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REVIEW

Role of nutrition and microbiota in susceptibility to inflammatory bowel diseases

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Inflammatory bowel diseases (IBDs), Crohn's disease (CD), and ulcerative colitis (UC) are chronic inflammatory conditions, which are increasing in incidence, prevalence, and severity, in many countries. While there is genetic susceptibility to IBD, the probability of disease development is modified by diet, lifestyle, and endogenous factors, including the gut microbiota. For example, high intakes of mono- and disaccharides, and total fats consistently increases the risk developing both forms of IBD. High vegetable intake reduces the risk of UC, whereas increased fruit and/or dietary fiber intake appears protective against CD. Low levels of certain micronutrients, especially vitamin D, may increase the risk of both diseases. Dietary patterns may be even more important to disease susceptibility than the levels of individual foods or nutrients. Various dietary regimes may modify disease symptoms, in part through their actions on the host microbiota. Both probiotics and prebiotics may modulate the microflora, and reduce the likelihood of IBD regression. However, other dietary factors affect the microbiota in different ways. Distinguishing cause from effect, and characterizing the relative roles of human and microbial genes, diet, age of onset, gender, life style, smoking history, ethnic background, environmental exposures, and medications, will require innovative and internationally integrated approaches.

Keywords:
Crohn's disease / Diet / Microbiota / Inflammatory bowel diseases / Ulcerative colitis

1 Introduction

The term "Inflammatory bowel disease" (IBD) includes Crohn's disease (CD) and ulcerative colitis (UC), both of which are characterized by chronic inflammation of the gastrointestinal tract. The two forms of the disease are distinguished by their location and severity. CD is more severe, can affect any part of the gastrointestinal tract, is typically discontinuous, and involves all layers of the intestinal wall [1]. In contrast, UC is continuous, restricted to the colon and the rectum, and affects only the mucosal layer of the intestinal wall. Despite the differences, an overlapping pathology suggests some common causes, as well as common potential treatments. Both forms of the disease profoundly affect quality of life, and have been increasing in incidence as well as prevalence in recent years [2].

There is no single, established cause of either form of IBD. Both appear to have a multi-factorial disease susceptibility, in which genetic predisposition, environmental factors (including diet), intestinal microbial flora, and the immune system are all involved [3–5] (Fig. 1). Around 100 susceptibility genes have been identified to date, some in common across both diseases and others specific to one or other form [6–8]. When a genetically predisposed individual is exposed to certain dietary, chemical, or pathogenic insults, this may result in an inappropriate immune response that leads to chronic inflammation and IBD.

Nutrition plays a causal role in both forms of IBD, interacting with various other factors to contribute to disease susceptibility [9, 10] (Fig. 1). Either the presence or absence of specific dietary components, in association with components of

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Abbreviations: ASA, 5-aminosalicylic acid; **CD**, Crohn's disease; **CFU**, colony forming units; **DHA**, docosahexaenoic acid; **DSS**, dextran sodium sulphate; **EPA**, eicosapentaenoic acid; **ERF**, enzyme-treated rice fiber; **FOS**, fructo-oligosaccharide; **IBD**, Inflammatory bowel disease; **LA**, linoleic acid; **MUFA**, monounsaturated fatty acids; ***n*-3 PUFA**, omega-3 polyunsaturated fatty acids; ***n*-6 PUFA**, omega-6 polyunsaturated fatty acids; **PUFA**, polyunsaturated fatty acids; **sIgA**, intestinal secretory IgA; **TLR**, toll-like receptor; **UC**, ulcerative colitis

