Chapter 5

Intestinal Microbiota and Celiac Disease

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Abstract

Intestinal microbiota is constituted by a particular assembly of bacteria that develop symbiotic relationships with their host, contributing to diverse physiological functions and determining resilience to disease. Diverse environmental and intrinsic factors can upset this symbiotic relationship, shifting the ecosystem from a state of eubiosis to one of dysbiosis, which causes functional modifications and promotes disease. Indeed, immune dysfunction frequently coincides with intestinal dysbiosis and one can occur as a result of the other, creating a vicious circle. On this basis, hypotheses suggest that a dysbiotic gut microbiota could influence the onset and progression of celiac disease (CD). Epidemiological studies indicate that common perinatal and early postnatal factors influencing CD risk also affect the intestinal microbiota structure. A recent prospective study of healthy infants at family risk of developing CD has also revealed that the HLA-DQ genotype influences the microbiota composition. Several studies have also shown imbalances in the intestinal microbiota of CD patients, which are not fully normalized despite their adherence to a gluten-free diet, thus suggesting that such imbalances are not just a secondary consequence of CD. Furthermore, two small intervention studies have recently reported potential interest in the use of specific bifidobacteria to improve CD treatment, although larger human trials are required to confirm the benefits. Altogether, findings indicate that gut microbiota composition and function may be one of the missing pieces in the CD puzzle that could help to fully explain disease pathogenesis and risk. Thus, it is interesting to investigate new

strategies for CD management that target gut microbiota within this research field.

Keywords

Microbiota, celiac disease, *Bifidobacterium*, probiotics.

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1. Introduction

Celiac disease (CD) is a chronic enteropathy triggered by cereal gluten proteins in genetically predisposed individuals. CD onset usually occurs in early childhood after the first exposure to dietary gluten; however, recent decades have witnessed an increase in the number of subjects experiencing gluten intolerance in their late adulthood[1]. This phenomenon is not fully explained by improvements in diagnosis and suggests changes in exposure to environmental factors that contribute to disease development.

The etiology of the disease is strongly associated with the genes of the human leukocyte antigen (HLA) that encode the HLA-DQ2 (HLA-DQ2.5 and HLA-DQ2.2) and HLA-DQ8 heterodimers expressed by antigen-presenting cells (APC). Gluten peptides bond to HLA heterodimers and are presented to T cells that trigger a complex immune response involving the innate and adaptive system. Most patients are carriers of the HLA-DQ2/DQ8 genes but this genotype is also present in about 40% of the general population and only a small percentage (2-5%) develops CD[2,3]. This indicates that the HLA-DQ genotype is necessary but not solely responsible for development of the disease. Gluten is the main environmental trigger of CD but its intake neither fully explains the onset nor its clinical manifestations. In recent years, other environmental factors that influence the early gut microbiota composition such as type of delivery at birth and milk-feeding, intestinal infections and antibiotic intake, have also been associated with the risk of developing CD[2-7].

Observational studies of children and adult patients with CD (untreated and treated with a gluten-free diet (GFD)) revealed imbalances in their intestinal microbiota as compared to control subjects, which could contribute to the pathogenesis of the disease[8,9]. This evidence suggests that the imbalances in gut microbiota are not only a secondary consequence of the inflammatory milieu characteristic of the active phase of CD but that they could also be a predisposing factor for disease development. However, the GFD *per se* also induced changes in gut microbiota composition of healthy adult subjects and could be partly responsible for the alterations detected in

treated CD patients[10]. Therefore, to understand whether gut microbiota imbalances could play a role in CD onset, a prospective study is underway to investigate the early features of the intestinal microbiome in infants at family risk of CD development.

Currently, CD is among the most prevalent chronic digestive disorders but the only treatment is life-long adherence to a GFD. However, compliance with this dietary restriction is complicated due to the presence of gluten in most processed foods and patients are continuously exposed to gluten. Therefore, the identification of modifiable environmental factors that contribute to CD onset is critical for the development of strategies that lead to a reduction in disease incidence. This may be the case for components of the intestinal microbiota, whose acquisition could be modulated by environmental and dietary factors.

Here, we summarize the current understanding of the role played by intestinal microbiota in the etiopathogenesis of CD. We also discuss the possibilities of contributing to disease prevention and treatment by modulating gut microbiota composition and function.

