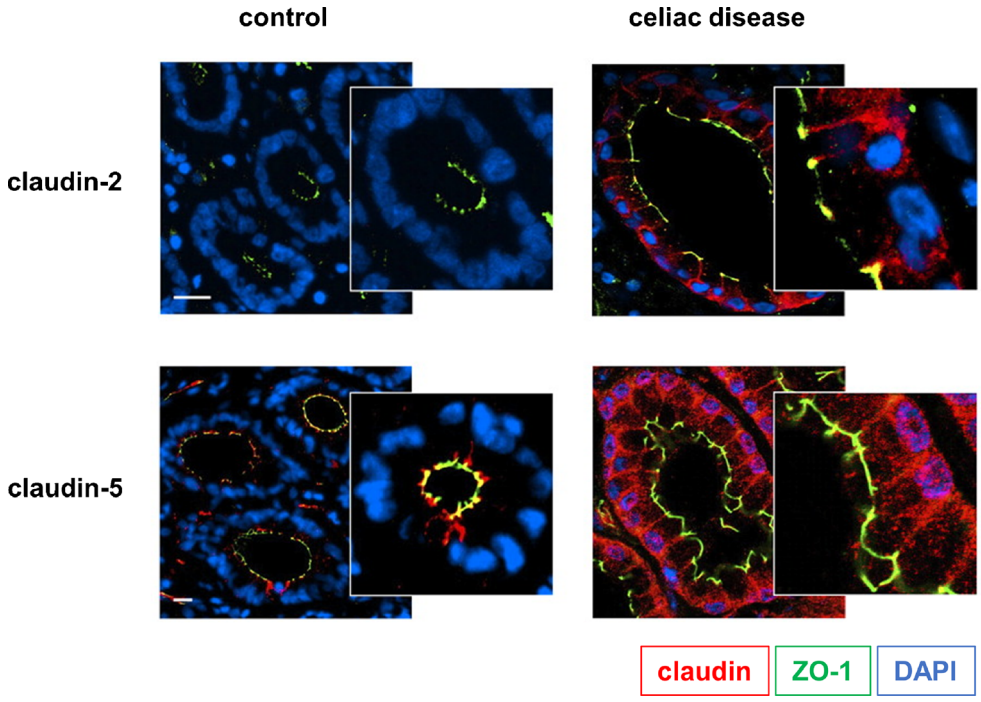

Fig. 2 Distribution and localization of claudin-2 and claudin-5 in the sigmoid colon of patients with active celiac disease. While not detectable in controls, claudin-2 increased in celiac disease but was restricted to TJs of crypt cells. Claudin-5 was localized to the TJ of both controls and patients with active celiac disease but in addition exhibited an intracellular vesicular pool only in active celiac disease. Bar = 20 μm. (reprinted and adapted from [86] with permission from BMJ Publishing Group Ltd. and British Society of Gastroenterology)

However, the authors demonstrated this finding only in T84 cells. Therefore, its relevance for celiac disease remains open.

Closing remarks

By presenting the function of intestinal claudins in detail and by describing their changes in four important intestinal diseases with special attention on the functional consequences, we tried to interpret these changes as being causative (active role) or coincident (passive role), in order to intensify the discussion of the functionality of distinct tight junction protein alterations.

