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Dietary Clues to the Pathogenesis of Crohn's Disease

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Abstract
Crohn's disease is a complex inherited disorder of unknown pathogenesis with environmental, genetic and microbial factors involved in the development of the disease. A remarkable feature of this disease in childhood is the effective response to exclusive enteral nutrition (EEN) therapy and the need for complete exclusion of normal diet required for success (principle of exclusivity). EEN or dietary interventions might act through removal of dietary components, which affect microbial composition, decrease a proinflammatory response and promote restitution of the epithelial barrier, likewise allowing termination of this vicious disease-forming cycle before a critical threshold is reached. Multiple traditional and nontraditional dietary components may affect the microbiome, mucous layer, intestinal permeability, or adherence and translocation of pathobionts. We review the epidemiological data, as well as data from animal models and cell lines, and propose a model for pathogenesis we have termed the 'bacterial penetration cycle', whereby dietary components such as animal fat, high sugar intake and gliadin, and consumption of emulsifiers, maltodextrin as well as low-fiber diets may be able to cause a localized acquired bacterial clearance defect, leading to bacterial adhesion and penetration, and subsequently inflammation in the gut.

Introduction

Crohn's disease (CD) is a complex inherited disease, arising at the interface between the luminal commensal bacteria and the intestinal epithelium. It arises in genetically susceptible individuals and involves an abnormal immune response to luminal or mucosal bacteria [1–11]. Under normal circumstances, intestinal epithelial cells serve as an efficient barrier to exclude viable enteric bacteria from penetrating or interacting with lamina propria immune cells.

The inflammatory response witnessed in CD involves primarily T helper 1 and 17 cells, which are characterized by the secretion of tumor necrosis factor-α, interferon-γ, interleukin (IL)-12 and IL-23 [2, 5, 6]. Though 163 loci have been identified in association with IBD, these loci account for only a small proportion of patients with CD [3], and suggest that genetic factors, when involved, are

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associated with host protection from bacteria. Bacteria play a major role in the pathogenesis of CD, as they are found in granulomas and are adherent to the intestinal mucosa [2, 5, 6]. Dysbiosis has been shown to be present in multiple studies (manifested as reduction in diversity, a reduction in Firmicutes, with increased Enterobacteriaceae and decreased clostridia [7–10]. The former have been found to play a role in CD, while the latter are short-chain fatty acid (SCFA)-producing bacteria [7]. Ruminococci, a mucolytic family of bacteria, has been demonstrated more frequently in CD [9]. Disease-specific inflammatory bacteria isolated from ileal tissue from patients with CD, such as adherent-invasive *Escherichia coli* (AIEC), are shown to replicate in macrophages and epithelial cells under certain circumstances and are abundant in patients with CD [11, 12].

The environment appears to play a major role in the pathogenesis of CD. Early exposure to antibiotics has been associated with CD [13]. Exposure to infections as risk factors has been the focus of interest of many studies. Similar to other autoimmune diseases, some epidemiological studies have suggested a role for the 'hygiene hypothesis', whereby exposure to infections in childhood confers protection against disease [13–15].

An environmental factor, which has not been adequately explored in CD, is diet. Diet has a dramatic effect on the composition of the intestinal microbiome and gut immune status [16–18]. Understanding the environmental component is important since it may have implications for the treatment of children with CD and it could provide the foundation for disease prevention. There are multiple lines of evidence from epidemiologic, clinical and animal studies demonstrating an impact of dietary exclusion on treatment, or dietary exposure on the pathogenesis of intestinal inflammation.

Evidence for Individual Dietary Components and Patterns and Susceptibility to IBD

CD is most prevalent in westernized countries with a clear north to south gradient [10]. Countries with the highest prevalence also have increased exposure to the Western diet, which includes more protein, animal fat, dairy products and industrialized food. Our daily diet consists of multiple nutrients, which can be easily grouped as carbohydrates, proteins, fats, vitamins and minerals. These components have been evaluated primarily in retrospective case-control or epidemiologic studies. However, Western diet often includes increased exposure to combinations of proteins, fat and sugars, with increased consumption of one component (for instance fats) often leading to increased consumption of another component (sugars), and evaluation is hampered by assessment of food frequency questionnaires at diagnosis. Thus, individualization may be misleading and may reflect dietary patterns.

