Microorganisms in the Pathogenesis and Management of Celiac Disease (CeD)

15

Arshdeep Singh, Harmeet Kaur, Vandana Midha, and Ajit Sood

Abstract

Celiac disease (CeD) is an autoimmune enteropathy caused by an aberrant immune response to gluten in genetically susceptible individuals. Though the data are heterogeneous, there is growing evidence to support that alterations in the composition and functions of the intestinal microbiota as well as infectious exposures in early childhood are associated with CeD. However, the mechanisms by which microorganisms contribute to the development of CeD remain elusive and the causal association between microorganisms and CeD has not been established. This chapter summarizes the available evidence on the interrelation between microorganisms and CeD describing the role of microorganisms in specific pathways involved in the pathogenesis of disease. Understanding the role of microorganisms in the pathogenesis of CeD would help to develop and refine microbiota modulating tools such as probiotics, prebiotics, microbe-derived gluten degrading enzymes, and fecal microbiome transfer, that can be used as therapeutic agents in CeD.

Keywords

Celiac disease (CeD) · Gastrointestinal microbiome · Gluten · Microbiota · Probiotics · Prebiotics

A. Singh (✉) · A. Sood
Department of Gastroenterology, Dayanand Medical College, Ludhiana, Punjab, India

H. Kaur
Department of Obstetrics and Gynaecology, Dayanand Medical College, Ludhiana, Punjab, India

V. Midha
Department of Internal Medicine, Dayanand Medical College, Ludhiana, Punjab, India

M. K. Dwivedi et al. (eds.), *Role of Microorganisms in Pathogenesis and Management of Autoimmune Diseases*,
https://doi.org/10.1007/978-981-19-4800-8_15

15.1 Introduction

Celiac disease (CeD) is a systemic, chronic, inflammatory, autoimmune enteropathy that affects approximately 1% of the global population (Singh et al. 2018). The disease is characterized by T-cell-mediated damage to small bowel mucosa caused by ingestion of gluten in genetically susceptible individuals. Though both genetic susceptibility and exposure to gluten are required, these alone are not sufficient, and an additional predisposing factor is necessary for the development of CeD. This is supported by the fact that nearly one third population of the world carries the celiac compatible HLA haplotypes (HLA DQ2 and/or HLA DQ8 genes), and yet only 1% of the population develops CeD (Yuan et al. 2013; Rostami-Nejad et al. 2014; Singh et al. 2018; Caio et al. 2019). Additional non-HLA genes have been identified in patients with CeD, though their clinical relevance is unclear. The other predisposing factors for CeD apart from genes and dietary gluten are the environmental influences, of which the intestinal microbiota has emerged as an important determinant. Given the myriad functions performed by the intestinal microbiota in maintaining gut homeostasis, facilitating digestion and absorption, and modulating the immune responses of the host by maintaining a balance between immune-competence and immune-tolerance, it is not surprising that alteration in the composition and function of microbiota is associated with the development of the disease (Feng et al. 2018). Implicated in disease pathogenesis, microbiota presents itself as an attractive target for use as a therapeutic agent. This chapter reviews the pathogenesis and management of CeD in light of the role played by intestinal microbiota.

15.2 Pathogenesis of Celiac Disease

Gluten, a protein composed of a complex group of alcohol-soluble gliadins and alcohol-insoluble glutenins, is the primary environmental trigger for CeD. Both the time of introduction of gluten in diet and the amount of gluten ingested have been implicated in the pathogenesis of CeD. Gliadins are digested partially by the proteolytic enzymes present in the intestinal lumen. These partially digested gliadin peptides (GP), via paracellular (through zonulin, that disintegrates intercellular tight junctions) and transcellular pathways, gain entry to the lamina propria, where, after deamidation by tissue transglutaminase (tTG2), they initiate aberrant innate [characterized by high interleukin 15 (IL15), interleukin 8 (IL8), and intraepithelial lymphocytes]; and adaptive and humoral (characterized by Th1- and Th17-mediated increased production and release of pro-inflammatory cytokines, and activation of B cells) immune responses. The pro-inflammatory milieu in the lamina propria damages the enterocytes, that further compromises and disrupts the intestinal barrier, and in the process compounds the immune-mediated injury to the enterocytes (Kupfer and Jabri 2012; Tye-Din et al. 2018).

CeD has a strong genetic component with high prevalence in monozygotic twins and first-generation relatives. The HLA genes play an important role in recognizing and presenting the deamidated GPs to T cells, activating the downstream cascade of

production of inflammatory cytokines. The non-HLA genes have also been hypothesized to regulate intestinal permeability, proliferation of B and T cells and pro-inflammatory cascade (Sharma et al. 2016). Figure 15.1 shows a schematic representation of the pathogenesis of CeD.

Intestinal microbiota plays an important role in the pathogenesis of CeD as it interacts with both gluten (the environmental trigger) and the genes (the susceptive host). Furthermore, the intestinal microbiota maintain the integrity of the intestinal epithelium, and the products of microbial metabolism modulate the inflammatory immune response. Though the intestinal microbiota comprises of bacteria, fungi, viruses, archaea, protozoa, and helminths; the bacteria are the most abundant and widely studied group of microorganisms. In the current chapter therefore, the term microbiota would primarily refer to intestinal bacteria. A detailed discussion on the role of fungi, viruses, and other microorganisms is beyond the scope of the chapter and has been discussed elsewhere in literature.

15.3 Dysbiosis in CeD

Using the gnotobiotic approach, the germ-free rats fed with gliadin were found to develop more severe intestinal damage as compared to the rats with normal intestinal microbiota (Stěpánková et al. 1996; Galipeau et al. 2011), suggesting that the intrinsic anti-inflammatory properties of the normal intestinal microbiota have the capacity to reduce the pro-inflammatory effects of gluten. Microbial dysbiosis (defined as a change in the structure and/or function of the resident intestinal microbiota) is therefore expected to predispose to the development and progression of immune-mediated damage in CeD. Duodenal mucosal biopsies as well as fecal samples from patients with CeD have demonstrated an altered microbial profile, though no specific group of microbes has been established as the cause.

Patients with active disease have shown increased abundance of *Clostridia* spp., *Bacteroides* spp., and *Prevotella* spp. and decreased abundance of *Lactobacillus* spp., *Enterococcus* spp., and *Bifidobacterium* spp. in the duodenal biopsy and fecal samples (Collado et al. 2009; De Palma et al. 2010b; Di Cagno et al. 2011). Further, Di Biase et al. reported *Enterobacteria* to be more abundant in the duodenal biopsies of untreated CeD patients as compared to *Bacteroidetes* or *Streptococcus*, whereas *Bacteroides-Prevotella*, *Akkermansia*, and *Staphylococcaceae* had lower abundances in the fecal samples of the same patients (Di Biase et al. 2021). Recently, a specific fecal microbiota signature for CeD, with decreased abundance of 11 operational taxonomic units (OTUs), the two most common being OTU_531 *Clostridium* sensu stricto and OTU_143 *Ruminococcus*, has been described in Scottish children, though it needs validation across larger and ethnically distinct populations (Zafeiropoulou et al. 2020). A low ratio of lactic acid bacteria-*Bifidobacterium* vs *Bacteroides-Enterobacteria* in feces or duodenal biopsy, as compared to healthy controls, characterizes CeD (Macfarlane et al. 1998; Di Cagno et al. 2009, 2011). High *Escherichia coli* and *Staphylococcus* and low *Lactobacillus* prevalence have been observed in individuals with CeD when compared to healthy controls (Schippa