2. Gut Microbiota Acquisition in Infants and CD Risk

The primary colonization of the intestinal microbiota begins at birth with the acquisition of microbes from the environment, mainly from the maternal vagina and the skin. It is a dynamic process that involves interactions of co-occurrence and exclusion between intestinal bacteria, reflecting life events of the newborn and undergoing changes until the first two-three years of age when the microbiome starts to converge toward a generic adult-like profile[11,12]. The intestinal colonization process leads to the acquisition and establishment of a protective microbiota that could modulate the risk of developing immune-mediated diseases in adulthood[13]. This influence is mediated by early gut microbiota and immune system interactions that are crucial for the development of tolerance towards harmless antigens from the diet and the microbiota.

2.1. Type of Delivery and Breast Feeding Practices

Perinatal and early postnatal environmental factors influencing the microbiota composition have been associated with CD susceptibility[14]. The greater risk of children born by caesarean section developing CD[15] might be attributed to the delay in intestinal colonization by bifidobacteria and the reduced bacterial diversity observed in caesarean-born compared to naturally-delivered infants[16]. Longer breast-feeding and particularly maintenance of breast-feeding when gluten is introduced seems to reduce the risk of developing CD or, at least, delays its onset in most case-control retrospective studies included in the meta-analysis by Akobeng et al.(2006)[17]. Also, feeding practices involving the gradual introduction of gluten simultaneous to breastfeeding were proposed as the protective agent responsible for reducing CD prevalence in one birth cohort compared to the "Swedish CD epidemic" cohort[18]. However, other prospective epidemiological and intervention studies failed to find a protective effect of breast-feeding in either CD autoimmunity or biopsy proven CD[19,20]. These inconsistencies could be due to the implication of non-controlled variables (type of delivery, incidence of infections, amount of gluten in the diet, etc.) that confound the statistical analysis on breastfeeding effects. Duration of breast-feeding could be associated with a reduced or delayed exposure of the newborn to dietary gluten, which might contribute to the protective effect of breast milk. Plausibly bioactive breast milk components may also be involved in the potential protective effect of breast-feeding on CD development. For the infant's gut, breast milk is a source of bacteria[21,22] and of human milk oligosaccharides (HMOs), which promote gut colonization by *Bifidobacterium* spp., possibly explaining the differences observed between the intestinal microbiota of breast-fed and formula-fed infants[23-25]. The beneficial properties of bifidobacteria on infants' health is widely accepted[26], and scarcity of these bacteria have been associated with the onset of inflammatory bowel disease (IBD)[27], type 1 diabetes (T1D)[28] and infant allergies[29]. Besides human milk provides many bioactive substances involved in passive immune protection and in immunological development of the neonate[30]. A complex network of chemo-attractants and cytokines in

human milk are thought to play a role in compensating the developmental delay of the neonate immune system and in preventing the development of immune-mediated diseases[31]. Recent research has analyzed differences between breast-milk composition of healthy mothers and mothers with CD on a GFD[32]. Mothers with CD presented a decrease in several immune markers (interleukin (IL)-12p70, transforming growth factor (TGF)-β1 and secretory IgA (sIgA) and in numbers of *Bifidobacterium* spp. in breast-milk[32]. Likewise, these differences in the breast milk of CD mothers might influence the protective effects of breast-feeding on infant health, partly explaining the controversy across studies[32]. Similarly imbalances characterized by a lower content of immune mediators (interferon (IFN)-γ, TGF-β2, IL-10 and sIgA) have been described and interpreted as a health risk factor for infants of allergic mothers[33,34]. Furthermore, wheat gliadins and other gluten peptides have been detected in breast milk using specific IgA-antibodies against gliadin[35,36] and the presence of gluten in breast milk has been suggested to play a role in the induction of oral tolerance of the breastfed infants. Thus, breast milk of mothers with CD following a GFD will lack this stimulus, and this might influence the future gluten tolerance of their offspring. However, as yet there is no evidence to support this hypothesis.

A number of epidemiological studies indicate that several perinatal factors participate in conjunction to modulate CD risk. However, there are no prospective studies revealing how differences in breast milk composition and in intestinal microbiota acquisition early in life might ultimately protect or contribute to CD onset.

