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REVIEW

Celiac Disease: Role of the Epithelial Barrier

Michael Schumann,[1,2,3] Britta Siegmund,[1] Jörg D. Schulzke,[4] and Michael Fromm[4]

[1]Department of Gastroenterology, Infectious Diseases and Rheumatology, [4]Institute of Clinical Physiology, Campus Benjamin Franklin, Charité–Universitätsmedizin Berlin, Berlin, Germany; [2]Berlin Institute of Health, Berlin, Germany; [3]Berlin-Brandenburg School for Regenerative Therapies, Berlin, Germany

SUMMARY

Recent findings have suggested that the mucosal barrier is a primary focus of disease activity in celiac disease. Alongside the well-established remodeling of the small intestinal architecture, focal epithelial barrier defects occur with increased apoptosis and an altered tight junction–mediated permeability. Barrier-forming claudins are down-regulated while the channel-forming claudins are up-regulated, both causing a loss of ions and water to the gut lumen. An intimately regulated transcellular passage of gliadin peptides is needed for celiac disease development. As a central organizer of proteins related to barrier function, the role of epithelial polarity regulators is discussed.

In celiac disease (CD) a T-cell–mediated response to gluten is mounted in genetically predisposed individuals, resulting in a malabsorptive enteropathy histologically highlighted by villous atrophy and crypt hyperplasia. Recent data point to the epithelial layer as an underrated hot spot in celiac pathophysiology to date. This overview summarizes current functional and genetic evidence on the role of the epithelial barrier in CD, consisting of the cell membranes and the apical junctional complex comprising sealing as well as ion and water channel-forming tight junction proteins and the adherens junction. Moreover, the underlying mechanisms are discussed, including apoptosis of intestinal epithelial cells, biology of intestinal stem cells, alterations in the apical junctional complex, transcytotic uptake of gluten peptides, and possible implications of a defective epithelial polarity. Current research is directed toward new treatment options for CD that are alternatives or complementary therapeutics to a gluten-free diet. Thus, strategies to target an altered epithelial barrier therapeutically also are discussed. *(Cell Mol Gastroenterol Hepatol 2017;3:150–162; http://dx.doi.org/10.1016/j.jcmgh.2016.12.006)*

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By definition, celiac disease (CD) is an immune-mediated small intestinal disorder with a strong genetic component. In genetically predisposed individuals it is triggered by the ingestion of gluten-containing food. It is assumed that gluten is responsible for CD induction together with one or more other, nondietary factors that have not been identified yet. Gluten is a mixture of proteins found in grains such as wheat, barley, and rye, and includes peptide sequences that have the potential to elicit a small intestinal HLA-DQ2– or HLA-DQ8–restricted T-cell response. The only accepted treatment of CD is the adherence to a strict gluten-free diet (GFD). Diagnosis is established on the basis of the following: (1) a positive transglutaminase-IgA serology; (2) a duodenal histology showing villous atrophy, crypt hyperplasia, and intraepithelial lymphocytosis; and (3) by documenting a clinical improvement after introduction of a GFD.[1,2]

As documented by large epidemiologic studies in Europe, North America, and India, the burden associated with CD on the society is significant because it affects approximately 1% of the population and is associated with significant morbidity secondary to malabsorption and a moderately increased risk of developing malignancy.[3–6]

This review focuses on what is known in CD pathophysiology with respect to the intestinal barrier. Barrier function has been shown to be altered in CD for many years already.[7–9] However, it has been a matter of debate regarding its significance ever since (ie, whether barrier function contributes to the development of CD or if it is merely a phenomenon secondary to the CD immune response). Thus, this article summarizes the factors that contribute to barrier function in CD, discusses its presumed functional outcome, and refers to current and future treatment strategies that can be deduced from these insights. In this review we refer to a hierarchy of components of the intestinal barrier. The *mucosal barrier* relates to the barrier the mucosa imposes as a whole (ie, including structures such as lamina propria cells). In contrast, the *epithelial layer* or *epithelial barrier* relates solely to the single layer of intestinal epithelial cells. The term *barrier*

Abbreviations used in this paper: aPKC, atypical protein kinase C; Bmp, bone morphogenetic protein; CBC, crypt base columnar cell; CD, celiac disease; EGF, epidermal growth factor; GFD, gluten-free diet; GI, gastrointestinal; GWAS, genome-wide association studies; IEC, intestinal epithelial cell; IL, interleukin; MIC-A, major histocompatibility complex class I chain–related gene-A; SNP, single-nucleotide polymorphism; TJ, tight junction; ZO, zonula occludens.

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function refers to the functional impact that a structure (eg, the tight junction) imposes on the mucosal immune system.

Genetics

The risk of first-degree relatives being affected by CD was shown to be 7.5% in a recently published meta-analysis.[10] Having at least 2 first-degree relatives with CD in the family increases CD risk to 17%.[11] Importantly, monozygotic twins show a concordance as high as 75% for CD development, which is considerably higher than respective figures in other autoimmune diseases such as multiple sclerosis, Crohn's disease, or type I diabetes mellitus.[12] Forty percent of the genetic risk is conferred by genes encoding for the HLA class II molecules HLA-DQ2 (DQ2.5-DQA1*0501-DQB1*0201 or DQ2.2-DQA1*0201-DQB1*0202) and HLA-DQ8 (DQA1*0301-DQB1*0302).[13–15] The remaining 60% are encoded by non-HLA genes, each of which is estimated to contribute only a small effect.[16]