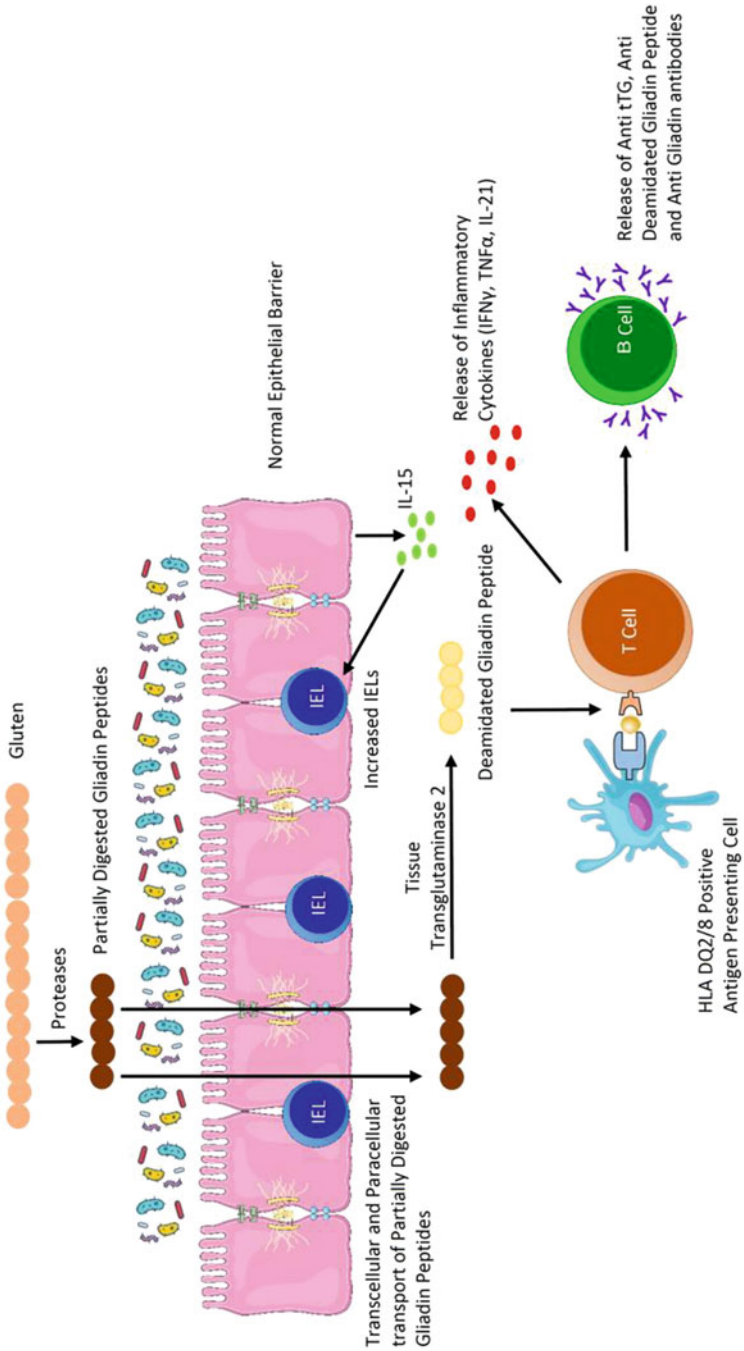

Fig. 15.1 Schematic representation of pathogenesis of celiac disease

Table 15.1 Intestinal bacterial dysbiosis in celiac disease

Increased abundance	Decreased abundance
Bacteroides spp.	*Lactobacillus* spp.
Escherichia coli	*Bifidobacterium* spp.
Staphylococcus spp.	*Akkermansia muciniphila*
Neisseria spp.	*Faecalibacterium*
Gram-negative bacteria	*Clostridium* cluster XIVa

et al. 2010; Sánchez et al. 2013; Lorenzo Pisarello et al. 2015). Pro-inflammatory microbial dysbiosis is also associated with symptoms of CeD. Overrepresentation of *Proteobacteria* has been reported to correlate with disease activity. Increased frequency of *Bacillaceae* and *Enterobaeriaceae* correlated with abdominal pain, whereas an increase in *Bacillaceae* and *Fusobacterium* and reduction in *Clostridium* cluster XIVa and *Akkermansia* correlated with diarrhea (Di Biase et al. 2021).

To summarize, the published studies on characterization of microbiota in CeD demonstrate an increased abundance of Bacteroides (*Bacteroides vulgatus* and *Bacteroides fragilis*), *Escherichia coli* and *Staphylococcus* spp. and decreased *Lactobacillus* spp., *Bifidobacterium* (*Bifidobacterium longum*), and *Akkermansia muciniphila* in the duodenal biopsy or fecal samples. The studies on prevalence of *Prevotella* in patients with CeD are conflicting and inconsistent. The bacterial dysbiosis observed in CeD is summarized in Table 15.1.

15.4 Role of Intestinal Microbiota in the Pathogenesis of CeD

Whether the association between bacterial dysbiosis in CeD is causal or not is a matter of debate. However, the intestinal microbiota dysbiosis remains at the center of disease pathogenesis (Fig. 15.2). The interplay between microbiota and specific pathways involved in the development of disease are discussed in the following sections.

15.4.1 Interactions Between HLA DQ Haplotypes and Microbiome

Associations between intestinal microbiota, host genetics, and single-nucleotide polymorphisms (SNPs), studied by two-sample Mendelian randomization analysis, have revealed that all the CeD SNPs identified themselves with *Firmicutes* and *Proteobacteria* phyla, thereby suggesting that the intestinal microbial composition in at-risk infants is influenced by the genetic make-up (García-Santisteban et al. 2020).

At phyla level, an increased abundance of *Firmicutes* and *Proteobacteria* and decreased abundance of *Bacteroidetes* and *Actinobacteria* have been seen in the fecal samples before the onset of disease in infants carrying celiac compatible HLA haplotypes (Sellitto et al. 2012). A higher *Bacteroides-Prevotella* proportion and an increased abundance of Gram-negative bacteria, *Streptococcus*, *Lactococcus*, and *Clostridium histolyticum* have been reported in high-risk infants in another study

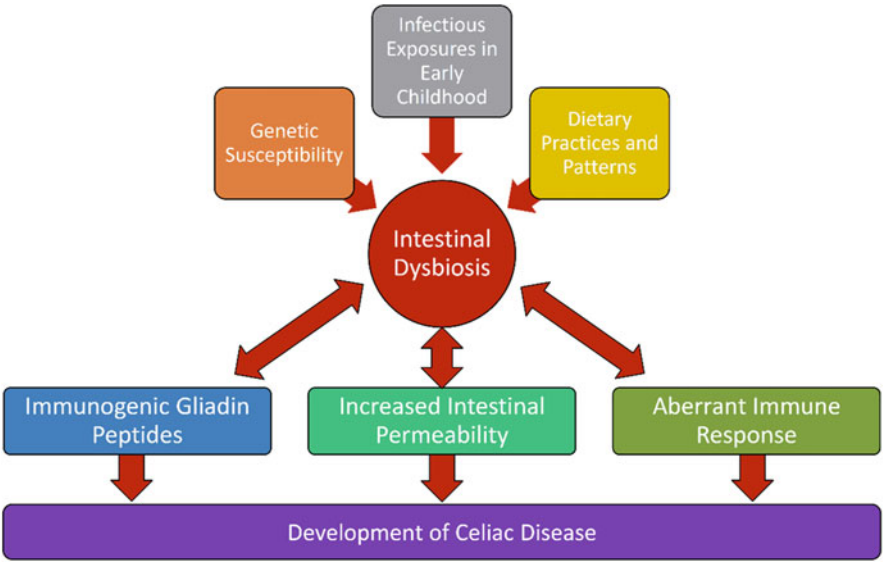

Fig. 15.2 Flow diagram depicting intestinal dysbiosis at the center of pathogenesis of celiac disease

(De Palma et al. 2010a). Other bacterial genera that are altered in at-risk patients include *Streptococcus*, *Coprococcus*, *Veillonella*, *Parabacteroides*, and *Clostridium perfringens* (Leonard et al. 2020).