2.2. Genotype and Intestinal Microbiota

Murine models using diverse mice strains congenic for major histocompatibility complex (MHC) genes indicate that MHC influences the composition of the faecal microbiota[37]. Recently a fish model using *Gasterosteus aculeatus* (threespine stickleback) has shown that the presence of certain MHC polymorphism is associated with altered abundance of some microbial families[38].

Over 30 years ago, Van de Merwe et al.[39] described that the faecal microbiota of monozygotic human twins was much more similar than that of dizygotic twins. Later a similar observation was reported for adults with varying degrees of relatedness[40] and identical twins, fraternal twins and unrelated controls[41].The most recent study compared microbiota of 416 twin pairs and identified many microbial taxa whose abundances were influenced by host genetics. The family Christensenellaceae showed the highest heritability, which formed a co-occurrence network with other heritable bacteria and Archaea in lean individuals[42]. This evidence suggests that host genetics influence the composition of the human gut and that this influences the phenotype[42]. In the case of CD, a prospective study in a cohort of 164 infants with a family history of the disease reported associations between genetic risk (HLA-DQ genotype) and alterations in intestinal microbiota composition[43-45]. The HLA-DQ2/8 genotype and the type of feeding (maternal or formula) influenced in conjunction the intestinal colonization analyzed by fluorescence *in situ* hybridization (FISH), real time PCR and denaturing gradient gel electrophoresis (DGGE) techniques[43-45]. In addition, specific decreases in *Bifidobacterium* spp. and *B. longum* and increases in *Staphylococcus* spp. were associated with higher genetic risk of developing CD irrespective of milk-feeding type[44]. The recent pyrosequencing analysis of the microbiota of a sub-cohort of 22 infants, all breast-fed and naturally delivered, confirmed that the HLA-DQ genotype influences *per se* the intestinal microbiota composition[46]. The high risk (HLA-DQ2 genotype) infant group showed an increase in the proportions of Firmicutes (*Clostridium* sensu stricto and unclassified Clostridiaceace and *Gemella*) and Protebacteria (*Raoultella* and unclassified Enterobacteriacea) and a reduction in Actinobacteria (*Bifidobacterium*). Associations have also been made between some *Clostridium* species, such as *C. difficile,* in ileal samples of human subjects and the NOD2 genotype and the phenotype of inflammatory bowel diseases[47]. A prospective study also reported that a reduction in the ratio of *Bifidobacterium* to *Clostridium* counts was associated with subsequent development of atopic dermatitis[48]. Another small study characterized the longitudinal changes in the microbial communities of genetically predisposed infants (HLA-DQ2/8)[5] and compared the results with

the data from another study on non-genotyped healthy infants[12]. The microbiota of HLA-DQ2/8 carriers was characterized by higher abundance of Firmicutes and lower abundance of Bacteroidetes (1% to undetectable) compared to that of healthy infants. However, the differences attributed by the authors to the HLA-DQ genotype could be due to their use of different methodologies for sampling, storage and processing of stool samples and for the taxonomic analyses (small subunit (SSU) rDNA microarray *vs* 454 pyrosequencing). This makes indeed the data incomparable.

The mechanisms by which the HLA-DQ genotype could selectively influence colonization and composition of gut microbiota remain unknown. However, we can speculate that MHC II presents phagocytized antigens of intestinal bacteria, which may then be presented to T cells. Depending on the antigen presented, effector T-cell activation could contribute to regulating the gut microbes colonizing the gut by activating B-cells to secrete protective antibodies directly into the gut mucosa and lumen[49]. Bacterial antigens presented via MHC II molecules, could also lead to T cell maturation into effector cells (Th1, Th2 or Th17) or Foxp3$^+$Treg cells with immunosuppressive activity, which could contribute to developing tolerance towards the intestinal microbiota. In this context, studies in rodents indicate that the repertoire of thymus-derived Treg cells, which constitute most Treg cells in all lymphoid and intestinal organs including the colon, is heavily influenced by microbiota composition, thus supporting this hypothesis[50].