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References

1. Ahmad R, Chaturvedi R, Olivares-Villagomez D, Habib T, Asim M, Shivesh P, Polk DB, Wilson KT, Washington MK, Van Kaer L, Dhawan P, Singh AB (2014) Targeted colonic claudin-2 expression renders resistance to epithelial injury, induces immune suppression, and protects from colitis. Mucosal immunology 7:1340–1353
2. Al-Sadi R, Khatib K, Guo S, Ye D, Youssef M, Ma T (2011) Occludin regulates macromolecule flux across the intestinal epithelial tight junction barrier. American journal of physiology Gastrointestinal and liver physiology 300:G1054–G1064
3. Amasheh M, Fromm A, Krug SM, Amasheh S, Andres S, Zeitz M, Fromm M, Schulzke JD (2010) TNFalpha-induced and berberine-antagonized tight junction barrier impairment via tyrosine kinase, Akt and NFkappaB signaling. J Cell Sci 123:4145–4155
4. Amasheh S, Meiri N, Gitter AH, Schoneberg T, Mankertz J, Schulzke JD, Fromm M (2002) Claudin-2 expression induces cation-selective channels in tight junctions of epithelial cells. J Cell Sci 115:4969–4976
5. Amasheh S, Milatz S, Krug SM, Bergs M, Amasheh M, Schulzke JD, Fromm M (2009) Na+ absorption defends from paracellular back-leakage by claudin-8 upregulation. Biochem Biophys Res Commun 378:45–50
6. Amasheh S, Schmidt T, Mahn M, Florian P, Mankertz J, Tavalali S, Gitter AH, Schulzke JD, Fromm M (2005) Contribution of claudin-5 to barrier properties in tight junctions of epithelial cells. Cell Tissue Res 321:89–96
7. Bagnat M, Cheung ID, Mostov KE, Stainier DY (2007) Genetic control of single lumen formation in the zebrafish gut. Nat Cell Biol 9:954–960
8. Barmeyer C, Erko I, Fromm A, Bojarski C, Allers K, Moos V, Zeitz M, Fromm M, Schulzke JD (2012) Ion transport and barrier function are disturbed in microscopic colitis. Ann N Y Acad Sci 1258:143–148
9. Barmeyer C, Erko I, Fromm A, Bojarski C, Loddenkemper C, Dames P, Kerick M, Siegmund B, Fromm M, Schweiger MR, Schulzke JD (2016) ENaC dysregulation through activation of MEK1/2 contributes to impaired Na+ absorption in lymphocytic colitis. Inflamm Bowel Dis 22:539–547
10. Barmeyer C, Schulzke JD, Fromm M (2015) Claudin-related intestinal diseases. Semin Cell Dev Biol 42:30–38
11. Barmeyer C, Troeger H, Bojarski C, Siegmund B, Fromm M, Schulzke JD (2014) Lymphocytic colitis-related diarrhea is caused by both, ERK1/2-dependent inhibition of the epithelial sodium channel (ENaC) and a claudin-induced barrier defect. Gastroenterology 146:S-475
12. Bertiaux-Vandaele N, Youmba SB, Belmonte L, Lecleire S, Antonietti M, Gourcerol G, Leroi AM, Dechelotte P, Menard JF, Ducrotte P, Coeffier M (2011) The expression and the cellular distribution of the tight junction proteins are altered in irritable bowel syndrome patients with differences according to the disease subtype. Am J Gastroenterol 106:2165–2173
13. Bodd M, Raki M, Tollefsen S, Fallang LE, Bergseng E, Lundin KE, Sollid LM (2010) HLA-DQ2-restricted gluten-reactive T cells produce IL-21 but not IL-17 or IL-22. Mucosal immunology 3:594–601
14. Bruewer M, Luegering A, Kucharzik T, Parkos CA, Madara JL, Hopkins AM, Nusrat A (2003) Proinflammatory cytokines disrupt epithelial barrier function by apoptosis-independent mechanisms. J Immunol 171:6164–6172
15. Burgel N, Bojarski C, Mankertz J, Zeitz M, Fromm M, Schulzke JD (2002) Mechanisms of diarrhea in collagenous colitis. Gastroenterology 123:433–443