Lower prevalence of *Bifidobacterium longum* has been reported in at-risk infants and infants with family history of CeD (Olivares et al. 2015, 2018). This suggests that genetic susceptibility to CeD inhibits the colonization of protective, anti-inflammatory bacteria such as *Bifidobacterium* in the gut, enhancing the risk for development of disease.

The presence of HLA haplotypes also influences the dynamics of intestinal microbiota. Various microbial metabolism and functional pathways have been reported deficient in infants between the ages of 4 and 6 months. The metabolites that are altered in genetically predisposed infants are butanoic acid (increased) and dihydroxyacetone (decreased) (Leonard et al. 2020).

The phylogenic diversity of the intestinal microbiota increases with age during infancy. However, it has been observed that in patients at risk for CeD, the phylogenic diversity does not increase significantly over time. This uncommon pattern of premature maturation of the intestinal microbiota during early stages of life is another way by which the genes influence the microbiome predisposing to CeD (Olivares et al. 2018). On the contrary, a delayed stabilization of the intestinal microbiota occurring beyond 12–24 months of age in genetically at-risk infants has also been described (Sellitto et al. 2012). The host genetics, apart from the environmental (e.g., feeding practices) influences, plays a major role in these abnormalities

in the time-related development, maturation, and stabilization of the intestinal microbiota.

These observations lead to the hypothesis that specific host genotype determines the composition of intestinal microbiota. The genetic make-up of the host selects specific microbial colonizers and rejects others to vest an increased risk of CeD.

15.4.2 Interactions Between Gluten and Microbiome

Pseudomonas aeruginosa (belonging to phyla *Proteobacteria*) has proteolytic activity against the immunogenic GPs (produced by partial digestion of gluten). Proteolysis of these GPs produces smaller peptides that rapidly cross the intestinal mucosal barrier and elicit a heightened immune response from gluten-specific T cells. On the contrary, *Lactobacillus* degrades and converts the immunogenic peptides (33-mer peptide) to non-immunogenic peptides (Caminero et al. 2016). *Lactobacillus* has also shown the ability to reduce intestinal inflammation by degrading the wheat amylase trypsin inhibitor in HLA $DQ8^{+}$ mice (Caminero et al. 2019a, b). *Bifidobacterium* strains also attenuate intestinal inflammation by modifying the toxic GPs generated during digestion (Laparra and Sanz 2010). Other oral (*Rothia*, *Streptococcus*, *Neisseria* and *Capnocytophaga*) and intestinal (*Bacteroides*, *Lactobacillus*, *Streptococcus*, *Staphylococcus*, and *Clostridium*) bacteria-derived enzyme activities also play an important role in the digestion of gluten (Herrán et al. 2017). Thus, intestinal bacteria have different activities and different metabolic patterns to handle digestion of gluten.

15.4.3 Interactions Between Microbiome and Intestinal Barrier

Maintaining intestinal barrier is an important step in preventing gluten sensitivity as increased mucosal permeability (both paracellular and transcellular) allows enhanced passage of immunogenic GPs into the lamina propria where they initiate the cascade of an aberrant immune response. The leaky intestinal barrier in CeD can be due to either activation of molecular pattern recognition receptors or modifications in the mucus layer or tight junctions.

The expressions of molecular pattern recognition receptors, Toll-like receptors (TLR), especially TLR2 and TLR9, and Toll-interacting protein (TOLLIP), are altered in duodenal biopsies of patients with CeD, suggesting the role of microbiota-associated factors in the pathogenesis of CeD (Kalliomäki et al. 2012). Also, pathogenic bacteria, via activation of TLR-4 and CD14, activate the innate immune system to produce pro-inflammatory cytokines. CXCL10 is a ligand for CXCR3 and the CXCL10/CXCR3 complex activates zonulin to increase the intestinal permeability. The CXCL10/CXCR3 axis can also be activated by bacterial antigens amplifying the gluten-induced inflammatory process (Heyman et al. 2012).

Animal studies have demonstrated the deleterious effects of *Enterobacteria* (*Escherichia coli* and *Shigella*) on the goblet cell numbers and integrity of tight

cell junctions in CeD (Cinova et al. 2011). *Bacteroides fragilis* and *Escherichia coli* also express metalloproteases that increase the intestinal permeability by the disruption of tight junctions (Glotfelty et al. 2014; Ménard et al. 2012). In contrast, *Bifidobacterium bifidum* and *Lactobacillus rhamnosus* are known to increase the number of goblet cells, produce metalloproteinase inhibitors, decrease the expression of NF-κB and chemokine CXCR3 receptors, contributing to maintenance of intestinal barrier and reduced translocation of GPs to lamina propria (Cinova et al. 2011; Orlando et al. 2014). Furthermore, *Akkermansia muciniphila*, a symbiont bacteria that regulates the synthesis of mucin, is deficient in CeD. The deficiency of *Akkermansia* impairs the mucin production to compromise the intestinal barrier (Bodkhe et al. 2019).

Short-chain fatty acids (butyrate in particular), produced by intestinal bacteria, have also been shown to regulate intestinal permeability and epithelial integrity by upregulating the gene expression of barrier proteins in CeD-derived organoids (Freire et al. 2019). *Lactobacillus rhamnosus* GG has been demonstrated to protect the epithelial barrier in Wistar rats from GP-induced enteropathy by favorably impacting the intestinal mucosal architecture and upregulating the expression of intercellular junction proteins (zonulin, occludin, claudin-1, β-catenin, and E-cadherin). However, the beneficial effects are observed only in animal models and that too once the enteropathy has set in. No protective or prophylactic effects of *Lactobacillus rhamnosus* have been elicited in human studies, as yet (Orlando et al. 2018).

The intestinal epithelial cells also maintain the sterility of the mucous layer by producing antibacterial C-type lectin (RegIIIγ). The production of RegIIIγ is regulated by the intestinal microbiota. The deficiency of RegIIIγ results in microbiota-dependent increase in Th1 cells in the lamina propria disrupting the immune homeostasis (Krishnareddy 2019). The microbes are therefore vital to maintain the functionality of the intestinal barrier. Disruption of the intestinal epithelium by dysbiotic microbes grants access to the GPs and other immunogenic bacterial peptides to gain entry to the lamina propria, favoring the development of CeD.

15.4.4 Interactions Between Microbiome and Immune Response in Celiac Disease

Microorganisms are vital for the maintenance of the architecture of the gastrointestinal immune system. Apart from maintaining the mucosal barrier between microbe-rich intestinal lumen and epithelial cells, the microbiota stimulate the formation of gut-associated lymphoid tissue (GALT) and regulate the immune responses by modulating the synthesis and function of various immune mediators.

The GALT synthesizes IgA in response to the identification of the non-commensal bacterial antigens in the intestinal lumen. The secreted IgA coats these bacterial antigens and limits the immune responses to the mucosal surface only, avoiding systemic inflammation. The secretion of IgA is dependent on the

microbiota. Germ-free (GF) mice have been shown to have reduced germinal centers in GALT, impairing the immunomodulatory properties intrinsic to GALT. *Bifidobacterium* species have been demonstrated to stimulate the synthesis of secretory IgA in Swedish infants, protecting against autoimmunity (Sjögren et al. 2009).

CeD is associated with increased intra-epithelial lymphocytes (IELs). The intestinal microbiota can affect the distribution and function of IELs through TLRs, NOD-2, or the aryl hydrocarbon receptors. *Pseudomonas aeruginosa* expresses gluten degrading proteases, which, through PAR-2 signaling, induce an innate immune response increasing the number of IELs. Animal studies have shown this induction of IELs by *Pseudomonas aeruginosa* to be independent of gluten, though the effect is synergistic (Caminero et al. 2019a).

The Paneth cells in the intestinal epithelium secrete defensins (the natural antimicrobials of the intestinal tract) and regulate the intestinal microbiota composition. The secretion of defensins is controlled by specific bacteria present in intestinal crypts. In patients with active CeD, the number of Paneth cells and levels of B-defensins are decreased. Treatment of CeD with gluten-free diet (GFD) restores the microbial dysbiosis as well as the normal levels of B-defensins, suggesting normalization of Paneth cell function (Tobi et al. 2021). There is currently a wide knowledge gap between Paneth cells, microbiome, and CeD, which needs to be bridged by performing focused research in future.