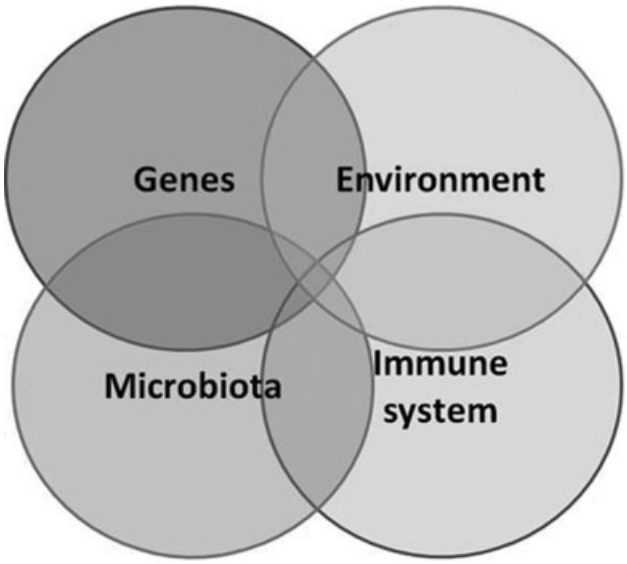

Figure 1. Key interactions involved in susceptibility to development of inflammatory bowel diseases. Twin studies and genome-wide association studies have provided evidence that there are more than 100 genes involved in susceptibility to IBD. These genetic risks have been classified functionally into several pathways, broadly categorized as abnormalities in innate immune function, immunoregulation, or barrier function. The dysfunctional innate immune regulation leads to changes in microbiota by several mechanisms, including altered secretion of antimicrobial peptides by Paneth cells (e.g. NOD2), or altered intracellular bacterial clearance by autophagy (e.g. ATG16L1). Variant genotypes have also been linked to bacterial dysbiosis in IBD patients. However, the observation that individuals can carry key risk genes, without developing the diseases, lends strong support to the involvement of other factors. Important dietary factors are summarized elsewhere in this review. These may affect immune function in their own right, but may also shift the gut microbiota by encouraging or discouraging the growth of certain bacteria, especially mucosally associated bacteria. Other environmental factors associated with risk include public health measures, infections, antibiotic exposure, and smoking.

the gut-associated microbial ecosystem (microbiome) [11–13] provides an environmental trigger for IBD development in genetically susceptible individuals. We review the causal role of both diet and microbiota, on their own and jointly, in susceptibility to these diseases.

2 Nutrition in the etiology of IBD

Evidence for a major role of dietary factors that may increase susceptibility to IBD is limited. Data summarized in Tables 1 and 22 reveal that, while a number of trends have been reported, much of the available data are inconsistent or of low statistical significance. Part of the reason for this may be that most of the published studies are case–control, relying on adequate recall of predisease diet. However, some data from prospective studies are now appearing (e.g. IBD in EPIC study [14]), and these will strengthen the relationships, as discussed in systematic reviews such as Hou et al. [15]. In Tables 1 and 2, we have gone back to the original literature from this systematic review, and added other information where this has become available.

Data from several case–control studies are available. For example, Bernstein and co-workers [21] performed an age, gender, and geographic residence matched case–control study on IBD risk factors, based on cases drawn from the population-based University of Manitoba IBD Research Registry and controls from the population-based Manitoba Health Registry. They reported that dietary risk factors for either form of the disease included having drunk unpasteurized milk or having eaten pork.

There are some general trends suggesting that high-refined sugar consumption, a high-caloric diet, and regular intake of processed fat might be risk factors for CD, whereas high fruit, vegetables, and dietary fiber consumption appear to decrease the risk [30–34]. Dairy products have been suggested as a risk factor, since IBD is more common in "dairy-based" countries, than in "soy-based" ones [35–37]. Additionally, high cheese intake appears as a risk factor [20]. Various studies have associated an increased intake of n-6 polyunsaturated fatty acids (PUFA) and a lower intake of *n*-3 PUFA with increased incidence of both CD and UC [9,14,16,36]. The variability in the data strongly implies differences among different types of carbohydrate. Simple sugars, or starches that are readily broken down to simple sugars, generally appear as a risk factor [18,19]. However, some types of mono- or disaccharide, and certain types of dietary fiber, may show protection [9, 16, 18, 19, 23, 24]. Much of the data, however, are equivocal and the roles of different types of fats, dietary fibers and other carbohydrate sources, alcohol, and proteins in IBD warrant further investigation. In addition, the role of processing and cooking should be taken into account.

