Chapter 11

Probiotics in Childhood Celiac Disease

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Abstract

Celiac disease (CD) is an autoimmune enteropathy induced by gluten ingestion in genetically susceptible individuals. Genetic predisposition plays an important role in the development of CD, but it is not sufficient by itself for the disease development. Although gluten proteins are the main environmental factor involved in CD pathogenesis and ingestion of gluten is necessary to manifest the disease, recent studies have suggested that alteration of the microbiota could be involved and, in particular, the interplay between gut microbiota and the mucosal immune system. Dysbiosis, the alteration of the microbiota, has been associated with a variety of intestinal pathologies including Crohn disease and CD. Most observational studies in children and adults with CD have shown alterations in the intestinal microbiota composition compared to control subjects, which is only partially recovered after treatment with a gluten-free diet (GFD). At this time, the only treatment for CD is lifelong adherence to a GFD, which involves the elimination of grains containing gluten, wheat, rye, and barley. However, it is difficult for many patients to follow a GFD. Abnormalities in the gut microbiome in CD patients have led to the use of probiotics as a promising alternative as a therapeutic or preventative approach.

Keywords: celiac disease, gluten free diet, intestinal microbiota, dysbiosis, probiotics

1. Introduction

Celiac disease (CD) is an autoimmune enteropathy induced by gluten ingestion in genetically susceptible individuals [1]. The major genetic risk factor for CD is represented by HLA-DQ genes. Ninety percent of affected individuals carry the HLA-DQ2 haplotype, 5% the DQ8 haplotype, and the remaining 5% carry at least one of the two DQ2 alleles [1, 2]. Genetic

predisposition plays an important role in the development of CD but it is not sufficient by itself for the disease development [3]. Approximately, 30% of the general population carry the HLA-DQ2/8 CD susceptibility genes, however, only 2–5% of these individuals will develop CD, suggesting that additional environmental factors contribute to disease development [4]. Although gluten proteins are the main environmental factor involved in CD pathogenesis and ingestion of gluten is necessary to manifest the disease, recent studies have suggested that potential factors such as birth delivery, breast-feeding, infectious agents, and antibiotic intake could contribute to the development of CD [5–7]. The alteration of the microbiota could also be involved and, in particular, the interplay between gut microbiota and the mucosal immune system [8].

The microbiota, the set of microorganisms that colonize the human body, has a fundamental role for the host. It is important for both physiological and metabolic factors, ranging from the absorption of nutrients to the regulation and development of the immune system [9]. Dysbiosis, the alteration of the microbiota, has been associated with a variety of pathologies like Crohn disease and obesity [10, 11]. Most observational studies in children and adults with CD have shown alterations in the intestinal microbiota composition compared to control subjects, which is partially recovered after treatment with a gluten-free diet (GFD) [12–14]. It has been demonstrated that levels of *Bifidobacteria* and *Lactobacilli* are reduced in CD patients [14, 15]. Specific alterations in the microbiota could contribute to the etiopathogenesis of CD by providing proteolytic activities that influence the generation of toxic and immunogenic peptides from gluten, and compromise the intestinal barrier function [16]. Probiotics are nonpathogenic live microorganisms, which, when orally administered in adequate amounts, alter the microflora of the host and have beneficial health effect [17].

At this time, the only treatment for CD is lifelong adherence to a GFD, which involves the elimination of grains containing gluten, wheat, rye, and barley. However, it is difficult for many patients to follow a GFD. Some probiotics have been found to digest or alter gluten polypeptides [18]. Abnormalities in the gut microbiome in CD patients have led to the use of probiotics as a promising alternative as a therapeutic or preventative approach.

Here we focus on the role of microbiota in the pathogenesis of CD and on the chances for probiotics to be involved in an alternative treatment strategy.

2. Microbiota composition in celiac children

Several research papers have suggested that an important risk factor involved in the etiology of CD could be the gut microbiota. Multiple studies investigating the role of gut microbiota in CD have been performed on fecal samples and, later, on duodenal biopsies.