Regarding possible pathogenicity of the microbiota alterations found in the CD genotype, the increase in *Staphylococcus* spp. described by De Palma et al.[44] is of particular interest. Some staphylococcal superantigens preferentially interact with HLA-DQ molecules, activating an inflammatory response that could increase the risk of developing CD[51]. This cohort of infants is being followed-up to monitor whether the intestinal microbiota alterations detected in early life are ultimately associated with CD onset. There is a strong association between CD and the expression of HLA-DQ2/DQ8 molecules compared to other HLA-linked diseases[52], but several non-HLA genes also contribute to the disease[53] and their influence on the intestinal microbiota composition should not

be discarded. For instance, non expression of the *FUT2* gene coding for fucosyltransferase 2, leading to a non-secretor phenotype, has been associated with an increased susceptibility of developing CD[54]. Fucosyltranferase 2 is responsible for synthesising ABH antigens in the mucus and other secretions and its expression has also been associated with reduced diversity, richness and abundance of bifidobacteria in the human intestinal tract[55]. Therefore, both HLA-DQ2/8 molecules and the non-secretor phenotype due to *FUT2* gene dysfunction have been linked with CD onset and also with reduced intestinal numbers of *Bifidobacterium* spp. This evidence, together with the reduced bifidobacteria levels detected in CD patients (described below; 9, 10), indicate this bacterial genus plays a role in CD risk.

3. Influence of Intestinal Microbiota in CD Pathogenesis

Several observational studies in children and adults with CD have shown alterations in the intestinal microbiota composition compared to control subjects. Our studies using molecular quantitative methods, such as FISH and quantitative PCR, found reduced numbers of *Bifidobacterium* spp. and *B. longum* and increased numbers of *Bacteroides* spp. in stools and duodenal biopsies of CD patients untreated and treated with a GFD compared to control subjects[8,9]. Also enterobacteria and staphylococci numbers were higher in untreated CD patients than in controls, but these differences were almost restored in CD subjects on a long-term GFD[9]. Likewise, other studies in children reported increased prevalence of *Bacteroides vulgatus* and *E. coli* in CD biopsies before and after the GFD by temporal temperature gradient gel electrophoresis (TTGE) compared to controls[56] and lower numbers of *Lactobacillu*s and *Bifidobacterium* and higher numbers of *Bacteroides, Staphylococcus* and enterobacteria in stools of children with CD compared to healthy controls[57]. Other studies performed by DGGE of the microbiota of adults with CD clustered the dominant microbial communities of healthy individuals together and separate from those of untreated CD patients[58]. However, the above study reported an increased prevalence in

Bifidobacterium bifidum in patients with active CD as opposed to the lower bifidobacteria numbers detected in CD patients in our studies[9,57,59,60] or the absence of differences reported in another study[61]. The analysis of metabolites derived from intestinal microbiota activity has also revealed significant differences between treated CD patients and healthy controls and suggests there is a metabolic signature for the CD microbiome[58,59]. One of the most recent studies has also reported that CD patients with gastrointestinal symptoms had different microbiota composition when compared with controls and patients with dermatitis herpetiformis, suggesting that the microbiota may play a role in the manifestation of the disease[62]. In Sweden, an early study with samples collected between 1985-1996 revealed that rod-shaped bacteria were frequently associated with the mucosa of CD patients, both in the active phase and treated with a GFD, as detected by scanning electron microscopy (SEM)[63]. Later, these SEM analyses were complemented with 16S rDNA sequencing to identify the bacterial communities detected in the samples of the Swedish epidemic (1985-1996) and in a new cohort of patients (2004-2007)[64]. Only one CD biopsy collected during 2004-2007 contained rod-shaped bacteria in contrast to the frequency described in the samples of the Swedish epidemic, invalidating the initial theory that these bacteria were causative factors of the CD epidemic[64]. The characterization of the microbiota from biopsies of CD patients from the Swedish celiac epidemic showed that SEM positive biopsies were significantly enriched in *Clostridium*, *Prevotella* spp. and *Actinomyces* compared to the SEM negative biopsies also from CD patients[64]. We also carried out a deeper characterization of the CD microbiota by isolating bacterial strains and analyzing their pathogenic features[65-67]. Specifically, *E. coli* clones belonging to virulent phylogenetic groups (B2 and D) isolated from untreated and treated CD patients presented a higher number of virulence genes encoding P fimbriae, capsule K5 and hemolysin than those isolated from healthy controls[65]. Furthermore, the abundance of the species *Bacteroides fragilis* coding for metalloproteases was increased in both untreated and treated CD patients, and thus could presumably play a pathogenic role in CD[66]. In fact, *Bacteroides fragilis* and the strains producing metalloproteases are frequently involved in opportunistic infections

and aggravate colitis in animal models[68]. The isolation and identification of clones belonging to the genus *Staphylococcus* also revealed that the species *S. epidermidis* carrying the *mecA* gene (methicillin resistant gene) was more abundant in the CD patients (treated and untreated) than in controls[67].