The immune responses in CeD are characterized by an upregulated Th1 cell-mediated immune response effected via macrophages, cytotoxic $CD8^+$ T cells, IFNγ and $CD4^+$ T cells. Studies have shown that increased expression of Th1 cytokines in CeD is partly contributed by microbial dysbiosis. The intestinal microbiota maintain the Th1/Th2 balance. *Bifidobacterium* and *Lactobacillus* species suppress the Th1-dependent pro-inflammatory cytokines (e.g., tumor necrosis factor-α, interleukin-1β, and IFNγ) abating the pro-inflammatory milieu and reinstating the Th1/Th2 balance (Medina et al. 2007, 2008; Baba et al. 2008; D'Arienzo et al. 2011; Palma et al. 2012). Reduced predominance of *Bifidobacterium* and *Lactobacillus* in CeD therefore augments the aberrant immune responses in CeD. Furthermore, the mucosal IL17A immune response is not seen uniformly in all the patients. The inconsistency of IL17A-driven immune response can be explained by the isolation of *Lachnoanaerobaculum* spp. and *Actinomyces* spp. in some patients of CeD. These bacteria trigger the IL17A-mediated immune response (Sjöberg et al. 2013).

Commensal *Clostridia* (Cluster IV and IVa) are inducers of regulatory T cells (Tregs). Reduced prevalence of these clusters in CeD affects the induction of Tregs, throwing the intestinal homeostasis into disarray (Cheng et al. 2013). Bacterial dysbiosis (predominance of *Escherichia*, *Neisseria*, and *Staphylococcus*) in CeD is also associated with increased expression of virulent genes and pro-inflammatory cytokines in vitro (Sánchez et al. 2012). The products of bacterial metabolism, specifically the SCFAs, also modulate the immune responses. Altered production of butyrate and lactate in CeD triggers posttranslational modifications (epigenetic switch between the two isoforms of the forkhead box protein 3) impairing the immune-suppressive functions of Tregs (Serena et al. 2017).

Furthermore, some intestinal bacteria express epitopes that mimic gliadin, and the molecular mimicry may potentially trigger immune responses. Two major peptides from *Pseudomonas fluorescens* have been demonstrated to cross react with immune stimulatory gliadin epitopes. This cross-reactivity is proposed to be based on functional molecular mimicry between peptide sequences from specific bacteria and T-cell immunogenic gluten peptides (Petersen et al. 2020). However, the translocation of the bacterial peptide sequences into the lamina propria, which houses the adaptive immune system, and the enterocyte damage induced by these peptides need to be evaluated in detail. The immune responses in CeD are thus shaped by, in addition to the immunogenic effects of gluten, the intestinal microbiota. Fig. 15.3 depicts the different steps at which microbiota interact with the host during the development of CeD.

15.5 Role of Infections in the Development of CeD

Infections during early childhood, especially during neonatal period, have been associated with the subsequent development of CeD. Repeated or persistent infections result in constant low-grade intestinal inflammation (through molecular mimicry, intestinal dysbiosis, or infection/inflammation-driven activation of immune responses) that breaks down gluten tolerance in genetically predisposed individuals. The common offenders are *Campylobacter jejunii*, *Clostridium difficile*, *Streptococcus pneumoniae*, *Rotavirus*, *Adenovirus*, *Reovirus*, and *Enterovirus* (Sánchez et al. 2021; Størdal et al. 2021). Interestingly, infections with cytomegalovirus (CMV), Epstein–Barr virus (EBV), and *Helicobacter pylori* have been reported to be protective against CeD (Lerner et al. 2017). The protective effects are hypothesized to be mediated through the alteration of host immune response, reduction of gluten immunogenicity, and modulation of intestinal permeability (Lerner et al. 2017). However, the long lag time between exposure to infectious agents and development of disease makes it difficult to establish the exact relationship between the two. Repeated longitudinal sampling is required to determine the differences between colonization and clinical disease. If the causal relationship is established, vaccination against the offending infectious agents could prevent the development of CeD.

15.6 Microbiota Targeted Therapy for CeD

The cross talk of intestinal microbiota with host genetics, gluten, intestinal epithelial barrier, and the immune system makes microbiota an attractive therapeutic target for the treatment of CeD. The modulation of intestinal microbiota can be done by diet (GFD for CeD), antibiotics, pre/probiotics, and fecal microbiome transfer (FMT). Each of these approaches has been explored as therapy for CeD, with variable success. Additionally, microbiota-derived enzyme-based therapy and specific microorganism-based therapy have also been tested (Table 15.2).

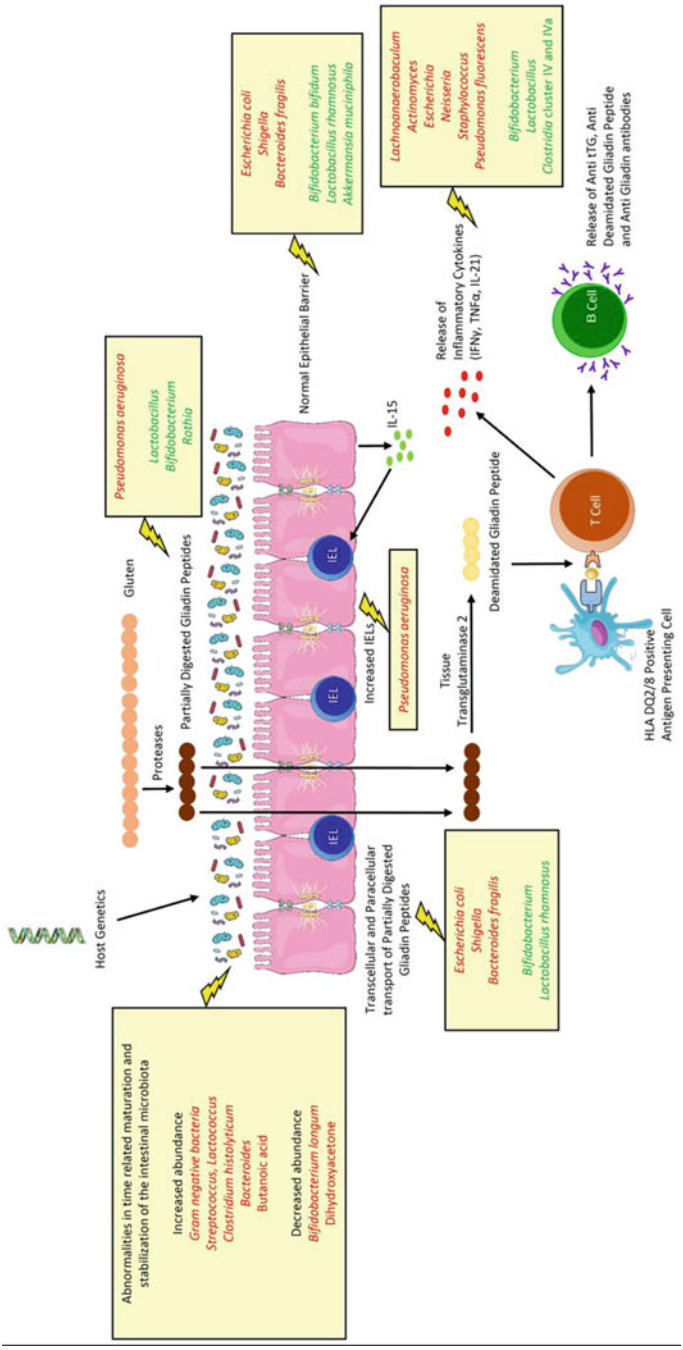

Fig. 15.3 Interactions of intestinal microbiota with specific pathways in the development of celiac disease (CeD). The microbes involved at a particular step in the pathogenesis of celiac disease are depicted in yellow boxes. Microbes written in red increase the susceptibility to develop celiac

15.6.1 Dietary Intervention: GFD

The composition of intestinal microbiota is determined by the dietary patterns and practices. The only accepted treatment of CeD to date is GFD. GFD reduces the clinical symptoms and reverses the enterocyte damage induced by gluten and microbial immunogenic peptides. However, the effect of GFD on the composition of the intestinal microbiota is inconsistent across different studies. Though the intestinal bacterial diversity has been demonstrated to improve on GFD, it is not completely restored. Between treated and untreated patients of CeD, differences in abundances of 39 OTUs have been observed (Zafeiropoulou et al. 2020). *Bifidobacterium* and *Lactobacillus* prevalence has been reported to be lower on GFD as compared to healthy controls, though the relative abundance was greater as compared to untreated CeD (Collado et al. 2008; Di Cagno et al. 2009; Golfetto et al. 2014).