More definitive data may be possible from prospective cohort studies, such as the large European prospective cohort study [14]. Attempts to determine the relationship between the intake of nutrients and the development of UC in 260 686 men and women aged 20–80 years were disappointing [24]. Only 139 subjects with incident UC were identified, and the only dietary association detected was a marginally significant positive association of UC risk with the highest quartile of intake of linoleic acid. Other data associate a high intake of red meat or other linoleic acid sources with high IBD risk [16, 17, 27, 28].

Overall fatty acid intake and/or the ratio of n-3:*n*-6 PUFA appears important risk or protective factors in a number of studies [9,14,16,25]. However, in our own review of the available literature on *n*-3 PUFA in IBD, we have raised a number of concerns about data interpretation in current studies [38]. There has been a lack of consistency as to whether original dietary sources of *n*-3 PUFA (such as wild salmon) have

Table 1. Dietary susceptibility to Crohn's disease. This table distinguishes the main dietary components that have been associated with the risk of developing the disease, as assessed using information about the predisease diet in either case control or cohort studies, in various populations

Food or food component	Positive (decreases risk)	Negative (increases risk)	Reference
Overall dietary fat intake		+	Sakamoto 2005 [16]
		+ (ns)	Amre 2007 [9]
		(ns)	Jantchou 2010 [17]
Saturated fats		+ (ns)	Sakamoto 2005 [16]
		+ (ns)	Amre 2007 [9]
Total MUFA		+	Sakamoto 2005 [16]
		+ (ns)	Amre 2007 [9]
Total PUFA		+ (ns)	Sakamoto 2005 [16]
		+ (ns)	Amre 2007 [9]
Total *n*-3 PUFA		+	Sakamoto 2005 [16]
		+ (ns)	Amre 2007 [9]
Long-chain *n*-3 PUFA	+		Amre 2007 [9]
n-6 PUFA		+	Sakamoto 2005 [16]
		+	Amre 2007 [9]
Total carbohydrate		+	Tragnone 1995 [18]
	+ (ns)		Sakamoto 2005 [16]
	+ (ns)		Amre 2007 [9]
		+ (ns)	Jantchou 2010 [17]
Mono- and disaccharides		+	Tragnone 1995 [18]
		+ (ns)	Reif 1997 [19]
Polysaccharides (starch)		+	Tragnone 1995 [18]
Dietary fiber	+ (ns)		Reif 1997 [19]
	+ (ns)		Sakamoto 2005 [16]
	+		Amre 2007 [9]
Fruit	+ (ns)		Reif 1997 [19]
	+ (ns)		Sakamoto 2005 [16]
	+ (ns)		Amre 2007 [9]
Vegetables		+ (ns)	Reif 1997 [19]
		+ (ns)	Sakamoto 2005 [16]
	+(ns)		Amre 2007 [9]
Total protein		+(ns)	Tragnone 1995 [18]
		+(ns)	Reif 1997 [19]
		+	Sakamoto 2005 [16]
		+ (ns)	Amre 2007 [9]
		+ (ns)	Jantchou 2010 [17]
Animal protein		+ (ns)	Jantchou 2010 [17]
Total meat		+ (ns)	Sakamoto 2005 [16]
Red meat		+	Maconi 2010 [20]
Chicken		+ (ns)	Bernstein 2006 [21]
Pork		+ (ns)	Bernstein 2006 [21]
Fish	+ (ns)		Reif 1997 [19]
		+ (ns)	Sakamoto 2005 [16]
	+ (ns)		Amre 2007 [9]
Dairy	+ (ns)		Sakamoto 2005 [16]
Cheese		+	Maconi 2010 [20]
Eggs		+ (ns)	Reif 1997 [19]
		+ (ns)	Sakamoto 2005 [16]
	+ (ns)		Halfarvson 2006 [22]
Vegetable proteins		+ (ns)	Jantchou 2010 [17]

+, relationship is statistically significant; + (ns) although the data are suggestive in the indicated direction, they fail to reach statistical significance.