Protein

Epidemiological studies have demonstrated that a diet high in animal protein is associated with a significantly higher risk of developing CD and IBD [19, 20]. In a French prospective study of 67,581 middle-aged women followed up by Jantchou et al. [20] for a mean time of 10.4 years, 77 cases of IBD were identified. High animal protein intake was associated with the risk of IBD. In particular, a high consumption of meat and fish but not of eggs or dairy products was associated with the risk of developing IBD (hazard ratio 1.87; 95% confidence interval, CI: 1.00–3.49; $p = 0.02$). However, animal protein consumption is usually associated with animal fat consumption, so it is unclear if this component can be adequately evaluated in isolation.

Carbohydrates

Several studies have shown that an increase in mono- and disaccharide consumption is associated with the risk of developing CD [21–24]. Tragnone et al. [23] found a significantly higher relative risk associated with sugar intake in CD (relative risk: 3.5; 95% CI: 1.5–8.1). Patients with CD who have increased sucrose intake have a relative risk of 2.6 (95% CI: 1.4–65.0) [25]. In a recently published Danish cohort, high sugar consumption was associated with an increased CD risk (odds ratio, OR: 2.9; 95% CI: 1.0–8.5) [26].

Fat

In epidemiological studies, increased consumption of monounsaturated fatty acids was associated with a higher risk for developing CD [27]. In a case-control study, D'Souza et al. [28] administered a semiquantitative questionnaire to collect information on 151 foods, including variety and portions. The frequency of consumption assessed ranged from 'never' or 'less than once per month' to '6 per day'. In girls, high intake of meat, fatty food and desserts was positively associated with CD (OR for the third vs. first tertile: 4.7; 95% CI: 1.6–14.2; $p = 0.006$). A dietary pattern characterized by high levels of vegetables, fruits, olive oil, fish, grains and nuts was inversely associated with CD in both genders [28]. On the other hand,

three different studies (Kasper and Sommer [29], Thornton et al. [30] and Tragnone et al. [23]) have shown that there is no effect of the type of fat consumed on the risk for CD, but it should be noted that these studies are dated.

Fibers, Fruits and Vegetables

Fibers can be divided into two groups: fermentable and nonfermentable. Fermentable fibers are fermented by the flora in the gut and SCFAs are produced as an end product. SCFA is considered an anti-inflammatory metabolite and lowers intestinal permeability (IP) [25]. Fibers are a component that has been shown to decrease the risk for CD in adults. Exclusive enteral nutrition (EEN) has been shown to alter SCFA production [31], and in particular to increase butyrate [32–34]. A Danish study demonstrated that whole meal bread that was rich in fiber may have a protective effect (OR: 0.4; 95% CI: 0.2–0.9). The main problem is that patients tend to reduce their fiber consumption after the first flare, which may lead to a low-fiber diet. Many studies show that fruit consumption has a protective affect and thereby lowers the risk for CD [27]. In a recently published Danish cohort, daily vegetable consumption lowered the risk for CD (OR: 0.3; 95% CI: 0.1–1.0) [25–27].

Western Diet and Susceptibility to Develop CD (Animal Models)