The studies that have addressed the relation between fecal microbiota and CD in the pediatric population are summarized in **Table 1** [13, 19–24]. In the earliest report involving a total of 49 children, 26 celiac patients aged 12–48 months and 23 age-matched controls, Collado et al. evaluated the composition of the fecal microbiota by both culture-dependent and culture-

independent methods using fluorescent in situ hybridization (FISH) [13]. They showed a high level of *Bacteroides*, *Clostridium*, and *Staphylococcus* in fecal samples from CD children compared to healthy subjects when analyzed by culture methods. The numbers of *Bacteroides-Prevotella*, *Clostridium histolyticum*, *Eubacterium rectale-Clostridium coccoides*, *Atopobium*, and sulfate-reducing bacterial groups were also significantly higher in fecal samples from CD children analyzed by FISH [13]. Subsequently, Sanz et al. [19], using polymerase chain reaction (PCR) and denaturing gradient gel electrophoresis (DGGE) in 10 CD children aged 15–45 months and 10 age-matched healthy controls, demonstrated that the presence of species such as *Lactobacillus curvatus*, *Leuconostoc mesenteroides*, and *Leuconostoc carnosus* were characteristic of coeliac patients, while the *Lactobacillus casei* group was characteristic of healthy controls. Moreover, the authors found a reduction in *Bifidobacterium* population diversity in CD patients. Collado et al. [20], using real-time PCR, evaluated duodenal and fecal microbiota in three groups of children: (1) untreated CD patients on a gluten-containing diet (GCD); (2) treated CD patients who had been on a GFD for a minimum of two years; and (3) healthy controls. They found that feces and biopsies of CD patients had an increased presence of *Bifidobacterium, Bacteroides, and Clostridium leptum* groups with respect to the control group; *Escherichia coli and Staphylococcus* were otherwise predominant in CD subjects on GFD. GFD determined a complete normalization of gut microbiota [20]. De Palma et al. examined fecal microbiology and immunoglobulin-associated features in active and non-active stages of CD in children and in age-matched controls [21]. They found that in CD patients there was an alteration in the type of fecal immunoglobulin-coated bacteria along with a shift in the composition of the microbiota. In fact, they demonstrated a reduction of the percentages of the IgA-coated bacteria in CD patients on a GFD and in those not following a GFD compared to the control group. They also found a reduction of the percentages of IgG- and IgM-coated bacteria in treated CD patients with respect to untreated CD subjects and control group. Moreover, treated and untreated CD subjects showed a predominance of *Bacteroides-Prevotella* as well as an impaired mucosal barrier, as suggested by the reduction of IgA-coated bacteria with respect to the controls [21]. Sanchez et al., in an attempt to determine whether intestinal *Staphylococcus* spp. and their pathogenic features differed between CD patients and healthy controls, studied 40 CD children (20 active CD and 20 non-active CD) and 20 healthy controls [22]. *Staphylococci* were isolated from feces and identified by PCR and DNA sequencing. CD was associated with alterations in species diversity and composition of the fecal *Staphylococcus* population. *Staphylococcus epidermidis* isolates carrying the *mecA* gene and both the *mecA* and *atIE* genes were more abundant in CD patients than in controls, most likely reflecting increased exposure of these subjects to opportunistic staphylococcal pathogens and antimicrobials, which in turn affected the composition/features of their intestinal microbiota [22]. Di Cagno et al. in a study including seven CD patients on GFD, seven CD patients on a GCD, and seven healthy controls, utilizing DGGE analysis and gas chromatography-mass spectrometry-solid-phase microextraction analysis of fecal volatile organic compounds (VOCs), found that the fecal microbiota and VOCs of CD patients on GFD were more similar to those of healthy patients than to those of CD patients on GCD [23]. Consequently, the authors speculated that *Lactobacillus* and *Bifidobacterium* strains isolated from healthy children could be a potential probiotic treatment to restore the balance of intestinal microbiota in treated and untreated CD

patients [23]. Similar conclusions have been reached by Lorenzo Pisarello et al. [24] in a very recent work. They found lower counts of *Lactobacillus* in the feces of CD compared to controls. Furthermore, the authors selected from feces of controls 5 *Lactobacillus* strains because of their high resistance percentages to gastrointestinal tract conditions. *Lactobacillus rhamnosus* (LC4) showed the highest percentage of autoaggregation and *Lactobacillus paracasei* showed high hydrophobicity suggesting a potential use of these strains as probiotics in CD [24].