Making use of single-nucleotide polymorphisms (SNPs) as markers of association, genome-wide association studies (GWAS) were performed to identify further genes responsible for CD. The first GWAS included 778 CD patients and 1422 controls and analyzed 310,605 SNPs, thereby identifying a locus on chromosome 4 harboring the interleukin (IL)2 and IL21 genes.[17] Further GWAS followed with increasing resolution secondary to recruitment of several thousand celiac patients and higher resolving chips that included more than 0.5 million SNPs. These recent chips focused on distinct regions of the genome, thereby uncovering a total of 39 loci that contained 115 genes.[18,19] Several conclusions can be drawn from these GWAS. First, most of the genes identified are implicated in the control of the adaptive immune response, including genes for T-cell activation as well as cytotoxicity, IL21 production, IgA response, and B cells. Second, the function of a considerable number of genes identified in the screen is to date unknown and will be the subject of future research as exemplified by recent work from Kumar et al.[20] The investigators applied a co-expression algorithm and thereby identified 4 CD-associated genes with as-yet unknown function, which now are predicted to be involved in intestinal barrier function, especially in the actin-cytoskeleton rearrangement and cell–cell adhesion pathways. In this regard, an insight published more than 20 years ago should be recalled, namely that healthy first-degree siblings of CD patients also show a significantly altered barrier function.[7] Albeit methodologically different, both studies came to the conclusion that mechanisms determining the intestinal barrier function in CD contribute to disease development rather than being secondary to it. Third, approximately 50% of the associated SNPs affect the expression of nearby genes (ie, expression quantitative traits loci), which implies that deregulated gene expression plays a significant role in CD pathogenesis. Fourth, there is a major overlap of genes involved in CD pathogenesis with genes involved in the development of other autoimmune pathologies including type I diabetes mellitus, rheumatoid arthritis, Crohn's disease, and ulcerative colitis.[21,22] This certainly reflects clinical experience because a high percentage of CD patients suffer from one or more additional autoimmune diseases.

Main Trigger of Celiac Disease: Gluten

Glutens are storage proteins occurring in grains of wheat, barley, rye, and archaic wheats. Although the alcohol-insoluble fraction is referred to as *glutenins* and is responsible for the baking properties of the respective dough secondary to its gluing and dispersing characteristics, the gliadins are alcohol-soluble and carry most of gluten's well-described antigenic properties. Wheat grains express α-, γ-, and ω-gliadins, as well as low- and high-molecular-weight glutenins. The proportion of glutamine and proline is remarkably high (30% and 15% of all amino acids, respectively), which leads to modification by tissue transglutaminase and then contributes to establishing strong interactions of gluten epitopes with the major histocompatibility complex II complex (Figure 1), and results in resistance to degradation by gastrointestinal endopeptidases, thus facilitating the advent of large immunogenic gluten fragments at the epithelial barrier.

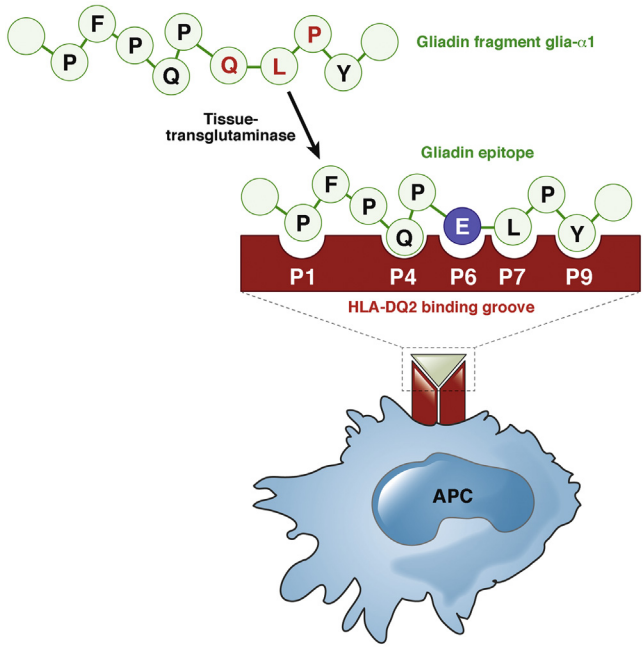


Figure 1. Binding of a gliadin epitope to the HLA complex. Gliadin fragments are deamidated by tissue transglutaminase (ie, a glutamine is transformed to a glutamate), thereby adding an additional negative charge to the epitope (*blue circle*). This facilitates binding to the DQ2 groove of the major histocompatibility complex molecule. Here, binding of the glia-α1 fragment to HLA-DQ2 is exemplified. However, this principle holds true for various oligopeptide sequences within α-, γ-, and ω-gliadin sequences, and also for binding to HLA-DQ8. Amino acids are shown in the 1-letter code with Q, glutamine and E, glutamate. The recognition motif of tissue transglutaminase within the unprocessed gliadin peptide (Q-X-P) is denoted in *red letters*. APC, antigen-presenting cell, P1…P9, binding positions within the DQ2 complex.

Additional Etiologic Factors

Both the genetic susceptibility and the presence of dietary gluten are essential factors for CD to develop. However, these 2 factors alone are not sufficient. Consistent with this notion, a significant number of CD patients are not diagnosed before age 40.[23] It has not yet been uncovered why these patients who are genetically prone to develop CD since their first day of life and who are consuming gluten since their first year of life do not develop overt disease much earlier.

In this regard, another poorly understood phenomenon related to CD etiology, known as the *Swedish epidemic*, is worth mentioning. Starting in the late 1980s, CD incidence in Sweden was increasing by 3-fold within less than 5 years and turned back to the initial incidence level in the late 1990s.[24] Surveys that monitored habits of the affected individuals of the epidemic were performed to engage potential causes for this transient shift in CD incidence and thereby to uncover additional CD etiologic factors.[25] Three major hypotheses were brought forward, as follows: (1) the protective function of breastfeeding and timing of weaning; (2) amount of gluten given during the introductory period; and (3) repeated infectious episodes.