16. Buschmann MM, Shen L, Rajapakse H, Raleigh DR, Wang Y, Wang Y, Lingaraju A, Zha J, Abbott E, McAuley EM, Breskin LA, Wu L, Anderson K, Turner JR, Weber CR (2013) Occludin OCEL-domain interactions are required for maintenance and regulation of the tight junction barrier to macromolecular flux. Mol Biol Cell 24:3056–3068
17. Caruso R, Marafini I, Sedda S, Del Vecchio BG, Giuffrida P, MacDonald TT, Corazza GR, Pallone F, Di Sabatino A, Monteleone G (2014) Analysis of the cytokine profile in the duodenal mucosa of refractory coeliac disease patients. Clin Sci 126:451–458
18. Coyne CB, Gambling TM, Boucher RC, Carson JL, Johnson LG (2003) Role of claudin interactions in airway tight junctional permeability. American journal of physiology Lung cellular and molecular physiology 285:L1166–L1178
19. De Benedetto A, Rafaels NM, McGirt LY, Ivanov AI, Georas SN, Cheadle C, Berger AE, Zhang K, Vidyasagar S, Yoshida T, Boguniewicz M, Hata T, Schneider LC, Hanifin JM, Gallo RL, Novak N, Weidinger S, Beaty TH, Leung DY, Barnes KC, Beck LA (2011) Tight junction defects in patients with atopic dermatitis. The Journal of allergy and clinical immunology 127:773-786–e771-777
20. Del Vecchio G, Tscheik C, Tenz K, Helms HC, Winkler L, Blasig R, Blasig IE (2012) Sodium caprate transiently opens claudin-5-containing barriers at tight junctions of epithelial and endothelial cells. Mol Pharm 9:2523–2533
21. Devriese S, Eeckhaut V, Geirnaert A, Van den Bossche L, Hindryckx P, Van de Wiele T, Van Immerseel F, Ducatelle R, De Vos M, and Laukens D (2016). Reduced mucosa-associated Butyricicoccus activity in patients with ulcerative colitis correlates with aberrant claudin-1 expression. J Crohns Colitis. doi:10.1093/ecco-jcc/jjw142
22. Dong CX, Zhao W, Solomon C, Rowland KJ, Ackerley C, Robine S, Holzenberger M, Gonska T, Brubaker PL (2014) The intestinal epithelial insulin-like growth factor-1 receptor links glucagon-like peptide-2 action to gut barrier function. Endocrinology 155:370–379
23. Edelblum KL, Turner JR (2009) The tight junction in inflammatory disease: communication breakdown. Curr Opin Pharmacol 9:715–720
24. Epple HJ, Schneider T, Troeger H, Kunkel D, Allers K, Moos V, Amasheh M, Loddenkemper C, Fromm M, Zeitz M, Schulzke JD (2009) Impairment of the intestinal barrier is evident in untreated but absent in suppressively treated HIV-infected patients. Gut 58:220–227

25. Fischer A, Gluth M, Pape UF, Wiedenmann B, Theuring F, Baumgart DC (2013) Adalimumab prevents barrier dysfunction and antagonizes distinct effects of TNF-alpha on tight junction proteins and signaling pathways in intestinal epithelial cells. American journal of physiology Gastrointestinal and liver physiology 304:G970–G979
26. Fischer A, Gluth M, Weege F, Pape UF, Wiedenmann B, Baumgart DC, Theuring F (2014) Glucocorticoids regulate barrier function and claudin expression in intestinal epithelial cells via MKP-1. American journal of physiology Gastrointestinal and liver physiology 306:G218–G228
27. Fujita H, Chiba H, Yokozaki H, Sakai N, Sugimoto K, Wada T, Kojima T, Yamashita T, Sawada N (2006) Differential expression and subcellular localization of claudin-7, -8, -12, -13, and -15 along the mouse intestine. The journal of histochemistry and cytochemistry : official journal of the Histochemistry Society 54: 933–944
28. 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 141:1539–1550
29. Furuse M, Furuse K, Sasaki H, Tsukita S (2001) Conversion of zonulae occludentes from tight to leaky strand type by introducing claudin-2 into Madin-Darby canine kidney I cells. J Cell Biol 153: 263–272