Author/ References	**Year**	**Country**	**Patients population and sample size**	**Methods**	**Main results**
Collado et al. [13]	2007	Spain	26 untreated CD (mean age, 26 months) 23 controls (mean age, 23.1 months)	Culture+ FISH	In untreated CD: ↑ *Bacteroides* ↑ *Staphylococcus* ↑ *Clostridium* ↑ *Bacteroides-Prevotella,* ↑ *Clostridium hystoliticum,* ↑ *Eubacterium rectale-C. coccoides,* ↑ *Atopobium, Staphylococcus* ↓ *Bifidobacterium*
Sanz et al. [19]	2007	Spain	10 untreated CD (mean age, 28 months) 10 controls (mean age, 24 months)	Culture+qPCR +DGGE	In untreated CD: High diversity of fecal microbiota ↑ *Leuconostoc carnosum,* ↑ *Leuconostoc mesenteroides,* ↑ *Lactobacillus curvatus* ↓ *Lactobacillus casei,* ↓ *Bifidobacterium adolescentis*
Collado et al. [20]	2009	Spain	30 untreated CD (mean age, 38.5 months) 18 treated CD (mean age, 37.7 months) 30 controls (mean age, 33.5 months)	qPCR	In untreated and treated CD: ↑ Bacterial count ↑ *E. coli*, ↑ B*acteroides,* ↑ *Clostridium leptum* *Staphylococcus* prevalence ↓ *Bifidobacterium* In treated CD: ↑ *Lactobacillus*
Di Cagno et al. [23]	2009	Italy	7 untreated CD (range, 6–12 years) 7 treated CD (range, 6–12 years) 7 (range, 6–12 years) controls	PCR+DGGE	In treated and untreated CD: ↓ Ratio of cultivable lactic acid bacteria and *Bifidobacterium* to *Bacteroides* and *Enterobacteria* In treated CD and in controls: *Lactobacillus brevis, Lactobacillus rossiae, Lactobacillus pentosus* Only in controls:

Author/ References	Year	Country	Patients population and sample size	Methods	Main results
					Lactobacillus fermentum, Lactobacillus delbrueckii subsp., *Lactobacillus bulgaricus, Lactobacillus gasseri*
De Palma et al. [21]	2010	Spain	24 untreated CD (mean age, 5.5 years) 18 treated CD (mean age, 5.5 years) 20 controls (mean age, 5.3 years)	FISH+ flow cytometry	In untreated CD: ↓ *Bifidobacterium,* ↓ *Clostridium histoliticum,* ↓ *Clostridium lituseburense,* ↓ *Fecalibacterium prausnitzii* ↑ *Bacteroides-Prevotella* In untreated CD and in controls: ↓ Levels of IgA coating the *Bacteroides-Prevotella*
Sanchez et al. [22]	2012	Spain	20 (mean age, 57.4 months) untreated CD 20 (mean age, 67.3 months) treated CD 20 (mean age, 54.0 months) controls	PCR+ DNA sequencing	In untreated CD: ↑ *Staphylococcus* spp. diversity ↑ *Staphylococcus haemolyticus* ↓ *Staphylococcus aureus* ↑ *mecA* and *atIE* genes in *S. epidermidis* clones
Lorenzo Pisarello et al. [24]	2015	Argentina	15 treated CD (mean age, 7.5 years) 15 controls (mean age, 6.5 years)	Culture (autoaggregation assay, hydrophobicity assay)	In treated CD ↓ *Lactobacilli* ↑ *Enterobacteria* *Lactobacillus rhamnosus and Lactobacillus paracasei* identified to improve sign and symptom in CD

CD celiac disease, *FISH* fluorescent in situ hybridization, *DGGE* denaturing gradient gel electrophoresis, *PCR* polymerase chain reaction, *qPCR* quantitative polymerase chain reaction.

Table 1. Fecal microbiota in celiac disease.