A Western diet is typically high in animal protein, total fat, n–6 polyunsaturated fat (PUFA) and sugar. This trend has been the focus of animal studies that have aimed to find a connection between the Western diet and CD etiology. In a recently published study by Martinez et al. [35], CEABAC10 mice were compared to wild-type mice, and both groups were fed a Western diet rich in fat and simple sugars (13.1% protein, 60.6% lipids and 26.3% carbohydrates) or standard chow for 12 weeks. One group was exposed to AIEC. After 12 weeks of diet, there was a decrease in the abundance of bacteria in the colonic mucosa of both groups. The Western diet promoted the growth of *Bacteroides, Prevotella* and mucin-degrading bacteria in both AIEC-positive and AIEC-negative groups, and increased IP. CEABAC10 mice treated with the Western diet had higher levels of cytokines, such as tumor necrosis factor-α. Fecal counts of AIEC were much higher in the CEABAC10 group consuming Western diet. The increase in IP as a result of Western high-fat diet was tested in a study conducted by Suzuki and Hara [36]. They compared mutant obese Long-Evans rats to lean rats fed two different diets: standard chow and a high-fat diet. After 3 weeks, IP was significantly increased in the rats on the high-fat diet compared to their controls ($p < 0.05$); IP increased further up to week 9 and was still increased after 15 weeks, which may suggest that the effect of dietary fat exposure is dose dependent. All these findings in cell and rodent models seem to indicate that Western/high-fat diet causes an increase in IP that can lead to increased bacterial entry into the epithelium [36]. In a study performed by Devkota et al. [37], three different diets were given to *IL 10–/–* mice: low fat (LF), PUFA and milk-derived fat (MF); the two high-fat diets were isocaloric. The mice on the PUFA diet and the mice on the MF diet had a lower diversity of the microbiome compared to the LF mice. Higher counts of Bacteroidetes and low counts of Firmicutes were found in the PUFA and MF groups. The most interesting finding is the presence of *Bilophila wadsworthia* in the MF diet group only. In the MF group, there was an increase in colitis from 25–30 to 60% in 6 months. In the PUFA and LF groups, the incidence of colitis was similar. The colitis score of the MF mice was significantly higher. *B. wadsworthia* is a proinflammatory bacterium that depends on sulfur and is not found in healthy subjects. It grows in the presence of taurine-conjugated bile acids that are rich in organic sulfur. MF induces the production of taurine-conjugated bile acids which create very strong micelles, which results in an optimal environment in the intestine for *B. wadsworthia* growth as it is sulfur dependent [37].

An additional dietary exposure that may have an effect is gluten, irrespective of its role in celiac disease. Gliadin is the toxic protein for celiac disease found in gluten, which is present in wheat and many other cereals. Lammers et al. [38] showed that gliadin increases IP by binding to CXCR3 on enterocytes and releasing zonulin, which in turn causes tight junction disassembly in mice.

Additional Dietary Factors That Alter IP and the Microbiome

The traditional approach to dietary components evaluates proteins, fats, carbohydrates, vitamins and minerals. However, the Western diet results in increased exposure to emulsifiers and food preservatives.

Swidsinski et al. [39] evaluated the effect of carboxymethylcellulose (CMC), a preservative commonly found in bread, ice cream and dairy products. As reported by the food industry, the amount of CMC (E466) used in the in-

dustry is growing every year. The model they used was *IL-10*-deficient mice that were given 2% CMC solution and a control group of mice receiving water. The results showed that the mice given CMC had bacterial overgrowth, especially between the villi, and some of the bacteria were adherent. In the CMC group, the epithelial bacterial count in the ileal mucosa was 30,000-fold higher than in the water-treated mice [39].

Roberts et al. [40] evaluated the effect of an emulsifier, polysorbate 80 (E433), as well as fibers on the translocation of AIEC across follicular epithelium and Caco2 cells. All AIEC isolated from CD patients translocated across M (membranous or microfold) cells in a high rater than through Caco2 cells (15.8-fold median increase). What managed to prevent the translocation was nonstarch polysaccharides (NSP) from plantain and broccoli ($p < 0.01$); this association was dose dependent. On the other hand, NSP from leeks and apple did not prevent the translocation. When examining the effect of NSP from plantain on AIEC translocation in human follicular-associated epithelial cells, it was found to be two times lower when the cells were treated with plantain NSP. The effect of polysorbate 80 on permeability was assessed and found to significantly increase the translocation in M cells ($p < 0.05$), follicular-associated epithelial cells ($p < 0.01$) and Caco2 cells ($p < 0.05$) in a dose-dependent manner [40].

As a result of these data, we may assume that as the Western diet has a large amount of emulsifiers, it may be possible that we are witnessing a rise in CD as a result of damage being done to the intestinal epithelium.

Another food additive that alters the intestinal function is carrageenan (E407). It is a sulfated polysaccharide that is extracted from red seaweed. It is used in the food industry as a stabilizer of dairy and meat products [41]. In a study by Choi et al. [41] in cell cultures, it has been shown that carrageenan diminishes the proinflammatory effect of nuclear factor-κB. By measuring the epithelial integrity as a response to carrageenan treatment, transepithelial electrical resistance was found to decrease as a result of carrageenan treatment, which was associated with discontinuous and irregular ZO-1 (zonula occludens) expression that increased IP [41].

Maltodextrin [42] has been found to promote AIEC biofilms and increase adhesion of AIEC strains to epithelial cells and macrophages via upregulation of expression of type 1 pili. Maltodextrin is a thickening and binding agent found in breakfast cereals as well as aspartame and sucralose, which are commonly used as artificial sweeteners.