30. Furuse M, Hata M, Furuse K, Yoshida Y, Haratake A, Sugitani Y, Noda T, Kubo A, Tsukita S (2002) Claudin-based tight junctions are crucial for the mammalian epidermal barrier: a lesson from claudin-1-deficient mice. J Cell Biol 156:1099–1111
31. Goswami P, Das P, Verma AK, Prakash S, Das TK, Nag TC, Ahuja V, Gupta SD, Makharia GK (2014) Are alterations of tight junctions at molecular and ultrastructural level different in duodenal biopsies of patients with celiac disease and Crohn's disease? Virchows Archiv : an international journal of pathology 465:521–530
32. Groschwitz KR, Hogan SP (2009) Intestinal barrier function: molecular regulation and disease pathogenesis. The Journal of allergy and clinical immunology 124:3–20 quiz 21-22
33. Grosse B, Cassio D, Yousef N, Bernardo C, Jacquemin E, Gonzales E (2012) Claudin-1 involved in neonatal ichthyosis sclerosing cholangitis syndrome regulates hepatic paracellular permeability. Hepatology 55:1249–1259
34. Gunzel D, Yu AS (2013) Claudins and the modulation of tight junction permeability. Physiol Rev 93:525–569
35. Hadj-Rabia S, Baala L, Vabres P, Hamel-Teillac D, Jacquemin E, Fabre M, Lyonnet S, De Prost Y, Munnich A, Hadchouel M, Smahi A (2004) Claudin-1 gene mutations in neonatal sclerosing cholangitis associated with ichthyosis: a tight junction disease. Gastroenterology 127:1386–1390
36. Hashimoto K, Oshima T, Tomita T, Kim Y, Matsumoto T, Joh T, Miwa H (2008) Oxidative stress induces gastric epithelial permeability through claudin-3. Biochem Biophys Res Commun 376: 154–157
37. Heller F, Florian P, Bojarski C, Richter J, Christ M, Hillenbrand B, Mankertz J, Gitter AH, Burgel N, Fromm M, Zeitz M, Fuss I, Strober W, Schulzke JD (2005) Interleukin-13 is the key effector Th2 cytokine in ulcerative colitis that affects epithelial tight junctions, apoptosis, and cell restitution. Gastroenterology 129:550–564
38. Hering NA, Andres S, Fromm A, van Tol EA, Amasheh M, Mankertz J, Fromm M, Schulzke JD (2011) Transforming growth factor-beta, a whey protein component, strengthens the intestinal barrier by upregulating claudin-4 in HT-29/B6 cells. J Nutr 141: 783–789
39. Hering NA, Schulzke JD (2009) Therapeutic options to modulate barrier defects in inflammatory bowel disease. Dig Dis 27:450–454
40. Hou J, Gomes AS, Paul DL, Goodenough DA (2006) Study of claudin function by RNA interference. J Biol Chem 281:36117–36123
41. Hou J, Renigunta A, Yang J, Waldegger S (2010) Claudin-4 forms paracellular chloride channel in the kidney and requires claudin-8 for tight junction localization. Proc Natl Acad Sci U S A 107: 18010–18015
42. Ikenouchi J, Furuse M, Furuse K, Sasaki H, Tsukita S, Tsukita S (2005) Tricellulin constitutes a novel barrier at tricellular contacts of epithelial cells. J Cell Biol 171:939–945
43. Inai T, Kobayashi J, Shibata Y (1999) Claudin-1 contributes to the epithelial barrier function in MDCK cells. Eur J Cell Biol 78:849–855
44. Ishizaki T, Chiba H, Kojima T, Fujibe M, Soma T, Miyajima H, Nagasawa K, Wada I, Sawada N (2003) Cyclic AMP induces phosphorylation of claudin-5 immunoprecipitates and expression of claudin-5 gene in blood-brain-barrier endothelial cells via protein kinase A-dependent and -independent pathways. Exp Cell Res 290:275–288
45. Jia W, Lu R, Martin TA, Jiang WG (2014) The role of claudin-5 in blood-brain barrier (BBB) and brain metastases (review). Mol Med Rep 9:779–785
46. Karaki S, Kaji I, Otomo Y, Tazoe H, Kuwahara A (2007) The tight junction component protein, claudin-4, is expressed by enteric neurons in the rat distal colon. Neurosci Lett 428:88–92