Although the first 2 hypotheses were related to the induction of oral tolerance, the third hypothesis was associated with infection-related alterations of the intestinal barrier. In a subsequent effort to identify infant feeding strategy as a major determining factor in CD development, 2 large prospective multicenter initiatives (PreventCD and CELIPREV) involving more than 1700 newborns were initiated and generated convincing evidence that neither breastfeeding habits nor the timing or dosing of gluten during the introductory phase were relevant for later CD development.[26,27] However, a recent case–control study involving a Swedish CD cohort realized a gluten dosage effect when they analyzed gluten consumption in children who were followed up until age 8.[28]

Remarkably, the remaining assumption that previous gastrointestinal (GI) infections could trigger CD development was much older and had its origin in studies by Kagnoff et al,[29] who identified sequence similarities between peptide sequences of gluten and adenovirus peptides. They also were substantiated epidemiologically by several studies including a recent one on a cohort of more than 70,000 children with an increased CD risk when experiencing more than 10 infections up to the age of 18 months, a study that suggested a role for rotavirus infection, and a third study that reported an association with *Campylobacter* and CD in military personal.[29–32] However, it still is unclear whether infections push celiac prevalence secondary to a mechanism resembling molecular mimicry or by alteration of the intestinal barrier, the latter of which being favored by recent studies.[33–35]

Intestinal Epithelial Cells: The Mainstay of the Intestinal Barrier

The histopathologic hallmark of CD is small intestinal villous atrophy and crypt hyperplasia. Given the fact that mucosal architecture is altered severely, epithelial cell turnover turns into the focus of CD pathobiology. Research from the past decade has identified the Lgr5-positive crypt base columnar cells (CBCs) as the intestinal stem cell generating absorptive as well as secretory enterocytes that then are pushed upward along the crypt–villous axis, reaching the villous tip within 5 days where they are extruded.[36] Fueling of this conveyor belt depends not only on the 15 CBCs situated in each crypt base, but also on direct contact of the CBCs to the 10 Paneth cells nursing the CBCs with growth factors of the epidermal growth factor (EGF), Wnt, and Notch signaling pathway and inhibitors of bone morphogenetic protein (Bmp) signaling.[37,38] In Crohn's disease, Paneth cell dysfunction is well established with autophagy and endoplasmic reticulum stress as 2 pivotal mechanisms that are altered genetically (Figure 2). In celiac disease Paneth cell dysfunction also is established, specifically lysozyme was found to be secreted at reduced levels into the crypt lumen resembling the Crohn's Paneth cell defect, where mutated ATG16L1 leads to altered secretory granule secretion and dysregulated autophagy. However, a mutation in ATG16L1 is not associated with celiac disease, leaving the mechanism open for future research.[39,40] Another measure of Paneth cell activity, expression of antimicrobial α-defensins, showed a reduction of α-defensin expression in complicated CD.[41] However, it currently is unclear whether these alterations are primary defects associated with a celiac genotype or if they are secondary to the small intestinal inflammation caused by the immune response of CD. Nevertheless, evidence was brought forward that growth factor–dependent signaling within the intestinal stem cell compartment is shifted significantly, including the Wnt- and EGF-dependent β-catenin–transcription factor signaling and downstream c-myc activation (Figure 2).[42–44] Wnt signaling is targeted indirectly by distinct activity modulation of the histone H3 methyltransferase polycomb repressive complex-2, which includes the members Suppressor of zeste 12 (SUZ12) and Enhancer of zeste homolog 2 (EZH2), the first being down-regulated in celiac disease.[45] Concurrent down-regulation of the Bmp receptor ligand Bmp4 presumably contributes to crypt proliferation because inhibition of Bmp signaling was shown to be associated with ectopic crypt formation.[43,46] Similarly, c-myc gene expression and expression of EGF signal transductors were found increased in celiac disease, which is in accordance with findings that conditional gene deletion of c-myc causes loss of intestinal crypts, and up-regulation of EGF signaling leads to an amplification of the stem cell niche.[42,44,47,48] Targeting the positioning of the zone where intestinal epithelia convert from crypt enterocytes to villous enterocytes is another potential mechanism that was discussed in celiac disease because ephrin-B2 expression was found to be increased. Interaction of Eph receptors (expressed in the crypts secondary to local Wnt signaling) and ephrin ligands (expressed in the transitory zone) contributes to positioning as a result of cell repulsion secondary to direct cell-to-cell interaction of these membrane proteins.[42,49]

In addition to developmental transformations of the crypt–villous axis, one major focus of CD research has been the occurrence of focal defects in the epithelial layer within celiac lesions. Many groups have reported an increased