been eaten, or supplements taken. In addition, there is inconsistency in the formulations considered, the method of presentation, and the storage of the supplements (where this was the method of administration). It is generally recognized that it is the long-chain *n*-3 PUFA that are most likely to be beneficial, but different sources of these will have different ratios of products, with varying chain lengths. Furthermore, since they are typically produced from marine sources, ac-

Table 2. Dietary susceptibility to ulcerative colitis. This table distinguishes the main dietary components that have been associated with the risk of developing the disease, as assessed using information about the predisease diet in either case control or cohort studies, in various populations

Food or food component	Positive (decreases risk)	Negative (increases risk)	Reference
Overall dietary fat intake		+ (ns)	Reif 1997 [19]
		+ (ns)	Geerling 2000 [23]
		+	Sakamoto 2005 [16]
		+ (ns)	Hart 2008 [24]
		+ (ns)	Jantchou 2010 [17]
Saturated fats		+ (ns)	Reif 1997 [19]
		+ (ns)	Geerling 2000 [23]
		+ (ns)	Sakamoto 2005 [16]
		+ (ns)	Hart 2008 [24]
Total MUFA		+	Reif 1997 [19]
		+	Geerling 2000 [23]
		+	Sakamoto 2005 [16]
		+ (ns)	Hart 2008 [24]
		± (ns)	IBD in EPIC study 2009 [14]
Total PUFA		+	Reif 1997 [19]
		+	Geerling 2000 [23]
		+ (ns)	Sakamoto 2005 [16]
		+ (ns)	Hart 2008 [24]
Total *n*-3 PUFA		+ (ns)	Sakamoto 2005 [16]
		+ (ns)	IBD in EPIC study 2009 [14]
			John 2010 [25]
Long-chain *n*-3 PUFA	+ (ns)		IBD in EPIC study 2009 [14]
	+ (DHA s, EPA ns)		John 2010 [25]
	+ (DHA s)		
n-6 PUFA		+ (ns)	Geerling 2000 [23]
		+ (ns)	Sakamoto 2005 [16]
		+ (ns)	IBD in EPIC study 2009 [14]
Linoleic acid		+	IBD in EPIC study 2009 [14]
Total carbohydrate		+	Tragnone 1995 [18]
		+ (ns)	Geerling 2000 [23]
	+ (ns)		Sakamoto 2005 [16]
		+ (ns)	Hart 2008 [24]
	+ (ns)		Jantchou 2010 [17]
Mono- and disaccharides		+	Tragnone 1995 [18]
		+	Reif 1997 [19]
		+ (ns)	Geerling 2000 [23]
	+ (ns)		Hart 2008 [24]
Polysaccharides (starch)		+	Tragnone 1995 [18]
	+ (ns)		Hart 2008 [24]
Non starch polysaccharides	+ (ns)		Geerling 2000 [23]
Dietary fiber	+ (ns)		Reif 1997 [19]
	+ (ns)		Sakamoto 2005 [16]
		+ (ns)	Hart 2008 [24]
Fruit	+ (ns)		Gilat 1987 [26]
		+ (ns)	Higashi 1991 [27]
	+ (ns)		Epidemiology Group of the Research 1994 [28]
	+ (ns)		Reif 1997 [19]
	+ (ns)		Sakamoto 2005 [16]
		+ (ns)	Halfvarson 2006 [22]
Vegetables	Fresh	Cooked	Higashi 1991 [27]
	+(ns)	+ (ns)	Reif 1997 [19]
	+ (ns)		Sakamoto 2005 [16]
	+ (ns)		Halfvarson 2006 [22]
		+ (ns)	
Total protein		+ (ns)	Reif 1997 [19]
	+(ns)		Geerling 2000 [23]
		+ (ns)	Sakamoto 2005 [16]
		+ (ns)	Hart 2008 [24]
		+ (ns)	Jantchou 2010 [17]