Duodenal microbial composition of pediatric CD patients was explored more extensively later on, with the main findings summarized in **Table 2** [20, 25–33]. Microbiota characterization from duodenal biopsy specimens was initially carried out on CD Spanish children by Nadal et al. [25] in 2007. The authors, in an attempt to identify the specific composition of the duodenal microbiota of celiac patients (with active and non-active disease), evaluated 20 CD patients on GCD, 10 CD patients on GFD for 1–2 years, and 8 healthy controls. Bacteriological analyses of duodenal biopsy specimens, carried out by fluorescent in situ hybridization coupled with flow cytometry, showed that the proportions of total and Gram-negative potentially pro-inflammatory bacteria were significantly higher in CD patients with active disease than in patients on GFD and controls. Although, the ratio of beneficial bacterial groups (*Lactobacillus*-

Bifidobacterium) to potentially harmful *Bacteroides-E. coli* was significantly reduced in CD patients on GFD, there was not a complete normalization of gut microbiota compared with controls [25]. Several subsequent Spanish studies confirmed these results [20, 26–28]. Particularly, these studies found that the *Bacteroides, E. coli, Bifidobacterium, Enterobacteriacae,* and *Staphylococcus* groups were significantly more abundant in GCD patients than in the controls with a greater diversity of these species [20, 26, 28], while, in contrast, members of the family Streptococcaceae were less abundant in CD patients [28]. Furthermore, the *Prevotella* genera were more frequent in healthy subjects than in celiac patients [27]. Ou et al. identified Clostridium, Prevotella and Actinomyces as predominant bacteria in the proximal small intestine biopsies from a cohort of 45 CD children and 18 healthy controls born during the so-called "Swedish CD epidemic" (2004-2007). This could explain the four-fold increase in the incidence of CD in children less than two years of age observed between 2004 and 2007 [29]. Schippa et al. [30] analyzed the mucosa-associated microbiota of CD children, before and after a GFD, and controls by temporal temperature gradient gel electrophoresis (TTGE). The most important findings of the study were: a demonstration of a presence of peculiar microbial TTGE profile and a significant higher biodiversity in CD pediatric patients' duodenal mucosa after 9 months of GFD compared to healthy controls. Di Cagno et al. [31], utilizing culture-dependent and culture-independent methods and metabolomics analyses, investigated the differences in the microbiota and metabolome of 19 treated CD patients and 15 controls. They confirmed the lower levels of *Lactobacillus* and increased levels of *Bacteroides* in CD patients. Moreover, the authors showed that a GFD lasting at least two years did not completely restore the microbiota and metabolome in CD patients [31]. A recent Spanish study demonstrated that the intestinal microbiota of patients with duodenal Marsh 3c lesions showed similarity of 98% and differed from that of CD patients with other type of histologic lesion as Marsh 3a, Marsh 3b, and Marsh 2 [32]. This indicated that the composition of duodenal microbiota differed depending on the grade of intestinal damage.

Authors/ references	Years	Country	Patients population and sample size	Methods	Main results
Nadal et al. [25]	2007	Spain	20 (untreated CD (mean age, 5.1 years) 10 treated CD (mean age, 5.6 years) 8 controls (mean age, 4.1 years)	FISH+ flow cytometry	In untreated CD: ↑ Total bacteria ↑ Gram-negative bacteria ↑ *Bacteroides* and *E. coli*, which normalized after GFD In treated and untreated CD: ↓ The ratio of *Lactobacillus-Bifidobacterium* to *Bacteroides*
Collado et al. [20]	2009	Spain	8 untreated CD (mean age, 56.4 months) 8 treated CD (mean age, 65.2 months)	qPCR	In untreated CD: ↑Bacterial counts ↑ *Lactobacillus* prevalence ↓ *C. coccoides* prevalence ↑ *Staphylococcus*