4. Potential Mechanism of Action of Intestinal Microbiota in CD

The microbiota and its alteration could contribute to the etiopathogenesis of CD by providing proteolytic activities that influence the generation of toxic and immunogenic peptides from gluten[66,69]; and by mediating-host-microbe interactions, which could influence the intestinal barrier[70] and the immune function[71] (Figure 1).

Some gluten peptides (gliadin) withstand gastrointestinal digestion and disturb the intestinal integrity by altering tight junction proteins, increasing epithelial intestinal permeability[73]. These may facilitate the access of gliadin peptides to the lamina propria and its interaction with infiltrated lymphocytes and APCs responsible for triggering the immune response. *B. fragilis* clones isolated from the intestinal microbiota of CD patients showed gliadin-hydrolyzing activity, and some of them generated peptides that maintain their immunogenicity, eliciting inflammatory cytokine production by Caco-2 cell cultures, and showing a greater ability to permeate the Caco-2 cell monolayer[66]. In contrast, different bifidobacteria and, particularly, *B. longum* CECT 7347 (also named *B. longum* IATA-ES1) reduced the cytotoxic and inflammatory effects of gliadin peptides generated during gastrointestinal digestion[69]. Thus, *in vitro* studies indicate that the proteolytic activity of the intestinal microbiota may modify gliadin peptides differently, increasing or reducing their toxicity. Similarly, Fernandez-Feo et al.[74] and Caminero et al.[75] isolated species from the oral cavity and faeces able to hydrolyse gluten peptides; however, their physiological effects have not been evaluated.