Table 2. Continued

Food or food component	Positive (decreases risk)	Negative (increases risk)	Reference
Total animal protein	+ (ns)		Geerling 2000 [23]
		+ (ns)	Jantchou 2010 [17]
Meat		+ (ns)	Higashi 1991 [27]
		+ (ns)	Epidemiology Group of the Research 1994 [28]
		+ (ns)	Sakamoto 2005 [16]
		+ (ns)	Jantchou 2010 [17]
Chicken		+ (ns)	Bernstein 2006 [21]
Pork		+ (ns)	Bernstein 2006 [21]
Fish		+ (ns)	Higashi 1991 [27]
	+ (ns)		Reif 1997 [19]
		+ (ns)	Sakamoto 2005 [16]
		+	Jantchou 2010 [17]
Dairy		+	Glassman 1990 [29]
		+ (ns)	Higashi 1991 [27]
		+ (ns)	Epidemiology Group of the Research 1994 [28]
	+ (ns)		Sakamoto 2005 [16]
	+ (ns)		Jantchou 2010 [17]
Margarine		+	Maconi 2010 [20]
Eggs		+ (ns)	Higashi 1991 [27]
		+ (ns)	Reif 1997 [19]
		+ (ns)	Sakamoto 2005 [16]
		+ (ns)	Halfarvson 2006 [22]
	+ (ns)		Jantchou 2010 [17]
Vegetable proteins		+ (ns)	Geerling 2000 [23]
		+ (ns)	Jantchou 2010 [17]

+, relationship is statistically significant; + (ns) although the data are suggestive in the indicated direction, they fail to reach statistical significance.

cumulation of pollutants may lead to adverse contaminants. Also, these products can be oxidized, and the way in which they are purified and stored is essential to their likely efficacy. Thus, while high intake of *n*-3 PUFA in relation to *n*-6 PUFA is suggested as protective against IBD by several studies, future work needs more controlled dietary input and also more careful data interpretation. Although a Mediterranean includes high MUFA intake and such diets have been associated with reduced CD risk [39], the only two studies directly considering high MUFA intake reported it as a risk, not a protective, factor [9, 16].

There is increasing evidence that it is not so much individual foods *per se*, but dietary patterns that are critical in enhancing or reducing disease risk. It is possible that nutritional factors suggested as risk factors for IBD may be merely an expression of a Westernized lifestyle, involving other risk factors that modify the pathogenesis of IBD. Recent reviews on this topic [39, 40] have concluded that a Western diet was associated with increased risk of IBD. The major constituents of such a diet may affect intestinal inflammation via several mechanisms [41]. These include the effects of insulin resistance, modification of intestinal permeability, the pro- or anti-inflammatory role of various types of PUFA, and the effect of various nutrients on host microbiota. Amre and co-workers [9, 10] also related IBD risk to dietary patterns, and concluded that imbalances in the consumption of PUFA, vegetables, and fruits are associated with an increased risk for CD in children. In their case–control study across three tertiary hospitals in Canada, D'Sousa et al. [10] established the nature of both risk and protective patterns. In girls, high intake of meats, fatty foods, and desserts was positively associated with CD. A more Mediterannean dietary pattern, characterized by high levels of vegetables, fruits, olive oil, fish, grains, and nuts, and was inversely associated with CD in both genders [39]. Encouraging uptake of a Mediterannean diet may be desirable in protecting against IBD and other forms of chronic disease [42].

The potential roles of vitamins in the etiology of IBD are even more elusive, because we do not have adequate data prior to disease development. For example, while there is clear evidence that children with IBD are very commonly severely vitamin D deficient [43], whether this is cause or effect of the disease is difficult to establish.

3 Microbiota in the etiology of IBD

There is reason to believe that changing public health practices are leading to altered microbial exposures over time, in New Zealand as well as in many other Westernized societies [3]. The hygiene hypothesis suggests that lack of early exposure to a range of microbes may negatively impact on

appropriate development of the adaptive immune response, and enhance sensitivity to certain diseases [21, 44]. In common with other autoimmune diseases, it appears that poor hygiene at an early stage of life may be a protective factor against CD [3]. It is well recognized that the human colonic microbiota is central to the induction of disordered immune function and inflammation [45–47]. The "pathogenic community" describes a condition in which the stability of the microbial ecosystem of the healthy human gut is disrupted in response to host genetics and destabilized immunity [47–49]. Clinical evidence for bacteria in the pathogenesis of CD is supported by the observations of several *in vitro* and *in vivo* studies [50]. This argument is confirmed by a number of IBD animal models showing that intestinal inflammation fails to develop when the animals are kept in a germ-free environment [51].