Authors/ references	Years	Country	Patients population and sample size	Methods	Main results
			8 controls (mean age, 45.0 months)		↑ *E. coli* ↓ *Bifidobacterium* In treated and untreated CD: ↑ *Bacteroides* ↑ *C. leptum*
Ou et al.[29]	2009	Sweden	33 untreated CD (median age, 5.9 years) 17 treated CD (median age, 7.5 years) 3 challenged CD (median age, 10.8 years) 18 controls (mean age, 3.2 years)	Culture +Scanning electron microscopy	In untreated CD ↑ *Streptococcus* ↑ *Neisseria*
Schippa et al. [30]	2010	Italy	20 CD (before and after GFD) (mean age, 8.3 years) 10 controls (mean age, 11.7 years)	TTGE	Differences in biodiversity between untreated CD and treated CD ↑*Bacteroides vulgatus* and *E. coli* in CD
Sanchez et al. [26]	2010	Spain	20 treated CD (mean age, 51.1 months) 12 untreated CD (mean age, 54.9 months) 8 controls (mean age, 50.1 months)	PCR-DDGE	In untreated and treated CD: ↓*Bacteroides* diversity In untreated CD: ↓ *Bacteroides dorei* ↑*Bifidobacterium* diversity ↑*Bifidobacterium adolescentis,* ↑ *Bifidobacterium animalis* ↓*Bacteroides diastonis,* ↓*Bacteroides fragilis* ↓ *Bacteroides thetaiotaomicron,* ↓ *Bacteroides uniformis* ↓ *Bacteroides Ovatus*
Di Cagno et al. [31]	2011	Italy	19 treated CD (mean age 9.7 years) 15 controls (mean age, 10.4 years)	PCR-DDGE	In treated CD: ↓ *Lactobacillus* ↓ *Enterococcus* ↓ *Bifidobacteria* ↑*Bacteroides,* ↑ *Staphylococcus,* ↑*Salmonella,* ↑ *Shigella, Klebsiella*
Sanchez et al. [28]	2013	Spain	32 untreated CD (mean age, 5.1 years) 17 treated CD (mean age, 5.9 years)	Culture +PCR	In untreated CD: ↑ *Proteobacteria, Enterobacteriaceae, Staphylococcaceae* (*Klebsiella oxytoca,*

Authors/ references	Years	Country	Patients population and sample size	Methods	Main results
			8 controls (mean age, 6.9 years)		*Staphylococcus epidermidis, Staphylococcus pausteri*) ↓ *Firmicutes* ↓ *Streptococcus anginosus,* ↓ *Streptococcus mutans*
Nistal et al. [27]	2012		8 untreated CD (mean age, 3.75 years) 5 controls (mean age, 7.2 years)	16SrRNA gene sequencing	↓ *Streptococcus* and *Prevotella*
De Meij et al. [32]	2013	Netherland	21 untreated CD (median age, 6.8 years) 21 controls (median age, 8.1 years)	IS-pro	In treated and untreated CD: ↑*Streptococcus* ↑ *Lactobacillus* ↑ *Clostridium*
Cheng et al. [33]	2013	Finland	10 untreated CD (median age 9.5 years) 9 controls (median age, 8.5 years)	qRT-PCR+ HIPchip microarray	No significant differences in the abundance of bacterial phylum-like groups between CD and controls The bacterial diversity was comparable between CD and controls In treated and untreated CD: ↑TLR2 expression ↑ IL-10, IFN-g, C-X-C chemokine receptor type 6 expression
Giron Fernandez-Crehuet et al. [34]	2015	Spain	11untreated CD (median age, 5.0 years) 6 controls (median age, 8.8 years)	DGGE	The intestinal microbiota of children with Marsh 3c lesion showed similarity of 98% and differs from other CD children with lesion as Marsh 3a, 3b and Marsh 2 In CD: ↓ Richness, diversity and abitability of *Lactobacillus* In untreated CD: ↓ *Streptococcus, Bacteroides*, *E. coli* In controls ↓ *Streptococcus, Bacteroides* ↑ *Bifidobacterium, Lactobacillus, Acinetobacter*

CD celiac disease, *FISH* fluorescent in situ hybridization, *DGGE* denaturing gradient gel electrophoresis, *GFD* gluten-free diet, *HIPchip* Human Intestinal Tract Chip, *IFN-g* interferon-gamma, *IL-10* interleukin-10, *IS-pro* 16S-23S interspacer, *PCR* polymerase chain reaction, *qPCR* quantitative polymerase chain reaction, *qRT-PCR* quantitative reverse-transcriptase-polymerase chain reaction, *TGGE* temporal temperature gradient gelelectrophoresis, *TLR2* toll-like receptor 2, C-X-C chemokine receptor type 6.