In children treated with GFD, high frequencies of *Bacteroides*, *Staphylococcus*, *Salmonella*, *Shigella*, and *Klebsiella* have also been documented in fecal samples, when analyzed by culture-dependent methods (Di Cagno et al. 2011). Mucosal healing occurring on GFD also determines the microbial composition. The differences in the composition of intestinal microbiota on GFD can be explained to some extent by changes in the dietary patterns after initiation of GFD (reduced intake of complex carbohydrates and fiber) and may not only be a result of the disease effect. Similar changes in the intestinal microbiota, in patients on GFD who received dietary interventions for diseases other than CeD, corroborate this hypothesis. The differences in the microbial signals on GFD, as compared to untreated disease, indicate toward modulation of microbiota with GFD, and identification of these bacteria could guide the development of immune modulating microbial therapeutics.

15.6.2 Probiotics

Probiotics are live microorganisms, which when ingested in adequate amounts, confer health benefit to the host. Acknowledging the direct as well as indirect role of microorganisms in the pathogenesis, probiotics have been tested as therapy in multiple in vitro and in vivo studies to assess the benefits of probiotics in regulating and harmonizing the microbial dysbiosis associated with CeD. Additionally certain probiotics are also known to have intrinsic anti-inflammatory properties that may lessen the gluten-derived inflammation, suggesting therapeutic benefits. The effects of probiotics on clinical symptoms, quality of life (QoL), levels of inflammatory cytokines, and intestinal permeability have been evaluated and reported in literature.

Fig. 15.3 (continued) disease, while those written in green have protective effect and have the potential to be used as therapy for celiac disease

Table 15.2 Microbiota targeted therapies for celiac disease

Therapy	Effect on					References
	Clinical symptoms	Quality of life	Inflammatory cytokines	Intestinal permeability	Mucosal healing	
Gluten-free diet	Improve	Improve	Decrease	Decrease	Occurs	
Probiotics	Improve	No effect	Decrease, the levels increase on stopping probiotics	Conflicting data	Not known	Seiler et al. (2020)
Prebiotics	Not known	Not known	Not known	Not known	Not known	Capriles and Arêas (2013), Krupa-Kozak et al. (2017)
Fecal microbiome transfer	Not known	Not known	Not known	Not known	Not known	ClinicalTrials.gov identifier NCT04014413
Microbiota-derived glutenases	Not known	Not known	Decrease	Not known	Not known	Lindfors et al. (2008), Mandile et al. (2017), Francavilla et al. (2019), Caminero et al. (2019a, b)
Helminth therapy	Not known	Not known	Not known	Not known	Not known	Croese et al. (2015)

Lactobacillus strains and *Bifidobacterium* spp., either alone or in combination, are the most widely studied probiotic bacteria in CeD.

Use of probiotics in CeD is associated with improvement in gastrointestinal symptoms (abdominal distension, bloating, diarrhea, constipation, abdominal pain, and vomiting) as compared to placebo (Smecuol et al. 2013; Olivares et al. 2014; Francavilla et al. 2019). A systematic review and meta-analysis found that ingestion of probiotics was associated with an improvement in GI symptoms in patients with CeD. However, the quality of evidence for overall improvement in gastrointestinal symptoms is low and has a high risk of being biased (Seiler et al. 2020). Only two studies have reported the effects of probiotics on QoL, and a pooled analysis did not demonstrate improvement in QoL of CeD patients with probiotics (Harnett et al. 2016; Francavilla et al. 2019).

The effect of probiotics on serum concentration of inflammatory cytokines has been reported. A mixture of GPs and *Bifidobacteria* has been reported to down regulate the synthesis and release of pro-inflammatory cytokines in vitro (Medina et al. 2008; Laparra and Sanz 2010). Probiotic *Saccharomyces boulardii* KK1 strain, in mice studies, has been associated with a decreased production of inflammatory cytokines and reversal of histological changes (Papista et al. 2012). In adults with CeD, *Bifidobacteria*-based probiotics decrease the concentration of serum TNF-α (Primec et al. 2019). In children, however, the values decrease initially, but the effect was seen to wane 3 months after stopping probiotics, suggesting continuous rather than intermittent administration of probiotics is beneficial (Klemenak et al. 2015).

In vitro studies have shown decreased intestinal permeability with the use of *Lactobacilli* and *Bifidobacterium* strains in a dose-dependent manner (Lindfors et al. 2008). However, in an RCT, the intestinal permeability, as measured using lactulose/mannitol ratio, did not decrease with the use of probiotics when compared to placebo (Smecuol et al. 2013). The probiotics (*Saccharomyces boulardii*, *Bifidobacteria*, and *Lactobacilli* strains) have also been demonstrated to alter the host immune response by blocking toxin receptors, secreting SCFA and other antimicrobial peptides, regulating intraluminal pH and promoting B-cell maturation in in vitro and animal studies (Chibbar and Dieleman 2019). However, studies evaluating these effects in humans are lacking at present.

Most of the human intervention studies on the role of probiotics in CeD have been carried out in patients on GFD, which itself is known to modulate the intestinal microbiota and restore integrity of the intestinal epithelial barrier. The effects of probiotics on the various pathophysiological mechanisms, independent of the GFD are unknown and need to be evaluated. The gluten content in commercially available probiotics is another concern (Nazareth et al. 2015).

15.6.3 Prebiotics

Prebiotics are substances that promote the growth of microorganisms that contribute to the health of the host. The dietary prebiotics are usually non-digestible fibers. However, various functional foods (such as fermented foods with live cultures,

garlic, and curcumin) also have prebiotic properties. Studies are ongoing to determine the daily serving dose of prebiotics. Also addition of prebiotics (inulin-type fructans) to GFD has been proposed to improve calcium absorption in patients with CeD (Capriles and Arêas 2013). In another study, addition of oligofructose-enriched inulin to GFD increased the total SCFAs in patients with CeD by providing a ready source of energy to the intestinal microbiota (Krupa-Kozak et al. 2017).

15.6.4 Microbiota-Derived Glutenases

Several bacterial enzymes such as prolyl endopeptidases (PEPs), cysteine proteases, and subtilisins can cleave gluten and GPs. The bacterial PEPs are derived from *Flavobacterium meningosepticum*, *Sphingomonas capsulate*, *Myxococcus xanthus*, and *Aspergillus niger*. The proenzyme for glutamine-specific cysteine protease has been isolated from *Escherichia coli*. Subtilisins are derived from *Rothia mucilaginosa* (Shan et al. 2004; Bethune et al. 2006; Zamakhchari et al. 2011; Wei et al. 2020).

