Celiac Disease and the Microbiome

Subjects: Nutrition & Dietetics
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Celiac disease (CD) has most often been perceived as a pediatric condition with a peak incidence in children younger than two years of age. Growing evidence supports the hypothesis that changes in both the composition and function of the intestinal microbiome are associated with a number of chronic inflammatory diseases including CD.

Keywords: celiac disease ; microbiome ; microbiota ; environmental factors ; at-risk infants

1. Introduction

Although the recognition of the causal link between gluten and celiac disease (CD) was unveiled in the 1950s [1], what factor (or factors) triggers the loss of immune tolerance to gluten in genetically predisposed subjects still remains unknown. Since its original description, CD has most often been perceived as a pediatric condition with a peak incidence in children younger than two years of age, with more recent data suggesting that most of the cases would manifest by five years of age [2].

The worldwide prevalence of CD ranges between 1% and 2% in the general population [3][4], with most patients remaining undiagnosed due to the subtle or multiform clinical manifestations of the disease [5]. Based on more recent epidemiological data, and contrary to the original paradigm, it is now appreciated that CD can present at any age with a broad range of intestinal and extra-intestinal symptoms [6][7]. Its prevalence, as in many other autoimmune diseases often found in comorbidity with CD [8], has increased over time in geographical regions characterized by a Western lifestyle [9]. This phenomenon was initially hypothesized to be secondary to the timing of gluten introduction at weaning [10], although two large, randomized, and prospective high-risk, birth cohort-controlled trials have disputed this premise by demonstrating that neither delayed nor early gluten introduction modified the risk of CD [2][11].

These findings raised doubts about another CD paradigm that suggested that genetic background and dietary gluten intake were necessary and sufficient to develop the disease. Besides the evidence that CD onset can occur years after gluten introduction into the diet [6], other evidence at odds with the old paradigm is the lack of 100% CD concordance among monozygotic twins [12]. Therefore, while genetic predisposition (including the required presence of HLA DQ2 and/or DQ8 haplotypes) and gluten exposure are necessary, they seem to be insufficient for the development of CD autoimmunity. Intestinal permeability is an additional element involved in CD pathogenesis, as a “leaky gut” might initiate the early phases of innate immune activation following the exaggerated trafficking of undigested gluten fragments from the intestinal lumen to the lamina propria [13].

Growing evidence supports the hypothesis that changes in gut microbiome composition and function are associated with a number of chronic inflammatory diseases including obesity [14], diabetes [15], inflammatory bowel disease [16] and cancer [17]. This might also be the case for CD.

In the last decades, one of the major advances in the field of microbiome studies has been the ability to apply culture-independent approaches to determine the microbiome’s composition [18]. These technologies allow for the identification and quantification of components of the human microbiota by studying nucleic acids (DNA and RNA) from fecal samples or other biological samples [19], which eliminates the need for tissue cultures and also allows the characterization of non-cultivable microbes.

The human gastrointestinal lumen contains a copious and diverse microbial ecosystem of over 100 trillion microorganisms [20]. More than 2 million genes are expressed by the human microbiome, and these genes encode for metabolic pathways that finally produce thousands of metabolites [21]. Conversely, it is striking to note that the human genome is composed of only 23,000 genes [22]. Consequently, the host and its microbial communities can be viewed as a “superorganism” with mutable immune and metabolic profiles [23].

Gut bacteria facilitate the digestion of insoluble fiber, produce vitamins such as vitamin K, and elaborate trophic and immunomodulating compounds such as short-chain fatty acids (SCFA) [24].

Moreover, they also display key immune-modulating functions within the gut. By competing for nutritional sources and producing anti-microbial molecules, beneficial gut bacteria counterbalance the growth of pathogenic bacteria and favor epithelial integrity [25][26]. Microbiome-derived SCFA can also modulate host histone deacetylase, therefore epigenetically influencing the function of innate and adaptive immune cells [27]. The impact of the gut microbiome on mucosal immunity is further demonstrated by the evidence of defects in lymphoid tissues (a decreased number of mucosal Peyer's patches and smaller mesenteric lymph nodes) and compromised antibody production in germ-free animals [28].

In early childhood, microbial diversity rises with age until it stabilizes with two major bacterial phyla: Firmicutes and Bacteroidetes, which represent roughly 90% of the whole gut microbiota. Actinobacteria, Proteobacteria, Fusobacteria and Verrucomicrobia are the next most-numerous describing a "healthy gut microbiota composition" [29]. At approximately three years of age, a child's gut microbiota composition and diversity are very similar to the adult microbiota [30]. While it is generally assumed that microbiome engraftment occurs at birth during the passage through the vaginal canal, or via maternal skin microbiota in case of cesarean section, there are a few reports showing that a specific microbiota colonizes the placenta [31] and is detectable in the meconium [32], suggesting that engraftment may start in utero.