47. Kinugasa T, Akagi Y, Yoshida T, Ryu Y, Shiratuchi I, Ishibashi N, Shirouzu K (2010) Increased claudin-1 protein expression contributes to tumorigenesis in ulcerative colitis-associated colorectal cancer. Anticancer Res 30:3181–3186
48. Kiuchi-Saishin Y, Gotoh S, Furuse M, Takasuga A, Tano Y, Tsukita S (2002) Differential expression patterns of claudins, tight junction membrane proteins, in mouse nephron segments. Journal of the American Society of Nephrology : JASN 13:875–886
49. Kong WM, Gong J, Dong L, Xu JR (2007) Changes of tight junction claudin-1,-3,-4 protein expression in the intestinal mucosa in patients with irritable bowel syndrome. Nan fang yi ke da xue xue bao = Journal of Southern Medical University 27:1345–1347
50. Krug SM, Schulzke JD, Fromm M (2014) Tight junction, selective permeability, and related diseases. Semin Cell Dev Biol 36:166–176
51. Kucharzik T, Walsh SV, Chen J, Parkos CA, Nusrat A (2001) Neutrophil transmigration in inflammatory bowel disease is associated with differential expression of epithelial intercellular junction proteins. Am J Pathol 159:2001–2009
52. Li WY, Huey CL, Yu AS (2004) Expression of claudin-7 and -8 along the mouse nephron. American journal of physiology Renal physiology 286:F1063–F1071
53. Liu LB, Xue YX, Liu YH, Wang YB (2008) Bradykinin increases blood-tumor barrier permeability by down-regulating the expression levels of ZO-1, occludin, and claudin-5 and rearranging actin cytoskeleton. J Neurosci Res 86:1153–1168
54. Matysiak-Budnik T, Candalh C, Dugave C, Namane A, Cellier C, Cerf-Bensussan N, Heyman M (2003) Alterations of the intestinal transport and processing of gliadin peptides in celiac disease. Gastroenterology 125:696–707
55. McCarthy KM, Francis SA, McCormack JM, Lai J, Rogers RA, Skare IB, Lynch RD, Schneeberger EE (2000) Inducible expression of claudin-1-myc but not occludin-VSV-G results in aberrant tight junction strand formation in MDCK cells. J Cell Sci 113(Pt 19):3387–3398
56. McLaughlin J, Padfield PJ, Burt JP, O'Neill CA (2004) Ochratoxin A increases permeability through tight junctions by removal of specific claudin isoforms. American journal of physiology Cell physiology 287:C1412–C1417
57. Mees ST, Mennigen R, Spieker T, Rijcken E, Senninger N, Haier J, Bruewer M (2009) Expression of tight and adherens junction

proteins in ulcerative colitis associated colorectal carcinoma: up-regulation of claudin-1, claudin-3, claudin-4, and beta-catenin. Int J Color Dis 24:361–368
58. Menard S, Lebreton C, Schumann M, Matysiak-Budnik T, Dugave C, Bouhnik Y, Malamut G, Cellier C, Allez M, Crenn P, Schulzke JD, Cerf-Bensussan N, Heyman M (2012) Paracellular versus transcellular intestinal permeability to gliadin peptides in active celiac disease. Am J Pathol 180:608–615
59. Michikawa H, Fujita-Yoshigaki J, Sugiya H (2008) Enhancement of barrier function by overexpression of claudin-4 in tight junctions of submandibular gland cells. Cell Tissue Res 334:255–264
60. Milatz S, Krug SM, Rosenthal R, Gunzel D, Muller D, Schulzke JD, Amasheh S, Fromm M (2010) Claudin-3 acts as a sealing component of the tight junction for ions of either charge and uncharged solutes. Biochim Biophys Acta 1798:2048–2057
61. Mishra A, Prakash S, Sreenivas V, Das TK, Ahuja V, Gupta SD, Makharia GK (2016) Structural and functional changes in the tight junctions of asymptomatic and serology-negative first-degree relatives of patients with celiac disease. J Clin Gastroenterol 50:551–560
62. Mitchell LA, Overgaard CE, Ward C, Margulies SS, Koval M (2011) Differential effects of claudin-3 and claudin-4 on alveolar epithelial barrier function. American journal of physiology Lung cellular and molecular physiology 301:L40–L49