Bifidobacteria, via proteolytic enzymes, have been demonstrated to degrade the proinflammatory GPs, reducing their immunogenicity (Lindfors et al. 2008). *Lactobacilli* also secrete degrading enzymes that digest other non-gluten wheat proteins and amylase-trypsin inhibitors (ATIs) resulting in decreased inflammatory stress and improved intestinal permeability (Caminero et al. 2019b). The oral commensal bacteria (*Rothia*, *Actinomyces*, *Neisseria*, *Capnocytophaga*, etc.) also synthesize and secrete enzymes that hydrolyze and degrade the toxic gluten epitopes rendering the GPs less or non-immunogenic (Fernandez-Feo et al. 2013). In an in vitro study, there was no increase in the levels of inflammatory cytokines in duodenal biopsies obtained from CeD patients who consumed hydrolyzed wheat bread produced with *Lactobacilli* strains (Rizzello et al. 2007). In vivo studies have also reported encouraging results with consumption of *Lactobacilli* pre-digested wheat bread (Mandile et al. 2017; Francavilla et al. 2019). These observations suggest the role of *Lactobacilli*-derived endopeptidases in mitigating the gluten toxicity for CeD patients.

However, until the specific immunogenic GPs are not cleaved and degraded rigorously, the peptides can cross the epithelial barrier and elicit an aberrant immune response. Therefore, identifying the precise and discrete gluten degrading enzymes (glutenases) and testing them systematically in large randomized studies holds the key for use of microbe-derived glutenases on a large scale.

15.6.5 Hookworms

In a pilot study, hookworm (*Necator americanus*) larvae were inoculated in adult CeD patients on GFD. Gluten challenge (gluten consumed as pasta) was given in escalating doses after hookworm inoculation. Experimental infection with hookworms did not result in elevation of anti-tTG titres or villous atrophy on

histology, implying improved gluten tolerance (Croese et al. 2015). Further evaluation is needed before classifying helminths as therapy for CeD.

15.6.6 Fecal Microbiome Transfer (FMT)

FMT has emerged as a novel tool to modulate the intestinal microbiota and has been used successfully for the treatment of *Clostridium difficile* infection and inflammatory bowel disease (IBD). At present only one clinical trial (NCT04014413) evaluating the role of FMT in CeD is underway.

15.7 Conclusion

CeD provides a unique model to study the impact of microorganisms in a noninfective chronic genetically mediated inflammatory disease. The strong influence of microorganisms on each step in the pathogenesis of CeD, starting from genetic predisposition to gluten digestion to elicitation of an aberrant immune response, has been demonstrated beyond doubt. Additionally, specific changes in the composition of intestinal microbiota have been reported in case–control and cross sectional studies. Microbiota targeted therapies such as probiotics have also been investigated with variable success. Though these observations are intriguing, a definite causal relationship cannot be commented upon, until prospective cohort studies demonstrate the causal association. The data derived from prospective studies would serve as a blueprint for integrating multi-omics into clinical decision-making. Utilization of multi-omics techniques would also enable evaluation of genetic and environmental influences on the functionality of the intestinal microbiome. Specific and targeted manipulation of microorganisms of interest may then be able to prevent or treat CeD.

References

Baba N, Samson S, Bourdet-Sicard R, Rubio M, Sarfati M (2008) Commensal bacteria trigger a full dendritic cell maturation program that promotes the expansion of non-Tr1 suppressor T cells. J Leukoc Biol 84(2):468–476. https://doi.org/10.1189/jlb.0108017

Bethune MT, Strop P, Tang Y, Sollid LM, Khosla C (2006) Heterologous expression, purification, refolding, and structural-functional characterization of EP-B2, a self-activating barley cysteine endoprotease. Chem Biol 13(6):637–647. https://doi.org/10.1016/j.chembiol.2006.04.008

Bodkhe R, Shetty SA, Dhotre DP et al (2019) Comparison of small gut and whole gut microbiota of first-degree relatives with adult celiac disease patients and controls. Front Microbiol 10:164. https://doi.org/10.3389/fmicb.2019.00164

Caio G, Volta U, Sapone A et al (2019) Celiac disease: a comprehensive current review. BMC Med 17:142. https://doi.org/10.1186/s12916-019-1380-z

Caminero A, Galipeau HJ, McCarville JL et al (2016) Duodenal bacteria from patients with celiac disease and healthy subjects distinctly affect gluten breakdown and immunogenicity. Gastroenterology 151(4):670–683. https://doi.org/10.1053/j.gastro.2016.06.041

Caminero A, McCarville JL, Galipeau HJ et al (2019a) Duodenal bacterial proteolytic activity determines sensitivity to dietary antigen through protease-activated receptor-2. Nat Commun 10(1):1198. https://doi.org/10.1038/s41467-019-09037-9

Caminero A, McCarville JL, Zevallos VF et al (2019b) Lactobacilli degrade wheat amylase trypsin inhibitors to reduce intestinal dysfunction induced by immunogenic wheat proteins. Gastroenterology 156(8):2266–2280. https://doi.org/10.1053/j.gastro.2019.02.028

Capriles VD, Arêas JAG (2013) Effects of prebiotic inulin-type fructans on structure, quality, sensory acceptance and glycemic response of gluten-free breads. Food Funct 4(1):104–110. https://doi.org/10.1039/c2fo10283h

Cheng J, Kalliomäki M, Heilig H et al (2013) Duodenal microbiota composition and mucosal homeostasis in pediatric celiac disease. BMC Gastroenterol 13:113. https://doi.org/10.1186/1471-230X-13-113

Chibbar R, Dieleman LA (2019) The gut microbiota in celiac disease and probiotics. Nutrients 11(10):2375. https://doi.org/10.3390/nu11102375

Cinova J, De Palma G et al (2011) Role of intestinal bacteria in gliadin-induced changes in intestinal mucosa: study in germ-free rats. PLoS One 6(1):e16169. https://doi.org/10.1371/journal.pone.0016169

Collado MC, Donat E, Ribes-Koninckx C, Calabuig M, Sanz Y (2008) Imbalances in faecal and duodenal bifidobacterium species composition in active and non-active coeliac disease. BMC Microbiol 8:232. https://doi.org/10.1186/1471-2180-8-232

Collado MC, Donat E, Ribes-Koninckx C, Calabuig M, Sanz Y (2009) Specific duodenal and faecal bacterial groups associated with paediatric coeliac disease. J Clin Pathol 62(3):264–269. https://doi.org/10.1136/jcp.2008.061366

Croese J, Giacomin P, Navarro S et al (2015) Experimental hookworm infection and gluten microchallenge promote tolerance in celiac disease. J Allergy Clin Immunol 135(2):508–516. https://doi.org/10.1016/j.jaci.2014.07.022

D'Arienzo R, Stefanile R, Maurano F et al (2011) Immunomodulatory effects of Lactobacillus casei administration in a mouse model of gliadin-sensitive enteropathy. Scand J Immunol 74(4):335–341. https://doi.org/10.1111/j.1365-3083.2011.02582.x

De Palma G, Capilla A, Nadal I et al (2010a) Interplay between human leukocyte antigen genes and the microbial colonization process of the newborn intestine. Curr Issues Mol Biol 12(1):1–10

De Palma G, Cinova J, Stepankova R, Tuckova L, Sanz Y (2010b) Pivotal advance: bifidobacteria and gram-negative bacteria differentially influence immune responses in the proinflammatory milieu of celiac disease. J Leukoc Biol 87(5):765–778. https://doi.org/10.1189/jlb.0709471

Di Biase AR, Marasco G, Ravaioli F et al (2021) Gut microbiota signatures and clinical manifestations in celiac disease children at onset: a pilot study. J Gastroenterol Hepatol 36(2):446–454. https://doi.org/10.1111/jgh.15183

Di Cagno R, Rizzello CG, Gagliardi F et al (2009) Different fecal microbiotas and volatile organic compounds in treated and untreated children with celiac disease. Appl Environ Microbiol 75(12):3963–3971. https://doi.org/10.1128/AEM.02793-08

Di Cagno R, De Angelis M, De Pasquale I et al (2011) Duodenal and faecal microbiota of celiac children: molecular, phenotype and metabolome characterization. BMC Microbiol 11:219. https://doi.org/10.1186/1471-2180-11-219