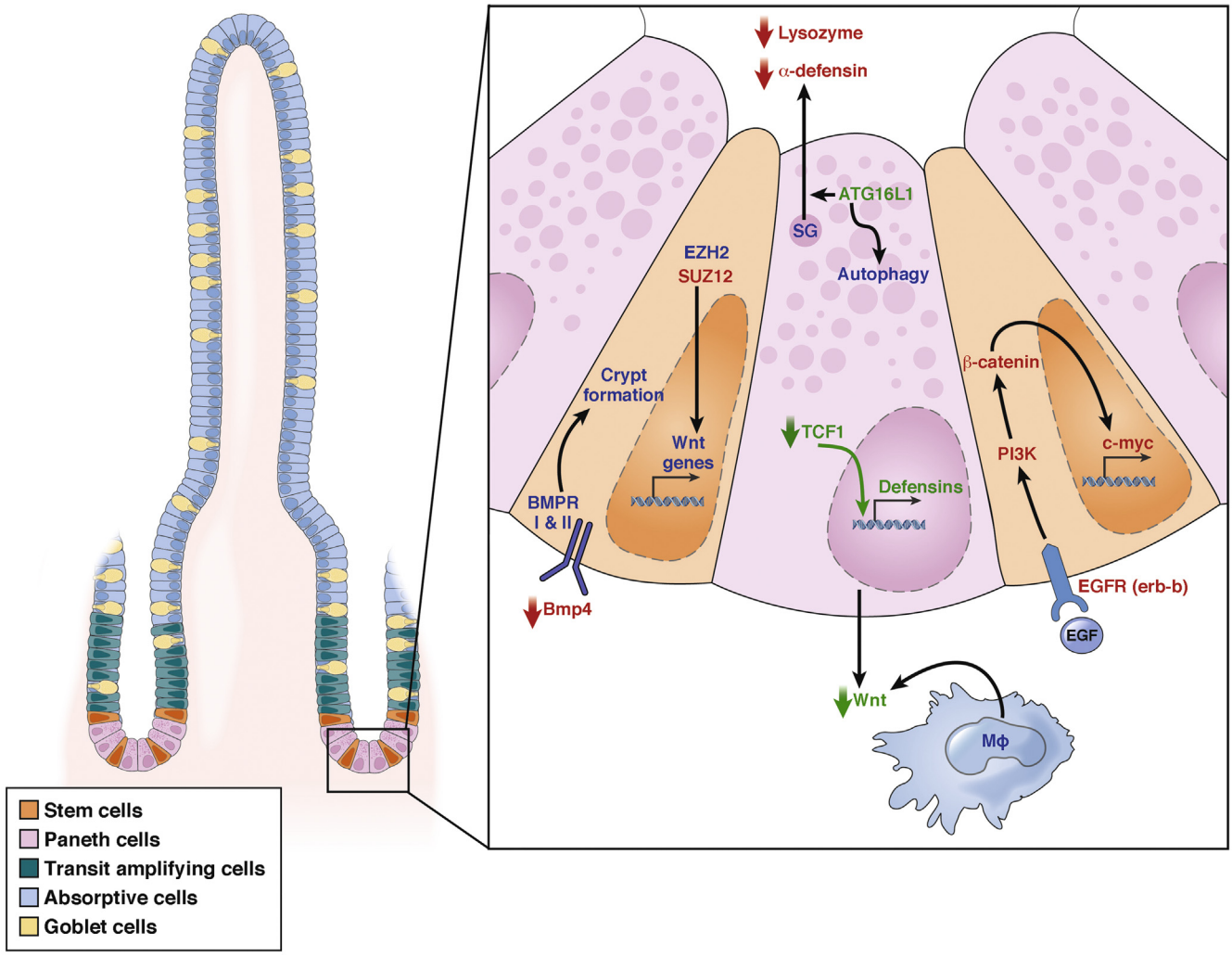


Figure 2. Crypt–villous axis of the small intestinal mucosa and alterations of the stem cell compartment found in celiac disease. For detailed explanations refer to the corresponding text. Altered signaling components relevant for celiac disease are highlighted in red, and those for Crohn's disease are shown in green. c-myc, Myc proto-oncogen; Mϕ, macrophage; SG, secretory granule; BMPR I and II, BMP receptor I and II; EZH22 and SUZ12, polycomb proteins (histone methyltransferases); ATG16L1, autophagy-related protein 16-1; TCF1, transcription factor-1; EGFR (erb-b), epidermal growth factor receptor; PI3K, phosphatidylinositol 3-kinase; β-cat, β-catenin.

number of apoptotic intestinal epithelial cells (IECs) in the celiac mucosa.[50–52] Various steps in the signal transduction initiating apoptosis are deferred, including the down-regulation of anti-apoptotic Bcl-2.[53] However, a major switch for celiac apoptosis is caused by IEC secretion of IL15 inducing up-regulation of FAS ligand in intraepithelial lymphocytes as well as epithelial FAS.[54,55] Second, IL15 cooperatively with an α-gliadin 19mer peptide elicits expression of major histocompatibility complex class I chain–related gene-A (MIC-A), a transmembrane protein that is categorized as an unconventional HLA class I molecule and functions as a stress signal on gastrointestinal epithelia.[56,57] MIC-A–expressing IECs are recognized by intraepithelial lymphocytes carrying an NKG2D-receptor, thereby causing IEC cytotoxicity. Another mechanism contributing to IEC apoptosis that also is independent of HLA class II restriction is triggered by a decapeptide sequence within α-gliadin and involves HLA A2–restricted, $CD8^+$ Fas ligand$^+$ T cells.[58]

Interestingly, an erroneous sequence of events in autophagy, which is the mechanism by which cells disassemble dysfunctional cellular components and thus specifically affects long-living cells such as, for example, Paneth cells, was shown to be a key mechanism responsible for epithelial dysfunction in Crohn's disease, another autoimmune inflammatory bowel disease affecting the small intestine. Although the respective defects of the autophagy machinery identified in Crohn's disease were not found to be relevant for CD pathogenesis,[59,60] recent work on CD biopsy specimens showed 107 of 214 autophagy-related genes to be expressed differentially, thereby suggesting that autophagy might in fact play a prominent role in CD.[61]

Tight Junction Defects in CD and Their Potential Causes

In the intestine, the epithelial monolayer constitutes a barrier consisting of the epithelial cell membranes and the apical junctional complex that defines the paracellular barrier. It is localized to the apical half of the lateral membrane of the IEC and is assembled from the tight junction (TJ) and the adherens junction (AJ). Although adherens junctions and desmosomes convey the mechanical linkage between adjacent cells, most TJ proteins seal the paracellular cleft against unlimited passage of solutes and water.[62,63] Contrary to that, some TJ proteins form—in different epithelial tissues—paracellular channels with a preference for cations (claudin-2, claudin-10b, claudin-15), anions (claudin-10a, claudin-17), and water (claudin-2).[64–67] In various pathologic conditions, the selective paracellular solute transport can be increased as a result of down-regulation of barrier-forming claudins and/or up-regulation of channel-forming claudins (Figure 3).[68]

Evidence for a defective epithelial barrier in CD comes from experimental approaches that determined ex vivo barrier function of CD mucosae by one-path impedance spectroscopy. This method allows for specific determination of the epithelial part of the transepithelial electrical resistance of the small intestinal mucosa.[52,69] Interestingly, epithelial barrier function was found to be reduced significantly, which was reversed partially in the group of GFD-adherent