Table 2. Duodenal-associated microbiota in celiac disease.

In contrast, two recent studies reached different results. De Meij et al. [33], analyzing the total microbiome profile in small bowel biopsies of 21 untreated CD and 21 age-matched controls, found that mucosa-associated duodenal microbiome composition and diversity did not differ between children with untreated CD and controls. The same results were obtained by Cheng et al. using bacterial phylogenetic microarray to comprehensively profile the microbiota in duodenal biopsies of 10 CD and nine healthy children, suggesting that the duodenal mucosa-associated bacteria do not play an important role in the pathogenesis of CD [34].

In summary, although the majority of the studies available have confirmed the presence of intestinal dysbiosis in CD children characterized by low levels of *Lactobacilli* and *Bifidobacteria* and increase in Gram-negative bacteria (*Bacteroides*), which were not completely normalized after GFD, some of them have failed to find a distinct signature that defines celiac microbiota. The available articles regarding the relationship between the gut microbiota and GFD, demonstrated that a GFD only allows a partial recovery of the gut microbiota in CD patients [30, 34, 35].

3. Pathogenetic role of intestinal dysbiosis in CD

The intestinal microbiota composition and function play a fundamental role in the balance between the host's health and disease by different mechanisms: (1) regulation of epithelial cell proliferation and expression of tight junction proteins which act on intestinal permeability; (2) influence on mucin gene expression by goblet cells and their glycosylation pattern; 3) secretion of antimicrobial peptides (defensins, angiogenins, Reg3γ, etc.) by intestinal cells, which contribute to control gut bacterial adhesion. Certain components of the gut microbiota also affect the expression and activation of pattern recognition receptors (PRR), such as toll-like receptors (TLRs), which are expressed by epithelial cells and innate immune cells. The mammalian TLR recognizes specific patterns of microbial components, called pathogen-associated molecular patterns (PAMPs). After the PRR-PAMP interaction, activated innate immune cells start the adaptive immune response by presenting the antigen and by producing cytokines, which leads to antigen-specific, protective immune response. In inflammatory and autoimmune diseases this response causes damage to host's tissues [36]. The gut microbiota impacts on adaptive immunity. Recently, specific commensal bacteria have been shown to influence T lymphocyte production (Th1, Th17) or anti-inflammatory regulatory T cells (Tregs) [36].

To date, human microbiota and mucosal barrier function are the key players in etiology of many inflammatory and autoimmune diseases [37]. Changes in mechanisms regulating mucosal immunity and tolerance, can lead to impaired mucosal barrier function, increased penetration of microbial components from lumen into the mucosa and circulation, and consequently lead exaggeration of aberrant immune responses and inflammation.

The exact mechanisms through which the gut microbiota might influence CD onset or progression is unknown, but could include activation of innate immune system, modulation

of the epithelial barrier, or exacerbation of the gliadin-specific immune response [38]. Moreover, the presence of microbiota can significantly influence the inflammatory effect of gluten. The microbiota may facilitate the access of gliadin peptides to the lamina propria and its interaction with infiltrated lymphocytes and antigen presenting cells (APCs) responsible for triggering the immune response via different mechanisms. In genetically predisposed individuals, gluten in association with microbial antigens can stimulate and modulate innate and adaptative immune response, sustaining a chronic mucosal inflammation, underlining this chronic disease [38].