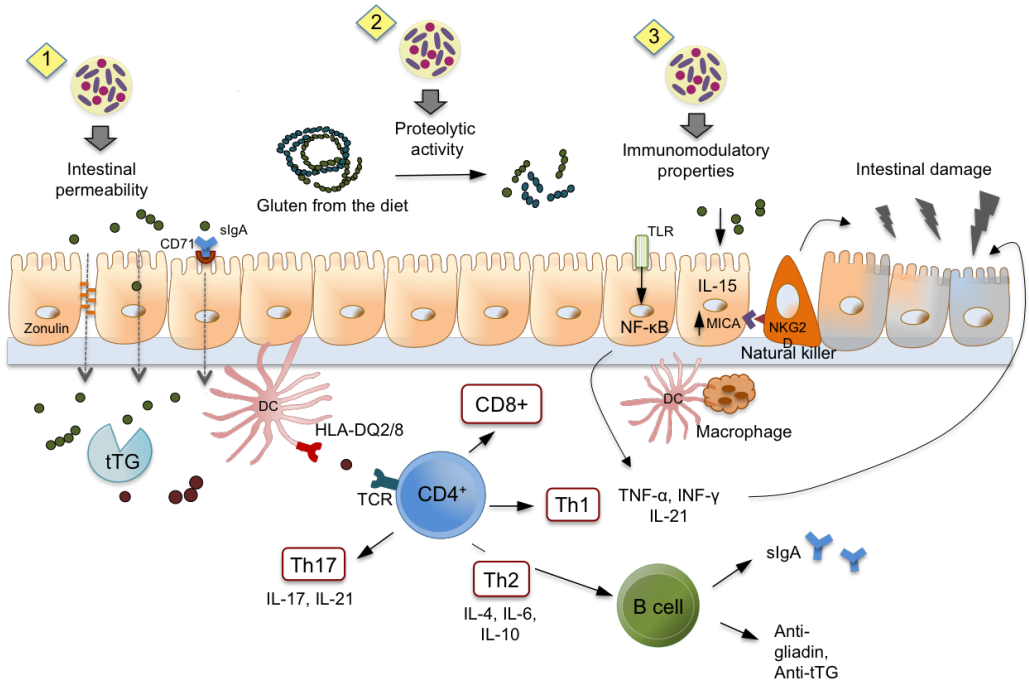

Figure 1. Schematic representation of CD pathogenesis and the potential role of intestinal dysbiosis. Some gluten peptides cross the intestinal epithelium and can be deamidated by the tissue transglutaminase (tTG), which increases their ability to bind the HLA-DQ2/8 molecules of antigen-presenting cells and to trigger an adaptive immune response, involving Th1, Th2 and Th17 cells that lead to the release of pro-inflammatory cytokines (IFN-γ, interleukin (IL)-21, etc.) and the production of CD antibodies; other gluten peptides activate the innate immune response by interacting with eptithelial cells and APCs and, thus, trigerring the activation of inflammatory pathways (NFκB) and the production of inflammatory cytokines such as IL-15. In particular, IL-15 increases the expression of the MICA molecule at epithelial cell surface and triggers activation of intraepithelial lymphocytes through engagement of NKG2D, leading to an innate-like cytotoxicity toward epithelial cells and enhanced CD8 T cell-mediated adaptive response, contributing to villous atrophy[72]. The microbiota could contribute to the etiopathogenesis of CD by (2) providing proteolytic activities that influence the generation of toxic and immunogenic peptides from gluten[66,69] and by mediating host-microbe interactions which could influence (1) the intestinal barrier[70] and (3) immune function[71].

Regarding the mechanism of action related to the intestinal barrier function, CD-triggers (gliadin and IFN-γ) decreased the goblet cell numbers in intestinal loops of inbred Wistar-AVN rats, and research shows the presence of enterobacteria isolated from CD patients, such as *Escherichia coli* CBL2 and *Shigella* CBD8, aggravate this effect[71]. Furthermore, exposure to these enterobacteria causes increased mucin secretion and greater disruption of tight junctions. By contrast, *Bifidobacterium bifidum* CECT 7365 (also named *B. bifidum* IATA-ES2) increased the number of goblet cells and the production of inhibitors of metalloproteinases, and also reduces gliadin translocation to the lamina propria, which could contribute to gut mucosal protection[71]. Other probiotic bacteria such as *Lactobacillus rhamnosus* GG have been shown to contribute *in vitro* to the maintenance of normal intestinal permeability in Caco-2 cell cultures exposed to gliadin[76].

The composition of the gut microbiota could also influence the release of pro-inflammatory cytokines triggered by gluten peptides. For instance, a mixture of isolated bacteria from CD patients (*Prevotella* sp., *Lachnoanaerobaculum umeaense* and *Actinomyces graevenitzii*) induced IL-17A mRNA expression in *ex vivo* biopsies of intestinal mucosa of CD patients[77]. Thus it was hypothesized that those bacteria could modulate the IL-17 response by helping to breakdown gluten tolerance[77]. By contrast, in gliadin-sensitized HLA-DQ8 transgenic mice, a strain of *Lactobacillus casei* reduced the TNF-α levels in jejunal tissue sections[78]. In a model of newborn rats sensitized with IFN-γ and orally administered gliadin, *B. longum* CECT 7347 reduced TNF-α and increased IL-10 concentration in intestinal tissue samples[79].

On the one hand, *B. longum* CECT 7347 and *B. bifidum* CECT 7365 reduced the inflammatory cytokines (IFN-γ and TNF-α) produced by the microbiota of CD patients, and, on the other, they increased IL-10 production, with anti-inflammatory effects in peripheral blood mononuclear cell (PBMC) cultures[80]. *Escherichia coli* CBL2 and *Shigella* CBD8 isolated from CD patients, boosted the production of IL-12 and IFN-γ, and the expression of HLA-DR and CD40 in co-cultures of monocyte-derived dendritic

cells (MDDCs) and Caco-2 cells compared to *B. longum* CECT 7347 or *B. bifidum* CECT 7365[81]. These responses could be mediated by the activation of toll-like receptors (TLRs), which play an important role in the recognition of microbial components, driving different transcription pathways involved in the immune response. So far it has been reported that biopsies from CD patients display increased TLR2 expression, which is a receptor responding to bacterial lipopeptides, and of TLR9, which is a receptor responding to bacterial DNA[61]. We could hypothesize that this increased TLR expression in biopsies of CD patients may intensify gut microbiota signalling and host response to intestinal dysbiosis although direct evidence is not available.