63. Miyoshi Y, Tanabe S, Suzuki T (2016) Cellular zinc is required for intestinal epithelial barrier maintenance via the regulation of claudin-3 and occludin expression. American journal of physiology Gastrointestinal and liver physiology 311:G105–G116
64. Monsuur AJ, de Bakker PI, Alizadeh BZ, Zhernakova A, Bevova MR, Strengman E, Franke L, van't Slot R, van Belzen MJ, Lavrijsen IC, Diosdado B, Daly MJ, Mulder CJ, Mearin ML, Meijer JW, Meijer GA, van Oort E, Wapenaar MC, Koeleman BP, Wijmenga C (2005) Myosin IXB variant increases the risk of celiac disease and points toward a primary intestinal barrier defect. Nat Genet 37:1341–1344
65. Morita K, Furuse M, Fujimoto K, Tsukita S (1999) Claudin multigene family encoding four-transmembrane domain protein components of tight junction strands. Proc Natl Acad Sci U S A 96:511–516
66. Morita K, Sasaki H, Furuse M, Tsukita S (1999) Endothelial claudin: claudin-5/TMVCF constitutes tight junction strands in endothelial cells. J Cell Biol 147:185–194
67. Nielsen HL, Nielsen H, Ejlertsen T, Engberg J, Gunzel D, Zeitz M, Hering NA, Fromm M, Schulzke JD, Bucker R (2011) Oral and fecal Campylobacter concisus strains perturb barrier function by apoptosis induction in HT-29/B6 intestinal epithelial cells. PLoS One 6:e23858
68. Nitta T, Hata M, Gotoh S, Seo Y, Sasaki H, Hashimoto N, Furuse M, Tsukita S (2003) Size-selective loosening of the blood-brain barrier in claudin-5-deficient mice. J Cell Biol 161:653–660
69. Ohtsuki S, Sato S, Yamaguchi H, Kamoi M, Asashima T, Terasaki T (2007) Exogenous expression of claudin-5 induces barrier properties in cultured rat brain capillary endothelial cells. J Cell Physiol 210:81–86
70. Osada T, Gu YH, Kanazawa M, Tsubota Y, Hawkins BT, Spatz M, Milner R, del Zoppo GJ (2011) Interendothelial claudin-5 expression depends on cerebral endothelial cell-matrix adhesion by beta(1)-integrins. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism 31:1972–1985
71. Oshima T, Miwa H, Joh T (2008) Changes in the expression of claudins in active ulcerative colitis. J Gastroenterol Hepatol 23(Suppl 2):S146–S150
72. Piche T, Barbara G, Aubert P, Bruley des Varannes S, Dainese R, Nano JL, Cremon C, Stanghellini V, De Giorgio R, Galmiche JP, Neunlist M (2009) Impaired intestinal barrier integrity in the colon of patients with irritable bowel syndrome: involvement of soluble mediators. Gut 58:196–201
73. Poritz LS, Harris LR 3rd, Kelly AA, Koltun WA (2011) Increase in the tight junction protein claudin-1 in intestinal inflammation. Dig Dis Sci 56:2802–2809
74. 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. Laboratory investigation; a journal of technical methods and pathology 85:1139–1162
75. Rahner C, Mitic LL, Anderson JM (2001) Heterogeneity in expression and subcellular localization of claudins 2, 3, 4, and 5 in the rat liver, pancreas, and gut. Gastroenterology 120:411–422
76. Raleigh DR, Marchiando AM, Zhang Y, Shen L, Sasaki H, Wang Y, Long M, Turner JR (2010) Tight junction-associated MARVEL proteins marveld3, tricellulin, and occludin have distinct but overlapping functions. Mol Biol Cell 21:1200–1213
77. Reiter B, Kraft R, Gunzel D, Zeissig S, Schulzke JD, Fromm M, Harteneck C (2006) TRPV4-mediated regulation of epithelial permeability. FASEB journal : official publication of the Federation of American Societies for Experimental Biology 20:1802–1812