The intraluminal microbiota affects the intestinal immune system and gut development, provides key nutrients, and modifies energy metabolism. Imbalances in bacterial functions, defective sensing and clearance of bacteria, impaired autophagy, and α-defensin and β-defensin production may have a role in the initiation of CD [52–54]. Compositional and functional changes in gut microbiota lead to invasion of epithelial cells of pathogenic bacteria, cytopathic effects, stimulation of pro-inflammatory cytokines, dysregulated immune response, and damage to the intestinal barrier [55, 56]. However, chronic inflammation is not only caused by compositional changes in gut microbiota but can also be seen as an initiation factor for favoring the growth of certain bacteria [57]. In addition, antimicrobial microbes such as intestinal secretory IgA (sIgA) that act as a defense mechanism against intestinal microorganisms have an impact on the composition of commensal bacteria [58]. Furthermore, pattern-recognition receptors of the innate immune system such as toll-like receptors (TLRs) and antimicrobial peptides (e.g. NOD2, ATG16L1, XBP-1) are secreted by Paneth cells and affect the composition and function of commensal bacteria. They may drive the onset of disease by stimulating the production of pro-inflammatory cytokines [52, 59–61]. These results indicate that defective sensing of bacteria may be associated with CD.

Where they have been studied, most individuals with IBD, especially CD, are characterized by "dysbiosis," in which one or a few potentially harmful microorganisms are dominant, creating a disease-prone situation [46, 48, 49]. The probability of this condition may be modulated according to genotype [48]. Although microbiota profiles in CD patients (with active or inactive disease) are very different from those of healthy individuals, they are more similar between healthy subjects and individuals with inactive UC [62]. This suggests different roles for the fecal microbiota in the pathophysiology of UC and CD.

With regard to CD location, changes in composition of commensal bacteria are different in the ileum and the colon [52]. However, clinical and experimental studies suggest that the relative balance of aggressive and protective bacterial species is altered in IBD, resulting in changes in intestinal microbial community [63–65]. The onset of inflammation may be associated with an imbalance of the intestinal microflora, with relative predominance of "aggressive" bacteria and an insufficient concentration of "protective" species. This results in a lower biodiversity of microbes, despite increased gut pathogen levels overall [50, 66, 67]. The results of various studies demonstrate that several commensal organisms, such as *Escherichia coli, Bacteroides, Enterococcus,* and *Klebsiella* species are involved in intestinal inflammation, while various *Lactobacillus* and *Bifidobacterium* species seem to be protective and have been suggested for therapeutic use as probiotics, by modulating gut microbial balance in the host [68–71]. A summary of the bacterial data is provided in Table 3.

Marchesi et al. [73] indicated that IBD patients have decreased levels of butyrate, acetate, methylamine, and trimethylamine in comparison to healthy controls, which might be due to a reduction of bacteria groups that are mainly responsible for production of short-chain fatty acids. This results in changes in pH, redox potential, and substrate availability. In addition, increased *Actinobacteria* (except the *Bifidobacteriales*), decreased *Firmicutes* (such as *Lachnospiraceae* and clostridial groups IV and XIVa including *Faecalibacterium prausnitzii*), increased *Proteobacteria* (*Enterobacteriaceae* such as *E. coli* and *Rumnicoccus gnavus*), and variable changes in *Bacteroides* are associated with human IBD [52, 60, 64, 74–82]. In CD patients, specifically, the B2 and D phylogenetic group of *E. coli* and adherent-invasive strains of *E. coli* play an important role [83–85]. Nevertheless, certain types of bacteria appear able to counter some of the effects of the bacteria mentioned above. For example, administration of *F. prausnitzii* in mice with colitis leads to the restoration of intestinal microbiota to a normal state, and decreased colitis [86]. This bacterium also appears to show some anti-inflammatory effects in CD patients [86].