5. Gluten Intake and Intestinal Microbiota

The only treatment for CD is adherence to a life-long GFD, which implies important dietary changes. Specifically, women on a GFD have a reduced dietary protein and fibre intake and an increased fat intake[82]. These dietary differences also seem to cause changes in the intestinal microbiota composition and in the immune response to the altered microbiota *in vitro*. After three months of adherence to the GFD, children with CD showed increases in the *B. fragilis* group and *Enterobacteriaceae* numbers and in sIgA levels in stools[83]. In healthy adults the GFD caused shifts in gut microbiota composition, characterized by reduced numbers of *Bifidobacterium* spp., *B. longum* and the *Lactobacillus* group, and increased numbers of *Enterobacteriaceae* and *E. coli*[10]. This led to the proposal that GFD should be considered as an environmental factor that may contribute to shaping the microbiota composition in treated CD patients[10].

In animal models, gut microbiota changes have also been related to the GFD but the data are not comparable to humans. For example, GFD-induced changes in the microbiota of NOD mice are characterized by higher numbers in *Bacteroides* and *Akkermansia* and a higher percentage of $CD4^+CD25^+$Foxp3 regulatory cells, and reduced T1D incidence[84]. By contrast, NOD mice fed a diet containing gluten had higher numbers of

Bifidobacterium, Tannerella and *Barnesiella* and increased T1D incidence[84]. Harsen et al., (2014)[85] also proposed that GFD-induced increases in *Akkermansia*, Protebacteria and TM7 abundance protected the offspring of NOD mice and reduced the incidence of diabetes[86]; however, direct evidence is lacking.

6. Role of Probiotics in CD: Human Studies

There are proposals to use of some probiotic bacteria in CD management based on the associations between CD and intestinal microbiota imbalances, and the role attributed to some bacterial strains in maintaining gut barrier function and regulating the immune response in certain chronic inflammatory diseases. To our knowledge, only two intervention trials have been conducted with probiotics in CD patients to date. Both were randomized, double-blind placebo-controlled trials, but differed in the aim, species and strain of bifidobacteria tested. In one of the interventions, *B. infantis* NLS was administered to untreated CD patients consuming gluten to evaluate the effect of the probiotic independently of the GFD[87]. The beneficial properties of *B. infantis* NLS included the reduction of some gastrointestinal symptoms, specifically indigestion, constipation and reflux with borderline significance. However, it did not improve diarrhoea or abdominal pain, nor modify intestinal permeability or the pro-inflammatory status, as reflected by the analysis of serum cytokines and chemokines[87]. Another study evaluated the influence of administering *B. longum* CECT 7347 to children with newly diagnosed CD following a GFD to assess whether it improved the efficacy of the GFD[83]. Inter-group comparisons revealed a decrease in peripheral $CD3^+$ T lymphocytes and TNF-α levels in the bifidobacterial group. The administration of *B. longum* CECT 7347 also reduced *Bacteroides fragilis* group numbers and sIgA in stools when compared to the placebo[83], which could presumably contribute to better recovery from the inflammatory status associated with the active phase of the disease. Despite the experimental differences, presumably the mechanism behind the effects of *B. infantis* NLS

differ from those of *B. longum* CECT 7347, as the latter influences inflammatory markers, gut microbiota and host-related defence mechanisms. Both studies suggest the potential interest of these probiotic bacterial strains for improving CD treatment, although larger human trials are required to confirm and strength of this evidence.

7. Conclusions

Most studies demonstrate associations between CD and shifts in the composition of intestinal microbiota. These alterations are not only consequence of the inflammatory status characteristic of the active phase of the disease because the ecological perturbations are not completely restored after adherence to a GFD, even though the GFD *per se* also influences the microbiota composition. In healthy infants at family risk of CD, prospective studies also indicate that alterations in gut microbiota composition are associated with the HLA-DQ genotype and could influence CD onset. The influence of gut microbiota composition on the etiopathogenesis of CD could be related to its proteolytic activity and ability to generate toxigenic and immunogenic peptides and, particularly, to its ability to regulate gut barrier function and the immune response to gluten. Further and larger studies are, however, necessary to confirm that gut microbiota modulation by the administration of specific bacterial strains could contribute to improving the health status of CD subjects, and to reducing the risk of CD development.

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