4. Probiotics in the treatment of CD

Probiotics are nonpathogenic live microorganisms, which when orally administered in adequate amounts, alter the microflora of the host and have beneficial health effects. Probiotics have shown to preserve the intestinal barrier promoting its integrity both in vitro and in vivo [39, 40] as well as regulating the response of the innate and adaptative immune system. The association of CD with intestinal dysbiosis and the evidence supporting a role for the microbiota and specific bacteria in maintaining gut barrier function and regulating the response of the innate and adaptive immune system, have supported the potential use of probiotics in CD treatment [41, 42]. Although the data regarding the use of probiotics for CD are encouraging, most of these data come from in vitro experimental models of CD [43, 44]. Studies regarding probiotics and CD in humans are very scarce [45–47]. Smecuol et al. evaluated the effect of the *Bifidobacterium* infantis natren life start (NLS) on gut permeability, the occurrence of symptoms, and presence of inflammatory cytokines in adult CD patients on GCD. Results have shown that probiotics did not modify intestinal permeability probably due to an insufficient dose or a short time of administration. However, probiotic administration improved gastrointestinal symptoms, alleviating and reducing constipation [47].

In children, the clinical trials performed on the effect of probiotics on CD are summarized in **Table 3**. In the earliest study Olivares et al. [45] evaluated the influence of *Bifidobacterium longum* CECT 7347 in addition to a GFD in children newly diagnosed with CD. They showed a decrease in peripheral CD3+ T lymphocytes and a trend in the reduction of tumor necrosis factor (TNF)-α serum levels, and a reduction in the *Bacteroides fragilis* group(pro-inflammatory bacteria) and in the content of IgA in stools. Klemenak et al. [46] evaluated the effect of a combination of the strains *Bifidobacterium breve* BR03 and *B. breve* B632, as compared to placebo. They reported that *B. breve* strains decreased the production of the pro-inflammatory cytokine TNF-α in children CD on a GFD.

At this time, the only treatment for CD is lifelong GFD, which involves the elimination of grains containing gluten, wheat, rye, and barley in addition to food products and additives derived from them [48]. To date, adherence to a diet is difficult for many patients. Studies have shown that dietary transgression in patients with CD is common and can occur anywhere from 32% to 55% [49]. Moreover, a GFD may be rich in high glycemic index foods which can increase

insulin resistance and, thus, the risk of obesity and cardiovascular disease. In the last decade, new therapies have been suggested to improve compliance to a GFD or to replace a GFD [50]. The use of probiotics appears to be able to reduce the damage caused by eating gluten-containing foods and may even accelerate mucosal healing after the initiation of GFD [50, 51]. A specific commercially available probiotic, VSL#3 (containing eight different bacteria), has been shown to reduce the toxicity of gluten when used in a fermentation process [52]. It is thought that the gut microbiota can be modified in its composition and function by probiotic administration. These may counteract or postpone the onset of CD, and it can be useful in patients on GFD, when the normal composition of the intestinal flora has not yet fully recovered.

Authors/ references	Years	Country	Study design	Patients population and sample size	Main results	Comments
Olivares et al. [45]	2013	Spain	DB, R, PC	18 CD (mean age, 6.8 years) received *B. longum* CECT 7347; 18 CD (mean age 8.5 years) received placebo for 3 months in parallel with the GFD	↓*B. fragilis* group ↓activated T-lymphocytes↓ TNF-α	*B. longum* CECT 7347 could improve the health status of CD patients
Klemenac et al. 2015 [46]	Italy Slovenia	DB, R, PC		22 CD (age, 10.43) daily received *B. breve* 25 CD (age, 10.81) daily received placebo for 3 months 18 (age, 8.83) controls	↓TNF-α levels on CD group	Probiotic intervention with *B. breve* strains has shown a positive effect on decreasing the production of pro-inflammatory cytokine TNF-α in children with CD on GFD

CD celiac disease, DB double-blind, R randomized, PC placebo controlled.

Table 3. Clinical trials on the effect of probiotics for CD.

5. Conclusions

An alternative treatment that can improve CD patients' quality of life may lie in probiotics. In particular, probiotics such as *Lactobacilli* and *Bifidobacterium* could be useful to reset altered gut microbiota, as well as reduce gliadin toxicity and immune activation. Their use as a primary prophylactic treatment for children at high risk of CD is also a potential consideration. However, their use in routine clinical practice is hindered by limited data from human studies. The role of specific probiotics and their mechanism of action need to be identified in a larger experimental population to confirm their effectiveness.

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