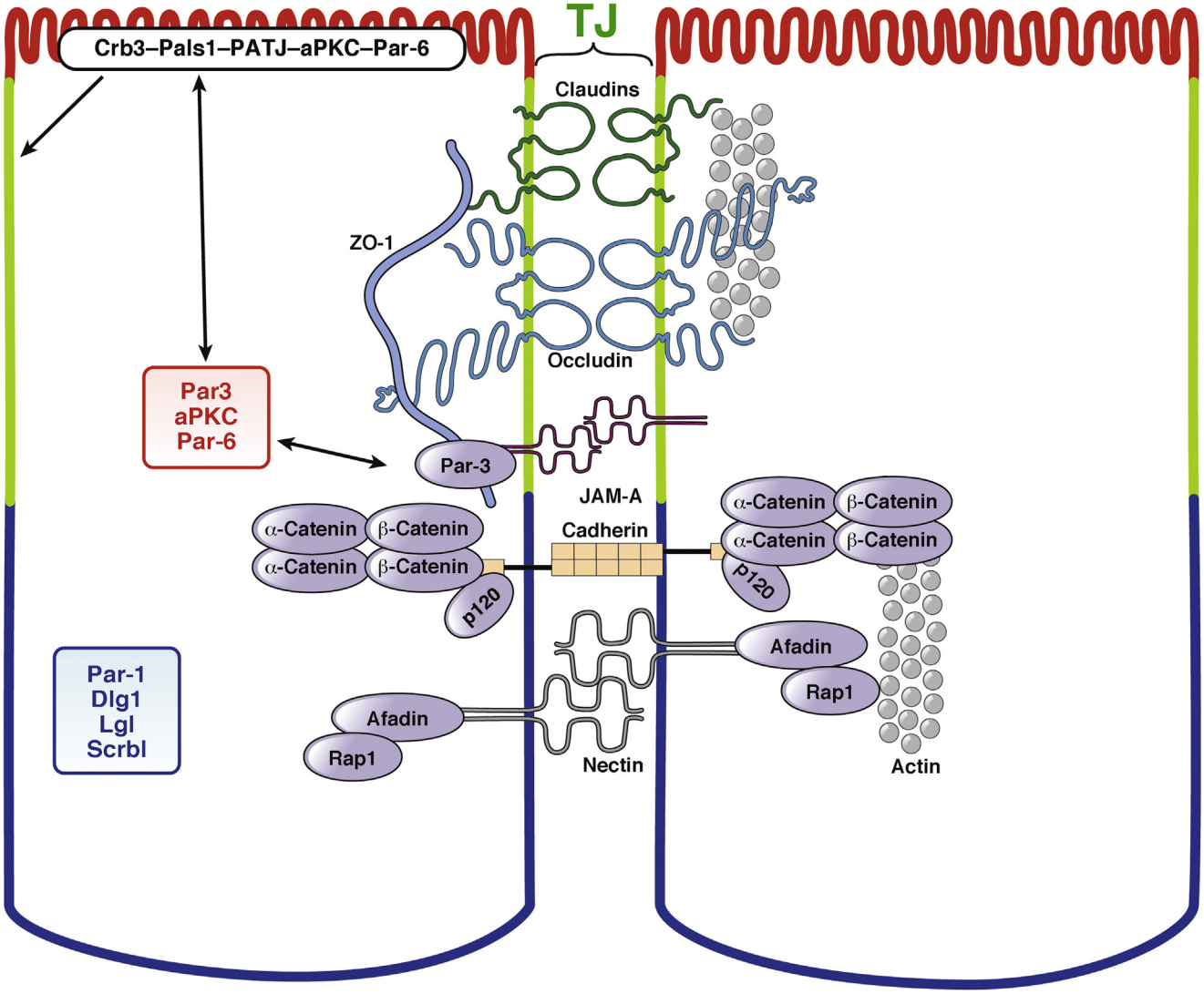


Figure 3. Composition of the apical junction and localization of polarity complexes in epithelia. The apical junction comprises the TJ and the adherens junction. Main mammalian TJ proteins are claudin-1 to claudin-27, occludin, and JAM-A, and important AJ proteins are cadherin and nectin. Although the proteins featuring 4 transmembrane domains (claudins, occludin) define the paracellular barrier or channel function for solute and water diffusion, the single-spanning proteins (JAMs, catenin, nectin) provide a mechanical linkage between neighboring cells. Note that JAMs are considered TJ proteins but do not convey barrier function. Virtually all TJ and adherens junction proteins are linked via intracellular proteins (ZO-1, Par-3, catenin, rap1) either interacting as scaffolds with the actin cytoskeleton (*grey dots*) and/or as polarity complexes with the membranes of the apical (red) or basolateral (blue) cell side.

Table 1. Total Transmural Electrical Resistance in Celiac Disease (R^t, Corresponding to Common Transmural Electrical Resistance), Consisting of Epithelial (R^{epi}) and Subepithelial Resistance (R^{sub}), as Determined by One-Path Impedance Spectroscopy