In recent years, research into the early development of the microbiome has highlighted the influences of delivery mode, maternal/infant nutrition and antibiotics on the engraftment and subsequent changes in intestinal microbiome composition [33][34]. This crucial initial symbiotic relationship between host and gut microbiome is instrumental in programming the immune system to distinguish between pathogens and commensals to achieve the proper strategies to unleash inflammation when necessary (for example fighting pathogens) or maintain anergy [35].

2. Microbiome, Environmental Factors and Gut Inflammation: Implications for Celiac Disease (CD)

Environmental factors strongly drive microbiota engraftment and subsequent composition. For example, vaginal delivery ensures the vertical mother–infant transmission for pivotal gut microbiome components such as Bacteroides and Bifidobacteria [36]. Conversely, cesarean (C) section-born infants show less Bacteroidetes, and the diversity of this specific phylum is lower [37]. However, while it is uncertain if these changes might explain some reports of an increased risk of CD for children born via C-section [38][39], it should be acknowledged that the association between C-section and CD is still controversial [40].

Diet is another key regulator of microbiome development and homeostasis. The human milk oligosaccharides (HMOs) select the growth of commensals such as Bifidobacteria and prevent the growth of potential pathogens such as Clostridium difficile [41][42]. Moreover, HMOs enhance overall barrier integrity by making enterocytes less vulnerable to bacterial-induced innate immunity [43]. Therefore, breast-feeding seems to be ideal for the engraftment of a symbiotic gut microbiome.

Some data also suggested that maternal antibiotic assumption during pregnancy shapes the gut microbiota in the offspring [44], albeit a cohort study found no statistically significant association between maternal use of antibiotics during pregnancy and CD risk in the offspring [45]. According to some reports, antibiotic exposure during the first year of life has been associated with an increased risk of developing CD [46][47], however, other studies did not confirm this finding [48][49][50]. A recent meta-analysis did not resolve these incongruences, albeit favoring a non-causal relationship between early antibiotics exposure and CD [51].

Early life infections may be involved in CD onset, and this issue is also supported by cohort studies [52][53]. Another study that looked at the effect of viral triggers and Th1 response recognized reovirus as a possible cofactor for both inappropriate immune activation and subsequent loss of tolerance to gliadin [54]. Patients with CD display higher antibody titers against human adenovirus serotype 2 [55][56]. This might go along with the clinical interpretation of in vivo data. A longitudinal prospective cohort of genetically at-risk children demonstrated that an increased rate of rotavirus gastroenteritis may strengthen the risk of CD in infancy [57]. However, the implementation of rotavirus vaccination did not prevent a rise in CD prevalence that has been recently reported in Italian children [58]. A role for Candida albicans in CD development has been hypothesized based on sequence similarities between a hyphal wall protein and several T-cell gliadin epitopes [59], albeit the only small study on mycobiome next-generation sequencing analysis of duodenal samples showed no difference between adult CD cases and controls [60]. A large cohort study from Sweden has shown that there is

a significantly higher hazard ratio of C. difficile infection in patients with CD when compared to age- and gender-matched controls [61], albeit study limitations leave open a few areas of uncertainties [62].

The physical isolation of microbes from the glycocalyx of the intestinal epithelium without evidence of overt inflammation suggests that preventing physical contact with the gut mucosa avoids activation of the immune system, therefore favoring a symbiotic relationship between the host and the gut microbiome [63]. A balanced gut microbiota also contributes to the maintenance of the mucous layer, especially due to bacteria such as Lactobacillus species and Akkermansia muciniphila [64][65]. A healthy microbiota additionally favors colonization resistance, namely, the capability of commensal bacteria to compete for nutrients with pathogens, thereby stimulating the epithelium to secrete antimicrobial molecules into the mucous layer and provide a better defense against pathogens [66]. Additionally, commensals contribute to this line of defense by synthesizing protective substances, such as acetate produced by Bifidobacterium, which prevents colonization by enterohemorrhagic E. coli O157:H7 [67]. IgAs produced by the gut-associated lymphoid tissue (GALT) also contribute to barrier maintenance, microbiome selection and decreased activation of innate immunity [68]. Related to this topic, Olivares et al. demonstrated that a reduction in IgA fecal level can precede CD development in infants [69].