The composition and distribution of microbial communities are affected by innate and adaptive immune response, environmental factors, antimicrobial peptides, and host genetic factors [87]. The commensal bacterial flora is shaped by the structure of the gastrointestinal tract and changes in lifestyle conditions such as hygiene, nutrition, and antibiotics, whereas changes are also observed also in the bacterial metagenome in IBD. In addition, polymorphisms in genes that are involved in CD pathogenesis may alter the nature of interactions between host and microbiota [48]. However, microorganisms that directly interact with the intestinal mucosa are poorly understood. Improvement in the understanding of the role of the gut microbiota has considerable public health implications by providing new therapeutical targets for the treatment of CD

Table 3. Increase and decrease of bacterial clades in CD phenotypes relative to healthy individuals

Phylum	Class	Order	Family	Genus
Actinobacteria	Ayctinobacteridae ⇑	Bifidobacteriales ⇑	Bifidobacteriaceae ⇑	Bifidobacterium ⇑
	Coriobacteridae ⇑	Coriobacteriales⇑	Coriobacteriaceae ⇑	Collinsella ⇑
→ An increase of Actinobacteria was generally observed in CD patients versus healthy controls				
Bacteroidetes ⇑⇓	Bacteroidetes	Bacteriodales	Porphyromonadaceae	un_Porphyromonadaceae↓
				Hallela⇓
↑↓			un_Bacterio-dales↓⇓	
→ Variable changes of Bacteroidetes were observed in CD patients versus healthy controls				
Firmicutes⇑	Clostridia ↓⇑	Clostridiales ↓⇑	Lachnospiraceae	Roseburia ↓
	Bacilli ↑	un_Clostridia ↓⇓	Ruminococca-ceae ↓⇑	un_Lachnospiraceae ↓
		Lactobacillales ↑	IncertaeSedisXIII	Ruminococcaceae Incertae Sedis ↓
			Peptococcaceae ↓	Faecalibacterium ↓⇑
			Un_Clostridiales ↓	Ruminococcus⇑
			Veillonellaceae	un_Ruminococcaceae ↓⇑
			Lactobacillaceae ↑	Anaerovorax ↑
				Peptococcus ↓
				Acidamino-coccus ↑
				Veillonella ↑⇓
				Lactobacillus↑
				Lactococcus⇓
→ A reduction of Firmicutes was generally observed in CD patients versus healthy controls				
Fusobac-teria ↑⇓	Fusobacteria ↑⇓	Fuso-bacteriales ↑⇓	Fuso-bacteriaceae ↑⇓	Fusobacterium ↑⇓
→ Variable changes of Fusobacteria were observed in CD patients versus healthy controls				
Proteo-bacteria↑	Gamma-proteobacteria ↑	Aermondales	Aeromonadaceae ↑	Aeromonas ↑
		Entero-bacteriales ↑	Enterobacteriaceae ↑	Citrobacter ↑
	Alpha-proteobacteria ⇓	un_Alpha-proteobacteria ⇓		Shigella ↑
				un_Enterobacteriaceae ↑
→ An increase of Actinobacteria was generally observed in CD patients versus healthy controls				
Teneri-cutes ↓⇑	Mollicutes ↓⇑	Anaero-plasmatales	Anaeroplasmataceae ↓⇑	Asterole-plasma ↓⇑
→ Variable changes of Fusobacteria were observed in CD patients versus healthy controls				

↑↓ Increase or decrease in CD localized in the ileum relative to healthy subjects.
⇑⇓ Increase or decrease in CD localized in the colon relative to healthy subjects.
Sources: [62, 72] compiled by the authors.