Dietary Intervention and Remission in CD

While multiple dietary components seem to induce inflammation in animal models, the most important link between diet and CD comes from dietary interventions in children with active CD. EEN is a well-documented method of treatment. It involves placing children on a standardized restricted diet composed of a single polymeric formula as the sole source of nutrition over 6–8 weeks. Use of this treatment method early in the disease (usually during the 1st year) results in clinical remission in 50–80% of children by week 8 with no additional pharmacological treatment. We now have evidence that in mild-to-moderate uncomplicated disease, EEN is as effective as steroids for the induction of remission and improves mucosal healing [43]. In a prospective study, Buchanan et al. [44] studied 114 children who were treated with EEN over 8 weeks (51.2% were fed orally and 48.8% were tube fed); 80% of the children achieved clinical remission by week 8 with significant reductions in erythrocyte sedimentation rate and C-reactive protein levels. The patients in remission also significantly improved the z-score for weight and body mass index [44]. In a study in adults by Yamamoto et al. [45] on the efficiency of EEN in maintaining remission, 40 patients treated with ileum or ileocolonic resection were allocated to one of two groups: one group received EEN for 1 year via overnight tube feeding and the other group (20 patients) was allowed to eat a nonrestricted diet. Only 5% of the EEN group versus 35% of the free-diet group had a clinical recurrence of the disease in the 1st year of the follow-up. After 1 year, endoscopy was performed, and 30% of the EEN group and 75% of the nonrestricted-diet group had an endoscopic recurrence. Rubio et al. [46] reviewed 106 EEN-treated pediatric patients either orally or by tube feeding over 8 weeks. They demonstrated that the route of feeding is not significant, as 75% of the patients receiving oral feeding versus 85% receiving tube feeding achieved clinical remission ($p = 0.157$) [46]. One last question regarding EEN is, does it really have to be exclusive? Is it the content of the formula that is beneficial, or is the exclusion of normal diet that leads to remission? In a study performed by Johnson et al. [47], 50 children with active CD were randomized into two groups, one was treated with EEN and the other group was given partial enteral nutrition with free diet, which meant that 50% of the diet came from formula and the remaining 50% was not restricted. The remission rate was low in the group with partial enteral nutrition combined with free diet compared with the EEN group (15 vs. 40%, respectively;

p = 0.035). Moreover, the children in the EEN group had improvement both in clinical symptoms and in inflammatory markers. This study was the first to show that the most important component is the principle of exclusivity. While the underlying mechanism of exclusivity is still unknown, it reinforces the concept that diet may be an important environmental trigger that should be further studied.

Fitting the Pieces to the Puzzle

An attractive but speculative hypothesis for the observed increase in the prevalence of IBD, and CD especially, may be that a persistent change in dietary intake leads to the breakdown of the epithelial barrier. This might allow adherence, translocation and penetration of bacteria that under normal conditions would be nonpathogenic, or of bacterial antigens, in susceptible individuals (e.g. genetically determined Paneth cell dysfunction or defective autophagy). Persistent exposure to adherent or penetrating bacteria may then trigger the adaptive immune system, resulting in inflammation, further breakdown of the epithelial barrier, increased migration and sensitization to these bacteria; as a result, this can induce a vicious cycle that we have termed the *'bacterial penetration cycle'*. EEN, especially in early stages of small intestinal disease, might act by decreasing exposure to offending agents which impair the barrier or bacterial clearance, and lead to a decline in penetrating or resident bacteria, with epithelial restitution [48], reversing this cycle. Alternatively, EEN or dietary interventions might act through removal of dietary components, which affect microbial composition, decrease proinflammatory responses and promote restitution of the epithelial barrier, likewise allowing termination of this vicious disease-forming cycle before reaching a critical threshold. In any case, the hypothesis that one dietary component is responsible for the rising incidence of CD is simplistic.

Our knowledge at present is far from complete, but multiple signals from epidemiological, animal model and interventional studies indicate that diet may have a profound effect on either the pathogenesis or management of CD. The challenges in identifying which components are important, how they affect pathogenesis, and how dietary manipulation may affect disease management are still extraordinary, but by identifying the important links in the chain, this can be resolved. True progress will require that more resources be focused on environmental and dietary components involved in the pathogenesis of the disease.

Disclosure Statement

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