Some studies have shown an increased expression of genes responsible for pathogen-associated molecular pattern (PAMPs) recognition, such as Toll-like receptors (TLR) in CD. For example, Szebeni et al. found higher expressions of TLR2 and TLR4 in untreated and treated CD patients versus controls, as shown both at mRNA and protein levels [70]. Furthermore, TLR2, TLR9 and TOLLIP, an intracellular protein that inhibits TLR, have been found as microbiota-associated factors in the possible development of CD [71]. Overexpression of TOLLIP in vitro offsets TLR pathways after lipopolysaccharide or lipotechoic acid stimulation. This phenomenon has been named “lipopolysaccharide tolerance” [72]. In fact, a reduced expression of TOLLIP in active CD might indicate that a failure to tolerate microbiota may contribute to CD immune activation.

It is well acknowledged that the host can tightly control the microbiota, however, the microbiota also exerts a strong programming on host metabolism and immunity [73]. SCFA synthesized by commensal bacteria condition regulatory T-cells (Treg cells), specifically, one member of the SCFA, butyrate, helps T-cells to differentiate toward Treg cells [74]. SCFA might inhibit histone deacetylases, provoking hyperacetylation of histones, which finally results in anti-inflammatory gene activation [75].

The role of gut microbiota and their metabolites in CD has been explored by a recent study showing their effects on Treg cells through epigenetic processes [76]. Specifically, CD patients showed an increased expression of a non-functional spliced form of FOXP3 (so increasing the risk of developing autoimmunity) which could be attributable to the altered intestinal microbiota and to its unbalanced butyrate production.

In another study, CD-derived organoids treated for 48 h with microbiota-derived compounds, such as lactate, butyrate and polysaccharide A, showed a significant improvement of intestinal permeability measured as transepithelial electrical resistance changes. Moreover, the same group also showed that butyrate significantly upregulated the expression of genes regulating epithelial integrity in CD organoids [77].

It has been noticed that the HLA-DQ genotype can affect early gut microbiota composition [78], and an increased occurrence of pathogenic bacteria such as enterotoxigenic Escherichia coli has also been described in infants genetically at risk for CD [79]. In a previous study from a Spanish group, higher numbers of Bifidobacterium spp. and Bifidobacterium longum were present in the gut microbiota of infants with the lowest HLA-DQ genetic risk for CD, whereas, for those with the highest genetic risk, higher Staphylococcus spp. and Bacteroides fragilis were identified. However, the method of infant-feeding influenced the composition of the microbiota, with breast milk favoring Clostridium leptum, Bifidobacterium longum and Bifidobacterium breve gut colonization, therefore slightly switching the fecal microbiome toward the one identified in infants with low HLA-DQ genetic risk [80].

3. Bifidobacteria and Lactobacilli Strains: Few “Paladins” in the Pathogenetic Joust?

In the pursuit of the best microbial candidate for disease immunomodulation, a few Bifidobacteria strains have been studied with considerable results. For example, in an in vitro model using peripheral blood mononuclear cell (PBMCs), both Bifidobacterium longum ES1 and Bifidobacterium bifidum ES2 have been shown to downregulate the Th1 pathway typical of CD [81].

In addition, Lindfors et al. assessed whether Bifidobacterium lactis is capable of neutralizing the toxicity of gliadin. In Caco-2 cells, they found that this strain was at least able to reduce the epithelial permeability triggered by gluten [82].

Laparra et al. evaluated Bifidobacterium longum CECT 7347 in a murine model of CD, and they found that this specific strain not only diminishes pro-inflammatory cytokine synthesis, such as tumor necrosis factor-alfa (TNF-alfa), but it also reduces jejunal architecture damage [83]. Another group has demonstrated that Bifidobacterium longum strain NCC2705 produces a serine protease inhibitor with immune-modulating features, i.e., attenuating gliadin-induced histological damage in NOD/DQ8 mice [84].

An alternative Bifidobacterium, B. infantis, seems to decrease Paneth cells and expression of alfa-defensin-5 on electronic microscopy of duodenal biopsy when administered in active CD [85]. Paneth cells are key masters of gut homeostasis in innate immunity against noxious pathogens through the release of defensins, lysozyme and phospholipase [86]. Furthermore, some evidence concerning the protective effect of Lactobacillus casei DN-114001 and E. Coli strain Nissen 1917 on gut barrier function has been reported [87].

D'Arienzo et al. analyzed the effect of Lactobacillus casei, Lactobacillus paracasei and Lactobacillus fermentum in a transgenic mouse model expressing human DQ8. They found that L casei reduces TNF-alfa secretion and related villous blunting, while both L paracasei and L fermentum determine increased antigen-specific TNF-alfa. This suggests that, depending on the strain and on the experimental model, probiotics may have either proinflammatory or immunomodulatory properties [88][89].