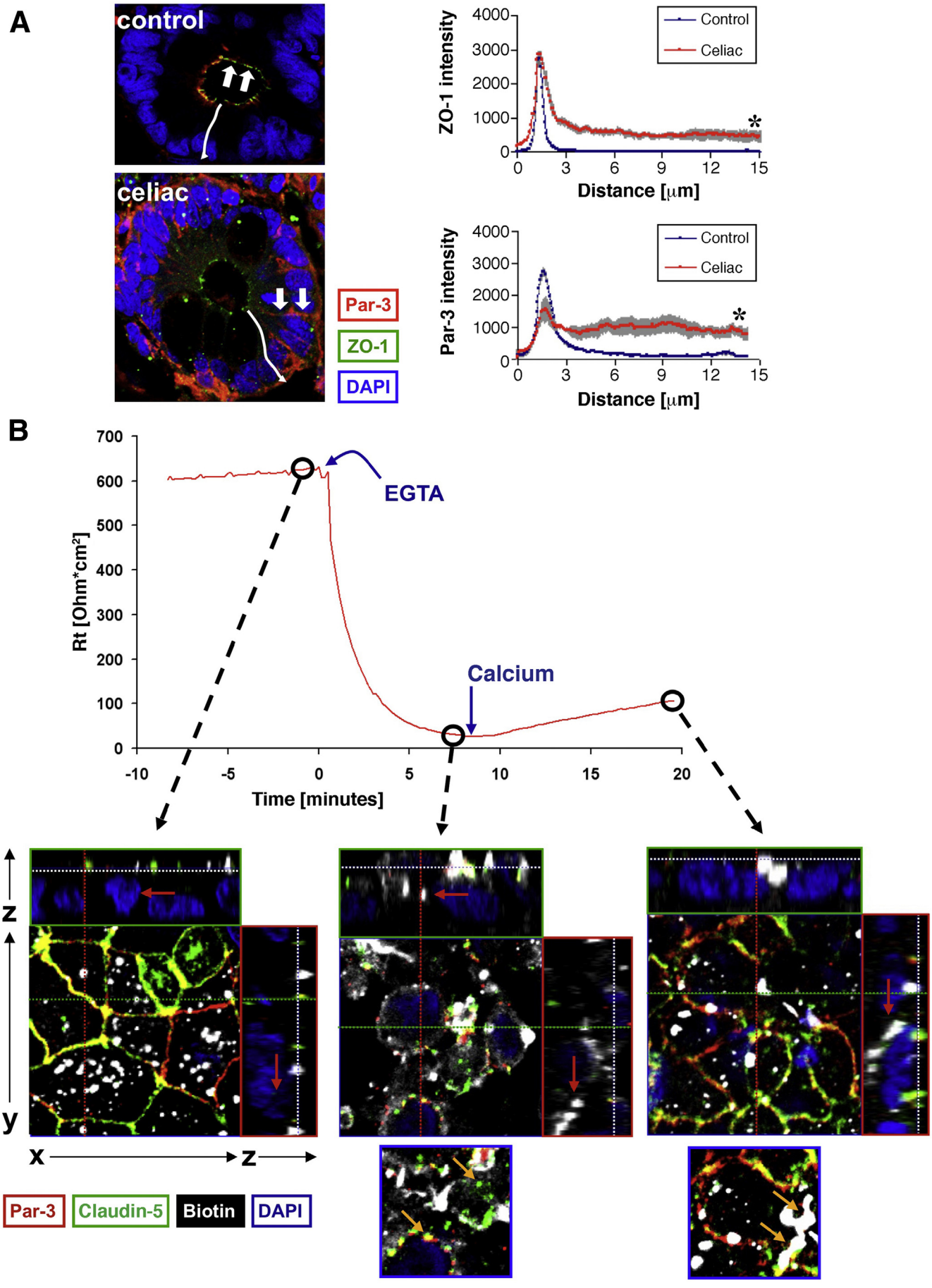
	R^t, $\Omega \cdot cm^2$	R^{epi}, $\Omega \cdot cm^2$	R^{sub}, $\Omega \cdot cm^2$	n
Control	59.8 ± 2.3	25.7 ± 1.3	34.2 ± 1.4	15
Active CD	36.6 ± 3.3[a]	13.3 ± 1.1[a]	23.4 ± 2.3[a]	8
Treated CD	52.1 ± 4.3[b]	21.6 ± 2.8[b]	30.5 ± 2.7[NS]	11

NOTE. All data represent means ± SEM. Control and active CD are data from Schumann et al[62] treated CD are original unpublished data.
[NS], not significantly different; R^{epi}, epithelial resistance; R^{sub}, subeppithelial resistance; R^t, common transmural electrical resistance.
[a]$P < .01$ (vs control).
[b]$P < .05$ (vs active CD).

CD patients (Table 1). Ultrastructurally, TJ strands were shown to be altered severely, showing a reduced number of strands as well as strand discontinuities. Again, a GFD was shown to reverse the defects only partially, introducing the potential of an intrinsic celiac barrier defect.[70] With regard to the molecular TJ composition, integral TJ proteins are differentiated from scaffolding proteins that are localized intracellularly and are associated with the TJ, mostly via PDZ-domains such as the zonula occludens (ZO) proteins.[71] The former are categorized further in single transmembrane proteins as junctional adhesion molecule proteins or tetraspanning proteins as either the claudins or the TJ-associated Myelin and lymphocyte And Related protein for VEsicle trafficking and membrane Link (MARVEL) proteins. In CD, the molecular counterpart to the TJ strand defects is a complex alteration of integral TJ proteins and includes the down-regulation of barrier-forming claudins including claudin-3, claudin-5, and claudin-7, as well as the up-regulation of channel-forming claudins such as claudin-2 and claudin-15.[52,72] The role of occludin is still not certain, with evidence pointing to a sealing function against macromolecular passage[73] and wound healing.[74] On the molecular level, the interaction of occludin and the TJ-associated molecule ZO-1 is defective, resulting in reduced levels of phosphorylated ZO-1.[75]

Epithelial Polarity as a Process to Regulate Tight Junction Permeability

The complex TJ defect described in CD mucosae suggests a coordinating master regulation. A genetic candidate approach showed 2 regulators of epithelial polarity, Partition-defective (Par-3) and Membrane-associated guanylate kinase, WW and PDZ domain-containing protein 2 (Magi2), to play a role in the CD barrier defect.[76] This insight raises the immediate question of how mechanisms of cell polarity might contribute to TJ integrity? Central to the process of epithelial polarity is the well-conserved partitioning defective protein (HUGO Gene Nomenclature: Par family cell polarity regulator) that was discovered in the nematode *Caenorhabditis elegans*, where it regulates the first asymmetric cell division of the zygote.[77,78] Intriguingly, most of the polarity proteins show a polarized distribution within the cell. Although Par-1, a serine/threonine kinase, together with the proteins Lgl and Scribble, is restricted to the basal cell compartment, Par-6, Cdc42, atypical protein kinase C (aPKC), Crumbs, and Pals1 are localized to the apical membrane and Par-3 is localized to the junctional complex (Figure 3).[79] Because membrane-associated Par proteins have the capability to diffuse freely between membrane domains, the system has to be regulated tightly by a mechanism of active exclusion (mediated by phosphorylation and by direct protein–protein interactions) to induce and maintain the functionally important polar distribution of polarity proteins.[80] Par-3, as a PDZ-domain–containing scaffolding protein, can associate with aPKC-λ and aPKC-ζ and Par-6 to form an apical polarity complex that orchestrates the formation of the apical junctional complex.[81,82] Although Ser-827 phosphorylation of Par-3 by aPKC induces association with the junctional membrane, Ser-144 and Ser-885 phosphorylation by Par-1 and consecutive binding to the 14-3-3 protein Par-5 ascertains exclusion of Par-3 from the lateral cell membrane.[83] Interestingly, the Par-3 defect found in epithelia of CD patients closely resembles the phenotype of drosophila cells that express a phosphorylation-defective mutant of the protein homologous to human Par-3. These cells are defective in performing lateral exclusion of Par-3.[52,84] As a presumptive consequence of this process in Drosophila flies as well as small intestinal mucosa from patients with CD, Par-3 is allowed to spread out to the lateral membrane and the cytosol rather than to focus at the apical junction, resulting in the aforementioned complex celiac TJ defect (Figure 4*A*). Moreover, protein phosphatase-1, mediating dephosphorylation of Par-3, is down-regulated, also contributing to reduced levels of Par-3 at the TJ.[52,79,85] Both processes add to an apparent deceleration of TJ formation that matches the phenotype of a defective celiac TJ. Interestingly, passage of the macromolecule biotin was found only in areas of disorganized Par-3 and claudin-5 membrane localization (Figure 4*B*). Another fascinating player in epithelial polarity is the serine/threonine kinase Par-4, better known under its tumor-suppressor name Lkb1. Par-4 phosphorylation conveys activity to the previously described Par-1 and thereby might play an initiator role in polarity.[86] Accordingly, activation of Par-4 was shown to be sufficient to induce polarity in intestinal epithelial cells, even in the absence of apical junctions.[87] Furthermore, Par-4/Lkb1 interacts in a kinase-independent fashion with the guanine exchange factor p114RhoGEF and, via activation of RhoA, contributes to maturation of primordial junctions to tight junctions.[88] On the other hand, loss of Par-4/Lkb1 by missense mutations results in Peutz–Jeghers syndrome, an autosomal-dominant disorder that is characterized by mucocutaneous pigmentation and gastrointestinal hamartomatous polyps, but also various cancers including small intestinal and colon cancer.[89] Thus, future research on Par-4 might uncover principles on polarity mechanisms that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Biotin
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contribute to a process such as epithelial-to-mesenchymal transformation and thus be important to pathologies as inflammation and tumorigenesis.