Feng Q, Chen W-D, Wang Y-D (2018) Gut microbiota: an integral moderator in health and disease. Front Microbiol 9:151. https://doi.org/10.3389/fmicb.2018.00151

Fernandez-Feo M, Wei G, Blumenkranz G et al (2013) The cultivable human oral gluten-degrading microbiome and its potential implications in coeliac disease and gluten sensitivity. Clin Microbiol Infect 19(9):E386–E394. https://doi.org/10.1111/1469-0691.12249

Francavilla R, Piccolo M, Francavilla A et al (2019) Clinical and microbiological effect of a multispecies probiotic supplementation in celiac patients with persistent IBS-type symptoms. J Clin Gastroenterol 53(3):e117–e125. https://doi.org/10.1097/MCG.0000000000001023

Freire R, Ingano L, Serena G et al (2019) Human gut derived-organoids provide model to study gluten response and effects of microbiota-derived molecules in celiac disease. Sci Rep 9:7029. https://doi.org/10.1038/s41598-019-43426-w

Galipeau HJ, Rulli NE, Jury J et al (2011) Sensitization to gliadin induces moderate enteropathy and insulitis in nonobese diabetic-DQ8 mice. J Immunol 187(8):4338–4346. https://doi.org/10.4049/jimmunol.1100854

García-Santisteban I, Cilleros-Portet A, Moyua-Ormazabal E et al (2020) A two-sample Mendelian randomization analysis investigates associations between gut microbiota and celiac disease. Nutrients 12(5):1420. https://doi.org/10.3390/nu12051420

Glotfelty LG, Zahs A, Hodges K, Shan K, Alto NM, Hecht GA (2014) Enteropathogenic E. coli effectors EspG1/G2 disrupt microtubules, contribute to tight junction perturbation and inhibit restoration. Cell Microbiol 16(12):1767–1783. https://doi.org/10.1111/cmi.12323

Golfetto L, de Senna FD, Hermes J, Beserra BTS, França F d S, Martinello F (2014) Lower bifidobacteria counts in adult patients with celiac disease on a gluten-free diet. Arq Gastroenterol 51(2):139–143. https://doi.org/10.1590/s0004-28032014000200013

Harnett J, Myers SP, Rolfe M (2016) Probiotics and the microbiome in celiac disease: a randomised controlled trial. Evid Based Complement Alternat Med 2016:9048574. https://doi.org/10.1155/2016/9048574

Herrán AR, Pérez-Andrés J, Caminero A et al (2017) Gluten-degrading bacteria are present in the human small intestine of healthy volunteers and celiac patients. Res Microbiol 168(7):673–684. https://doi.org/10.1016/j.resmic.2017.04.008

Heyman M, Abed J, Lebreton C, Cerf-Bensussan N (2012) Intestinal permeability in coeliac disease: insight into mechanisms and relevance to pathogenesis. Gut 61(9):1355–1364. https://doi.org/10.1136/gutjnl-2011-300327

Kalliomäki M, Satokari R, Lähteenoja H et al (2012) Expression of microbiota, toll-like receptors, and their regulators in the small intestinal mucosa in celiac disease. J Pediatr Gastroenterol Nutr 54(6):727–732. https://doi.org/10.1097/MPG.0b013e318241cfa8

Klemenak M, Dolinšek J, Langerholc T, Di Gioia D, Mičetić-Turk D (2015) Administration of Bifidobacterium breve decreases the production of TNF-α in children with celiac disease. Dig Dis Sci 60(11):3386–3392. https://doi.org/10.1007/s10620-015-3769-7

Krishnareddy S (2019) The microbiome in celiac disease. Gastroenterol Clin N Am 48(1):115–126. https://doi.org/10.1016/j.gtc.2018.09.008

Krupa-Kozak U, Drabińska N, Jarocka-Cyrta E (2017) The effect of oligofructose-enriched inulin supplementation on gut microbiota, nutritional status and gastrointestinal symptoms in paediatric coeliac disease patients on a gluten-free diet: study protocol for a pilot randomized controlled trial. Nutr J 16(1):47. https://doi.org/10.1186/s12937-017-0268-z

Kupfer SS, Jabri B (2012) Celiac disease pathophysiology. Gastrointest Endosc Clin N Am 22(4):639–660. https://doi.org/10.1016/j.giec.2012.07.003

Laparra JM, Sanz Y (2010) Bifidobacteria inhibit the inflammatory response induced by gliadins in intestinal epithelial cells via modifications of toxic peptide generation during digestion. J Cell Biochem 109(4):801–807. https://doi.org/10.1002/jcb.22459

Leonard MM, Karathia H, Pujolassos M et al (2020) Multi-omics analysis reveals the influence of genetic and environmental risk factors on developing gut microbiota in infants at risk of celiac disease. Microbiome 8:130. https://doi.org/10.1186/s40168-020-00906-w

Lerner A, Arleevskaya M, Schmiedl A, Matthias T (2017) Microbes and viruses are bugging the gut in celiac disease. Are they friends or foes? Front Microbiol 8:1392. https://doi.org/10.3389/fmicb.2017.01392

Lindfors K, Blomqvist T, Juuti-Uusitalo K et al (2008) Live probiotic Bifidobacterium lactis bacteria inhibit the toxic effects induced by wheat gliadin in epithelial cell culture. Clin Exp Immunol 152(3):552–558. https://doi.org/10.1111/j.1365-2249.2008.03635.x

Lorenzo Pisarello MJ, Vintiñi EO, González SN, Pagani F, Medina MS (2015) Decrease in lactobacilli in the intestinal microbiota of celiac children with a gluten-free diet, and selection

of potentially probiotic strains. Can J Microbiol 61(1):32–37. https://doi.org/10.1139/cjm-2014-0472

Macfarlane S, Quigley ME, Hopkins MJ, Newton DF, Macfarlane GT (1998) Polysaccharide degradation by human intestinal bacteria during growth under multi-substrate limiting conditions in a three-stage continuous culture system. FEMS Microbiol Ecol 26(3):231–243. https://doi.org/10.1111/j.1574-6941.1998.tb00508.x

Mandile R, Picascia S, Parrella C et al (2017) Lack of immunogenicity of hydrolysed wheat flour in patients with coeliac disease after a short-term oral challenge. Aliment Pharmacol Ther 46(4): 440–446. https://doi.org/10.1111/apt.14175

Medina M, Izquierdo E, Ennahar S, Sanz Y (2007) Differential immunomodulatory properties of Bifidobacterium logum strains: relevance to probiotic selection and clinical applications. Clin Exp Immunol 150(3):531–538. https://doi.org/10.1111/j.1365-2249.2007.03522.x

Medina M, De Palma G, Ribes-Koninckx C, Calabuig M, Sanz Y (2008) Bifidobacterium strains suppress in vitro the pro-inflammatory milieu triggered by the large intestinal microbiota of coeliac patients. J Inflamm (Lond) 5:19. https://doi.org/10.1186/1476-9255-5-19

Ménard S, Lebreton C, Schumann M et al (2012) Paracellular versus transcellular intestinal permeability to gliadin peptides in active celiac disease. Am J Pathol 180(2):608–615. https://doi.org/10.1016/j.ajpath.2011.10.019

Nazareth S, Lebwohl B, Voyksner JAS, Green P (2015) 108 widespread contamination of probiotics with gluten, detected by liquid chromatography-mass spectrometry. https://doi.org/10.1016/S0016-5085(15)30097-4

Olivares M, Castillejo G, Varea V, Sanz Y (2014) Double-blind, randomised, placebo-controlled intervention trial to evaluate the effects of Bifidobacterium longum CECT 7347 in children with newly diagnosed coeliac disease. Br J Nutr 112(1):30–40. https://doi.org/10.1017/S0007114514000609

Olivares M, Neef A, Castillejo G et al (2015) The HLA-DQ2 genotype selects for early intestinal microbiota composition in infants at high risk of developing coeliac disease. Gut 64(3): 406–417. https://doi.org/10.1136/gutjnl-2014-306931