4. Focus on Other Species and Strains: The Joust Gets Hectic

Several other bacterial species and specific strains have been studied in regard to their possible link with CD pathogenesis. As Bacteroides fragilis clones expressing metalloproteases were often reported in patients with CD, this might underscore an anticipated role played in CD pathogenesis. B. fragilis strains carrying metalloprotease genes may lead to increased intestinal permeability and production of gliadin immunogenic peptides. Furthermore, these peptides could maintain or even intensify their ability to provoke an inflammatory response mediated by TNF-alfa. These increases in TNF-alfa production by epithelial cells could have deleterious effects that fuel both innate and adaptive immunity in CD onset [90].

Some Prevotella species, Lachnoanaerobaculum umeaense and Actinomyces Graevenitzii, were isolated from CD jejunal biopsies. These species could trigger an IL-17A-driven immune response [91]. This emphasizes the possibility that the increased IL-17A response seen in active CD could be in part attributable to host-microbiota interactions, and this might also explain why the IL-17A mucosal response in CD is not consistent in some CD patients [92].

Neisseria flavescens determines inflammation and induces disturbances in the mitochondrial chain processes of Caco-2 epithelial cells. This latter metabolic alteration seems to be partly corrected when Lactobacillus paracasei CBA is administered [93]. Another study involving N. flavescens showed that five different strains isolated from adults with untreated CD led to an inflammatory activation of both human and murine dendritic cells (DC) [94]. Nevertheless, it is not clear whether N. flavescens causes inflammation, or the inflammatory process occurring in the gut of CD patients may favor its colonization, which then simply maintains an activated proinflammatory response.

In addition, it has been demonstrated by Galipeau et al. that gut microbiota can either reduce or exacerbate gliadin-induced damage in a mouse model of CD [95]. In this study, the expansion of the Proteobacteria phylum caused more severe intestinal damage induced by gluten. This could possibly be explained by the fact that the intestinal mucus layer is more penetrable to bacteria and toxins where Proteobacteria prevail [96]. Similar evidence comes from a study on Caco-2 cells from Spain. Enterobacteriaceae (belonging to the Proteobacteria phylum) were found to act similarly to gliadins regarding DC maturation, i.e., attachment, spreading and pro-inflammatory cytokine polarization. On the other hand, Bifidobacterium longum CECT 7347 counterbalanced IFN-production as a consequence of gliadin stimulation and increased IL-10 release [97]. Altogether, these evidences underline the importance of the biological milieu of the intestinal lumen for disease advancement.

5. Microbiome- Derived Gluten-Degrading Enzymes: Opportunity for Prevention and Alternative Treatment

Another issue to consider is the capacity of the enzyme machinery belonging to the gut microbiome to completely digest gluten. To this extent, it is of note that, after the bacterial proteolytic degradation of gliadin, peptides could still be toxic and eventually cross the intestinal barrier more easily [98]. However, few in vitro studies have revealed that microbiota components, specifically Bifidobacteria, can degrade proinflammatory gluten peptides in the small intestine, thus reducing their immunogenic potential [82][99]. In one recent study, some Lactobacilli were able to digest in vitro amylase-trypsin

inhibitors (ATIs), non-gluten wheat proteins that induce an innate immune response through the Toll-like receptor 4 (TLR4)–MD2–CD14 mechanisms. It is of note that the administration of Lactobacilli species (Lactobacillus salivarius H32.1, Lactobacillus mucosae D5a1 and Lactobacillus rhamnosus LE3) decreased both inflammation and permeability stimulated by ATIs [100].

Along with the bacterial component of the gut microbiome, enzymes able to digest gluten can also be elaborated by some eukaryotes. Papista et al. studied the influence of Saccharomyces boulardii KK1 supplementation in an animal model of gluten enteropathy (BALB/c mice). This intervention allowed the hydrolyzation of toxic gliadin peptides and counterbalanced both enteropathy and pro-inflammatory cytokine production [101]. In line with these data, another group has shown degrading activities toward toxic gluten epitopes by oral commensal bacteria such as Rothia spp, Actinomyces odontolyticus, Neisseria mucosa and Capnocytophaga sputigena [102]. Currently, some drugs based on degrading enzymes from bacteria and fungi have been used in clinical trials with diverse results [103].

It is well established that compliance to the GFD is difficult [104], and, for this reason, there is a great expectation among CD patients for drug-based therapies [105]. In light of these challenges, these findings on gluten-degrading activities by specific microbial strains might pave the way for a probiotics-based complementary therapy of CD in the years to come.

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