Impact of Zonulin on Tight Junction Permeability in Celiac Disease

An intensely discussed mechanism of modulating TJ permeability was brought forward by Alessio Fasano's group,[90] who identified zonulin as an endogenous homologue to the ZO toxin of *Vibrio cholerae*. Evidence was presented that ZO toxin as well as zonulin reversibly opens small intestinal TJs by a signaling pathway that includes the transactivation of the EGF receptor.[91] Furthermore, recent data have indicated that AT-1001, a peptide with the terminal amino acid sequence of ZO toxin, affects the gliadin-dependent F-actin rearrangement.[92]

Zonulin was shown to be secreted by IECs and LP macrophages upon gliadin exposure and thus might be part of a general inflammatory gut reaction.[91,93,94] Consistent with this, zonulin also has been associated with bacterial gastrointestinal infections, rheumatoid arthritis, systemic lupus erythematosus, and type 1 diabetes mellitus.[95,96] Thus, zonulin is neither specific for CD nor is zonulin unique because other proinflammatory mediators of barrier and TJ down-regulation have been found to be released in active CD, namely tumor necrosis factor α and interferon-γ.[97,98]

Therefore, definition of the role of zonulin in CD pathogenesis remains controversial because almost 2 decades after the first publication on zonulin, (1) no zonulin inhibition approach has been presented that completely blocked the barrier defect in CD patients, (2) zonulin concentrations affecting barrier function are surprisingly high, and (3) the exact identity of zonulin that has been proposed to be identical to prehaptoglobin-2 still is controversially discussed.[91] Nevertheless, this concept recently has reached the clinical bedside as larazotide, a drug targeting the zonulin system, which was explored in CD patients as a supplement to a gluten-free diet (see later).

Functional Impact of a Defective Paracellular Barrier in Celiac Disease

The integrity of the epithelial barrier including normal TJ function guarantees that transcellularly absorbed solutes do not leak back into the lumen via the paracellular route. However, in the case of a defective barrier, an uncontrolled paracellular flux of solutes and water into the intestinal lumen may occur and contribute to what therefore has been named a leak-flux diarrhea. This has been functionally implicated in a number of gastrointestinal disorders, including *Giardia lamblia* infection, human immunodeficiency virus enteropathy, *Clostridium difficile* colitis, and also to Crohn's disease and ulcerative colitis.[99–105]

In CD, leak-flux diarrhea appears to be a major contributing mechanism in diarrhea pathophysiology because additional secretory activity could not be identified.[106] One additionally should consider osmotic mechanisms, especially for the case of lactose, which is maldigested secondary to a loss of small intestinal lactase.

However, it is still a matter of debate whether the increased paracellular permeability contributes to the uptake of gliadin peptides or if this occurs exclusively transcellularly.

Uptake of Gliadin by the Intestinal Mucosa

Secondary to the lack of prolyl and glutamyl endopeptidases in the human gastrointestinal tract, human beings are incapable of completely digesting gliadins to a dipeptide or tripeptide level. Thus, oligopeptides and polypeptides as large as the α-gliadin 33mer remain in the intestinal lumen and are detectable even in the colon.[107] The question arises, how peptides such as the α-gliadin 19mer that induce the innate MIC-A response or the immunodominant 33mer are taken up by IECs? Two decades ago, after having shown the severely altered celiac TJ structure by freeze fracture electron microscopy, a paracellular pathway seemed to be obvious.[70] However, many researchers challenged this hypothesis, doubting that macromolecules as large as 2–4 kilodaltons would pass an epithelial barrier that merely is altered with regard to its TJs. More recent research provided evidence for a transcellular pathway instead. Most significantly, Heyman's laboratory uncovered a soluble immunoglobulin A (sIgA)-dependent uptake pathway for gliadin peptides.[33,108,109] Although CD71, a transferrin receptor, usually is expressed basolaterally in IECs, it is expressed at the apical IEC membrane in CD mucosae. An elegant study using intramucosal fluorescence resonance energy transfer proved binding of gliadin peptides that