78. Rokkam D, Lafemina MJ, Lee JW, Matthay MA, Frank JA (2011) Claudin-4 levels are associated with intact alveolar fluid clearance in human lungs. Am J Pathol 179:1081–1087
79. Rosenthal R, Milatz S, Krug SM, Oelrich B, Schulzke JD, Amasheh S, Gunzel D, Fromm M (2010) Claudin-2, a component of the tight junction, forms a paracellular water channel. J Cell Sci 123:1913–1921
80. Saeedi BJ, Kao DJ, Kitzenberg DA, Dobrinskikh E, Schwisow KD, Masterson JC, Kendrick AA, Kelly CJ, Bayless AJ, Kominsky DJ, Campbell EL, Kuhn KA, Furuta GT, Colgan SP, Glover LE (2015) HIF-dependent regulation of claudin-1 is central to intestinal epithelial tight junction integrity. Mol Biol Cell 26:2252–2262
81. Sandle GI (2005) Pathogenesis of diarrhea in ulcerative colitis: new views on an old problem. J Clin Gastroenterol 39:S49–S52
82. Sandle GI, Higgs N, Crowe P, Marsh MN, Venkatesan S, Peters TJ (1990) Cellular basis for defective electrolyte transport in inflamed human colon. Gastroenterology 99:97–105
83. Schmitz H, Barmeyer C, Fromm M, Runkel N, Foss HD, Bentzel CJ, Riecken EO, Schulzke JD (1999) Altered tight junction structure contributes to the impaired epithelial barrier function in ulcerative colitis. Gastroenterology 116:301–309
84. Schulzke JD, Bentzel CJ, Schulzke I, Riecken EO, Fromm M (1998) Epithelial tight junction structure in the jejunum of children with acute and treated celiac sprue. Pediatr Res 43:435–441
85. Schulzke JD, Schulzke I, Fromm M, Riecken EO (1995) Epithelial barrier and ion transport in coeliac sprue: electrical measurements on intestinal aspiration biopsy specimens. Gut 37:777–782
86. Schumann M, Gunzel D, Buergel N, Richter JF, Troeger H, May C, Fromm A, Sorgenfrei D, Daum S, Bojarski C, Heyman M, Zeitz M, Fromm M, Schulzke JD (2012) Cell polarity-determining proteins Par-3 and PP-1 are involved in epithelial tight junction defects in coeliac disease. Gut 61:220–228
87. Schumann M, Richter JF, Wedell I, Moos V, Zimmermann-Kordmann M, Schneider T, Daum S, Zeitz M, Fromm M, Schulzke JD (2008) Mechanisms of epithelial translocation of the alpha(2)-gliadin-33mer in coeliac sprue. Gut 57:747–754
88. Shoar S, Saber AA, Aladdin M, Bashah MM, AlKuwari MJ, Rizwan M, Rosenthal RJ (2016) Bariatric manipulation of gastric arteries: a systematic review on the potential concept for obesity treatment. Int J Surg 36:177–182
89. Stamatovic SM, Keep RF, Wang MM, Jankovic I, Andjelkovic AV (2009) Caveolae-mediated internalization of occludin and

claudin-5 during CCL2-induced tight junction remodeling in brain endothelial cells. J Biol Chem 284:19053–19066
90. Steed E, Rodrigues NT, Balda MS, Matter K (2009) Identification of MarvelD3 as a tight junction-associated transmembrane protein of the occludin family. BMC cell biology 10:95
91. Stio M, Retico L, Annese V, Bonanomi AG (2016) Vitamin D regulates the tight-junction protein expression in active ulcerative colitis. Scand J Gastroenterol 51:1193–1199
92. Szakal DN, Gyorffy H, Arato A, Cseh A, Molnar 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 Archiv : an international journal of pathology 456:245–250
93. Tamura A, Hayashi H, Imasato M, Yamazaki Y, Hagiwara A, Wada M, Noda T, Watanabe M, Suzuki Y, Tsukita S (2011) Loss of claudin-15, but not claudin-2, causes Na + deficiency and glucose malabsorption in mouse small intestine. Gastroenterology 140:913–923
94. Tamura A, Kitano Y, Hata M, Katsuno T, Moriwaki K, Sasaki H, Hayashi H, Suzuki Y, Noda T, Furuse M, Tsukita S, Tsukita S (2008) Megaintestine in claudin-15-deficient mice. Gastroenterology 134:523–534