Olivares M, Walker AW, Capilla A et al (2018) Gut microbiota trajectory in early life may predict development of celiac disease. Microbiome 6(1):36. https://doi.org/10.1186/s40168-018-0415-6

Orlando A, Linsalata M, Notarnicola M, Tutino V, Russo F (2014) Lactobacillus GG restoration of the gliadin induced epithelial barrier disruption: the role of cellular polyamines. BMC Microbiol 14:19. https://doi.org/10.1186/1471-2180-14-19

Orlando A, Linsalata M, Bianco G et al (2018) Lactobacillus rhamnosus GG protects the epithelial barrier of Wistar rats from the pepsin-trypsin-digested gliadin (PTG)-induced enteropathy. Nutrients 10(11):1698. https://doi.org/10.3390/nu10111698

Palma GD, Kamanova J, Cinova J et al (2012) Modulation of phenotypic and functional maturation of dendritic cells by intestinal bacteria and gliadin: relevance for celiac disease. J Leukoc Biol 92(5):1043–1054. https://doi.org/10.1189/jlb.1111581

Papista C, Gerakopoulos V, Kourelis A et al (2012) Gluten induces coeliac-like disease in sensitised mice involving IgA, CD71 and transglutaminase 2 interactions that are prevented by probiotics. Lab Investig 92(4):625–635. https://doi.org/10.1038/labinvest.2012.13

Petersen J, Ciacchi L, Tran MT et al (2020) T cell receptor cross-reactivity between gliadin and bacterial peptides in celiac disease. Nat Struct Mol Biol 27(1):49–61. https://doi.org/10.1038/s41594-019-0353-4

Primec M, Klemenak M, Di Gioia D et al (2019) Clinical intervention using Bifidobacterium strains in celiac disease children reveals novel microbial modulators of TNF-α and short-chain fatty acids. Clin Nutr 38(3):1373–1381. https://doi.org/10.1016/j.clnu.2018.06.931

Rizzello CG, De Angelis M, Di Cagno R et al (2007) Highly efficient gluten degradation by lactobacilli and fungal proteases during food processing: new perspectives for celiac disease. Appl Environ Microbiol 73(14):4499–4507. https://doi.org/10.1128/AEM.00260-07

Rostami-Nejad M, Romanos J, Rostami K et al (2014) Allele and haplotype frequencies for HLA-DQ in Iranian celiac disease patients. World J Gastroenterol 20(20):6302–6308. https://doi.org/10.3748/wjg.v20.i20.6302

Sánchez E, Ribes-Koninckx C, Calabuig M, Sanz Y (2012) Intestinal Staphylococcus spp. and virulent features associated with coeliac disease. J Clin Pathol 65(9):830–834. https://doi.org/10.1136/jclinpath-2012-200759

Sánchez E, Donat E, Ribes-Koninckx C, Fernández-Murga ML, Sanz Y (2013) Duodenal-mucosal bacteria associated with celiac disease in children. Appl Environ Microbiol 79(18):5472–5479. https://doi.org/10.1128/AEM.00869-13

Sánchez D, Hoffmanová I, Szczepanková A, Hábová V, Tlaskalová-Hogenová H (2021) Contribution of infectious agents to the development of celiac disease. Microorganisms 9(3):547. https://doi.org/10.3390/microorganisms9030547

Schippa S, Iebba V, Barbato M et al (2010) A distinctive "microbial signature" in celiac pediatric patients. BMC Microbiol 10:175. https://doi.org/10.1186/1471-2180-10-175

Seiler CL, Kiflen M, Stefanolo JP et al (2020) Probiotics for celiac disease: a systematic review and meta-analysis of randomized controlled trials. Am J Gastroenterol 115(10):1584–1595. https://doi.org/10.14309/ajg.0000000000000749

Sellitto M, Bai G, Serena G et al (2012) Proof of concept of microbiome-metabolome analysis and delayed gluten exposure on celiac disease autoimmunity in genetically at-risk infants. PLoS One 7(3):e33387. https://doi.org/10.1371/journal.pone.0033387

Serena G, Yan S, Camhi S et al (2017) Proinflammatory cytokine interferon-γ and microbiome-derived metabolites dictate epigenetic switch between forkhead box protein 3 isoforms in coeliac disease. Clin Exp Immunol 187(3):490–506. https://doi.org/10.1111/cei.12911

Shan L, Marti T, Sollid LM, Gray GM, Khosla C (2004) Comparative biochemical analysis of three bacterial prolyl endopeptidases: implications for coeliac sprue. Biochem J 383(Pt 2):311–318. https://doi.org/10.1042/BJ20040907

Sharma A, Liu X, Hadley D et al (2016) Identification of non-HLA genes associated with celiac disease and country-specific differences in a large, international pediatric cohort. PLoS One 11(3):e0152476. https://doi.org/10.1371/journal.pone.0152476

Singh P, Arora A, Strand TA et al (2018) Global prevalence of celiac disease: systematic review and meta-analysis. Clin Gastroenterol Hepatol 16(6):823–836.e2. https://doi.org/10.1016/j.cgh.2017.06.037

Sjöberg V, Sandström O, Hedberg M, Hammarström S, Hernell O, Hammarström M-L (2013) Intestinal T-cell responses in celiac disease—impact of celiac disease associated bacteria. PLoS One 8(1):e53414. https://doi.org/10.1371/journal.pone.0053414

Sjögren YM, Tomicic S, Lundberg A et al (2009) Influence of early gut microbiota on the maturation of childhood mucosal and systemic immune responses. Clin Exp Allergy 39(12):1842–1851. https://doi.org/10.1111/j.1365-2222.2009.03326.x

Smecuol E, Hwang HJ, Sugai E et al (2013) Exploratory, randomized, double-blind, placebo-controlled study on the effects of Bifidobacterium infantis natren life start strain super strain in active celiac disease. J Clin Gastroenterol 47(2):139–147. https://doi.org/10.1097/MCG.0b013e31827759ac

Stěpánková R, Tlaskalová-Hogenová H, Sinkora J, Jodl J, Fric P (1996) Changes in jejunal mucosa after long-term feeding of germfree rats with gluten. Scand J Gastroenterol 31(6):551–557. https://doi.org/10.3109/00365529609009127

Størdal K, Kahrs C, Tapia G, Agardh D, Kurppa K, Stene LC (2021) Review article: exposure to microbes and risk of coeliac disease. Aliment Pharmacol Ther 53(1):43–62. https://doi.org/10.1111/apt.16161

Tobi M, Talwar H, McVicker B (2021) The celiac disease microbiome depends on the Paneth cells of the puzzle. Gastroenterology 161(1):359. https://doi.org/10.1053/j.gastro.2021.02.023

Tye-Din JA, Galipeau HJ, Agardh D (2018) Celiac disease: a review of current concepts in pathogenesis, prevention, and novel therapies. Front Pediatr 6:350. https://doi.org/10.3389/fped.2018.00350

Wei G, Helmerhorst EJ, Darwish G, Blumenkranz G, Schuppan D (2020) Gluten degrading enzymes for treatment of celiac disease. Nutrients 12(7):E2095. https://doi.org/10.3390/nu12072095

Yuan J, Gao J, Li X et al (2013) The tip of the "celiac iceberg" in China: a systematic review and meta-analysis. PLoS One 8(12):e81151. https://doi.org/10.1371/journal.pone.0081151

Zafeiropoulou K, Nichols B, Mackinder M et al (2020) Alterations in intestinal microbiota of children with celiac disease at the time of diagnosis and on a gluten-free diet. Gastroenterology 159(6):2039–2051.e20. https://doi.org/10.1053/j.gastro.2020.08.007

Zamakhchari M, Wei G, Dewhirst F et al (2011) Identification of Rothia bacteria as gluten-degrading natural colonizers of the upper gastro-intestinal tract. PLoS One 6(9):e24455. https://doi.org/10.1371/journal.pone.0024455