Figure 4. (***See previous page***). **Distribution of TJ and polarity complex proteins in CD.** (*A*) Confocal LSM recordings of ZO-1 and Par-3 fluorescence signals in control and CD small intestinal mucosae. ZO-1 and Par-3 signals spread out along the lateral membrane (*small arrows*). The diagrams in the right row present apical-to-basal signal profiles along lateral membranes as indicated by the *long arrow* in the laser scanning microscopy (LSM) figure. Scale means ±SEM, $^*P < .05$. (*B*) Caco-2 cells were subjected to a combined calcium switch and biotin translocation experiment with transepithelial resistance (TER) being monitored throughout the experiment. Filters were biotinylated apically at the indicated time points to uncover local barrier defects and then immunostained (Par-3, red; claudin-5, green; biotin/streptavidin, white; and 4′,6-diamidino-2-phenylindole [DAPI], blue). Orthogonal views are presented to illustrate differences in apico–basal biotin passage associated with the integrity of the TJ expressing Par-3 and claudin-5. Before ethylene glycol-bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid (EGTA) treatment, biotin is excluded from the basal compartment. Biotin deposits appear underneath dysfunctional TJs in EGTA-treated cells (*red arrows*). After calcium replenishment the TJ partially is reorganized (ie, belt-like TJs impermeable for biotin are found next to disorganized TJ strands permeable for biotin as seen in the x-y-z collapsed stack projection; inserted panels, *arrows*). Reprinted with permission from Schumann et al.[52]

previously had been complexed by soluble IgA to apical CD71.[109] Inhibition experiments using an array of immunoglobulin competitors showed functionality and specificity of luminal-to-subepithelial gliadin transport.[33] Studies in IEC lines have suggested that trafficking of apically internalized gliadin involves the apical endosome and basolateral secretion.[52,110,111] The mechanism underlying the ectopic expression of CD71 at the apical membrane is unclear. However, one should keep in mind that trafficking of CD71 is considered to be organized within polarity processes.[87]

One as yet unresolved issue is whether a specific trigger exists that can induce peaks of gliadin translocation and thereby potentially induce active disease periods. In this regard it is worthwhile to hypothesize that again polarity of epithelia might be a key determinant. Because established triggers of depolarization include infections (or merely colonization?) with specific microbiota and a switch in polarity is sufficient to boost transcytosis of macromolecules, this mechanism appears to be an attractive hypothesis to explain outbreaks of CD activity.[112,113]

Role of Barrier Dysfunction in Celiac Disease for the Liver

It has been proposed that liver function is compromised as a consequence of CD because the celiac intestinal barrier defect is assumed to lead to the development of a reversible hepatitis that frequently is found in active CD patients with increased transaminase levels that normalize upon introduction of a GFD.[114] Of note, using an in vivo permeability test, it already was shown early on that the occurrence of an intestinal barrier defect and the development of a transaminitis are correlated significantly in CD patients.[115] This finding corresponds to current ideas on the existence of an amplifying loop in liver diseases that is initiated after proinflammatory cytokine secretion by hepatocytes and consecutively leads to an intestinal barrier defect.[116] This defective barrier function is mechanistically complex and allows for paracellular passage of bacterial products and transcellular passage of whole bacteria via the portal vein to the liver, where it aggravates the pre-existing liver damage.[117] However, another explanation for the transaminase levels in CD could be that circulating serum proinflammatory cytokines intensify hepatocyte apoptosis.

Treatment Options for Celiac Disease That Target Barrier Defects

The only treatment for CD established to date is a strict and life-long gluten-free diet. This induces mucosal healing with regard to the small intestinal architectural defects within weeks to months, presumably by abolishing T-cell–mediated immune activation. In vivo and ex vivo data have indicated that barrier function partially is restored with normalizing lactulose/mannitol permeabilities after implementation of a GFD, as well as partial remission of resistance defects measured in the Ussing chamber–based one-path impedance analysis on mucosal explants of CD patients on GFD (Table 1).

However, new therapies for CD are anticipated by the community, because adherence to a GFD results in a reduced quality of life, and various studies have reported alarmingly high percentages of CD patients being unable for various reasons to adhere to the diet sufficiently. Promising approaches include preparations of endopeptidase enzymes that effectively cleave gliadin peptides and thereby circumvent gliadin-associated immune activation, vaccination for relevant gliadin epitopes to induce oral tolerance to gluten, and transglutaminase inhibitors that will reduce affinity of gliadin peptides for T cells and thereby might ameliorate the T-cell response to gluten. It is to date unknown if the intestinal barrier is targeted directly or indirectly by these therapies. Nevertheless, with larazotide acetate (formerly named AT-1001), a barrier-targeting drug approaches the market. Previous in vitro data have suggested that larazotide is capable of stabilizing TJs and in vivo data included evidence for improving barrier function.[118,119] However, subsequent studies on larazotide in CD patients failed regarding the primary goal to stabilize the intestinal barrier as assessed by the lactulose-to-mannitol ratio.[120,121] Moreover, larazotide treatment does not revert a flat celiac mucosa into an intestinal mucosa with normal architecture. On the other hand, a low dose of larazotide reproducibly improved GI symptoms. Thus, the last study published on larazotide in CD suggested that patients suffering from persistent GI symptoms despite adherence to a GFD might benefit from GFD plus larazotide.[122]

Summary and Future Directions

Almost certainly the development of new treatment concepts for CD-related barrier defects requires a fundamental understanding of the mechanisms triggering the barrier defect including disclosure of the function of those genes that are believed to convey a defective epithelial barrier in CD. However, uncovering these mechanisms not only might open the door to new, barrier-effective therapeutics, but also identify as yet unknown additional triggers that contribute to CD development in genetically predisposed individuals.

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Correspondence
Address correspondence to: Michael Fromm, MD, Institute of Clinical Physiology, Charité Berlin, Campus Benjamin Franklin, Hindenburgdamm 30, 12203 Berlin, Germany. e-mail: michael.fromm@charite.de; fax: (49) 30 450-514962; or Michael Schumann, MD, Department of Gastroenterology, Infectious Diseases and Rheumatology, Charité Berlin, Campus Benjamin Franklin, Hindenburgdamm 30, 12203 Berlin, Germany. e-mail: michael.schumann@charite.de; fax: (49) 30 450-514962.

Conflicts of interest
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