95. Thuijls G, Derikx JP, de Haan JJ, Grootjans J, de Bruine A, Masclee AA, Heineman E, Buurman WA (2010) Urine-based detection of intestinal tight junction loss. J Clin Gastroenterol 44: e14–e19
96. Tian R, Luo Y, Liu Q, Cai M, Li J, Sun W, Wang J, He C, Liu Y, Liu X (2014) The effect of claudin-5 overexpression on the interactions of claudin-1 and -2 and barrier function in retinal cells. Curr Mol Med 14:1226–1237
97. van Elburg RM, Uil JJ, Mulder CJ, Heymans HS (1993) Intestinal permeability in patients with coeliac disease and relatives of patients with coeliac disease. Gut 34:354–357
98. Van Itallie C, Rahner C, Anderson JM (2001) Regulated expression of claudin-4 decreases paracellular conductance through a selective decrease in sodium permeability. J Clin Invest 107: 1319–1327
99. Van Itallie CM, Fanning AS, Anderson JM (2003) Reversal of charge selectivity in cation or anion-selective epithelial lines by expression of different claudins. American journal of physiology Renal physiology 285:F1078–F1084
100. Wang F, Daugherty B, Keise LL, Wei Z, Foley JP, Savani RC, Koval M (2003) Heterogeneity of claudin expression by alveolar epithelial cells. Am J Respir Cell Mol Biol 29:62–70
101. Wapenaar MC, Monsuur AJ, van Bodegraven AA, Weersma RK, Bevova MR, Linskens RK, Howdle P, Holmes G, Mulder CJ, Dijkstra G, van Heel DA, Wijmenga C (2008) Associations with tight junction genes PARD3 and MAGI2 in Dutch patients point to a common barrier defect for coeliac disease and ulcerative colitis. Gut 57:463–467
102. Watari A, Hasegawa M, Yagi K, Kondoh M (2016) Checkpoint kinase 1 activation enhances intestinal epithelial barrier function via regulation of claudin-5 expression. PLoS One 11:e0145631
103. Watson RE, Poddar R, Walker JM, McGuill I, Hoare LM, Griffiths CE, O'Neill CA (2007) Altered claudin expression is a feature of chronic plaque psoriasis. J Pathol 212:450–458
104. 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. Laboratory investigation; a journal of technical methods and pathology 88:1110–1120
105. Willemsen LE, Hoetjes JP, van Deventer SJ, van Tol EA (2005) Abrogation of IFN-gamma mediated epithelial barrier disruption by serine protease inhibition. Clin Exp Immunol 142:275–284
106. Wisner DM, Harris LR 3rd, Green CL, Poritz LS (2008) Opposing regulation of the tight junction protein claudin-2 by interferon-gamma and interleukin-4. J Surg Res 144:1–7
107. Wray C, Mao Y, Pan J, Chandrasena A, Piasta F, Frank JA (2009) Claudin-4 augments alveolar epithelial barrier function and is induced in acute lung injury. American journal of physiology Lung cellular and molecular physiology 297:L219–L227
108. Yu AS, Enck AH, Lencer WI, Schneeberger EE (2003) Claudin-8 expression in Madin-Darby canine kidney cells augments the paracellular barrier to cation permeation. J Biol Chem 278: 17350–17359
109. Yuan L, Le Bras A, Sacharidou A, Itagaki K, Zhan Y, Kondo M, Carman CV, Davis GE, Aird WC, Oettgen P (2012) ETS-related gene (ERG) controls endothelial cell permeability via transcriptional regulation of the claudin 5 (CLDN5) gene. J Biol Chem 287:6582–6591
110. Zeissig S, Burgel N, Gunzel D, Richter J, Mankertz J, Wahnschaffe U, Kroesen AJ, Zeitz M, Fromm M, Schulzke JD (2007) Changes in expression and distribution of claudin 2, 5 and 8 lead to discontinuous tight junctions and barrier dysfunction in active Crohn's disease. Gut 56:61–72
111. Zhou W, Cao Q, Peng Y, Zhang QJ, Castrillon DH, DePinho RA, Liu ZP (2009) FoxO4 inhibits NF-kappaB and protects mice against colonic injury and inflammation. Gastroenterology 137: 1403–1414