4 Dietary modulation of the gut microflora

Although it makes biological sense that different dietary regimes may affect IBD risk through affecting the composition of the gut microbiome, data are somewhat limited, because this endpoint has not usually been included in human studies to date. In animal models, manipulation of both the type and level of both fat and carbohydrates have led to dramatic effects on the gut microbiota [88–90]. Similarly, mouse models showed strong effects of different sources and different levels of dietary selenium on selenoproteome expression of the host, through influencing the gut microbiota [91]. In humans, Werner [92] showed that depletion of luminal iron alters the gut microbiota and prevents some CD-like symptoms. Probiotics are living commensal microorganisms of the intestinal tract, that are commonly sold through the health food market under the claim that, "if ingested regularly, they are capable of modulating the gut microbiota." There is evidence that they affect the gastrointestinal tract and associated immune system, and have numerous effects on intestinal function and immune responses [93, 94]. It is important to recognize that the most compelling evidence comes from animal, rather than human studies [49]. For example, administration of *F. prausnitzii* in mice with colitis leads to the restoration of intestinal microbiota to a normal state, and decreased colitis [84, 86].

There is no current evidence that early intake of either pre- or probiotics can protect against the development of IBD. The current evidence base would seem to support but not prove the possibility that some probiotic mixtures are beneficial for immune function, gut microbiota modulation, and maintenance of remission, in at least some forms of IBD. One study considered whether a probiotic mixture (Probio-Tec AB-25, composed of *Lactobacillus acidophilus La-5* and *Bifidobacterium animalis subsp. lactis BB-12*) would affect the maintenance of remission in patients with UC [49]. Although minor differences in time to remission were seen, there were no statistically significant diferences between probiotic-treated and control patients in a small randomized double-blind placebo-controlled trial. Effects of rectal with oral administration of *L. casei* DG were compared in relation to the faecal microflora composition in the colonic mucosa of patients with mild UC [95]. Rectal but not oral administration led to an increase in *Lactobacillus* spp. and reduction in *Enterobacteriaceae*.

The most convincing human data come from the use of VSL#3™, which contains 450 billion colony forming units (CFU) of lactic acid bacteria (*B. breve, B. longum, B. infantis, L. acidophilus, L. plantarum, L. paracasei, L. delbrueckii subspecies bulgaricus,* and *Streptococcus thermophilus*). A total of 144 consecutive patients were randomly treated for 8 weeks with VSL#3 at a dose of 3600 billion CFU/day (71 patients) or with placebo (73 patients) [96], to consider the potential benefit of treatment of relapsing mild-to-moderate relapsing UC with the probiotic VSL#3, as adjunctive to standard treatments. The primary endpoint of the study was to assess the effects of supplementation with VSL#3 in patients with UC who are already under treatment with 5-aminosalicylic acid (ASA) and/or immunosuppressants at stable doses. The study achieved statistically significant differences in several parameters. This particular mix has been used successfully in the treatment of pouchitis.

A prebiotic can be defined as: "the selective stimulation of growth and/or activity(ies) of one or a limited number of microbial genus(era)/species in the gut microbiota that confer(s) health benefits to the host" [97]. Animal studies support the action of such compounds in IBD. For example, a new prebiotic, enzyme-treated rice fiber (ERF) protected against inflammation in three distinct murine models of colitis and modulated their gut microbiota [97]. The murine models were all induced by the oral administration of dextran sodium sulphate (DSS). The preventive effect of ERF on colitis was significantly superior to that of the original rice bran, or the control group.

Unfortunately, such animal data do not at present extrapolate to beneficial effects by prebiotics on IBD in humans. For example, although preliminary studies had suggested that a prebiotic (fructo-oligosaccharide [FOS]) could modulate the intestinal microflora and reduce disease activity in CD, this was not the outcome of the single adequately powered, double blind placebo-controlled trial of FOS in active CD [98].

5 Moving beyond probiotics and prebiotics

The studies in the previous section drew attention to some of the problems of extrapolating from animal studies to humans, especially in predicting modulation of the gut microflora by diet. The physiological effects of food are influenced in part by an individual's gut microbiota, and genes of both the human and the microbiota. The interrelations among dietary type and eating frequency, the composition of the gut microbiota, and harvest of nutrients and energy is further impacted by variations in human environmental exposures and genotype.

In order to separate out some of these factors, Turnbaugh et al. [89] created a humanized gnotobiotic mice, as a well-defined, representative animal model of the human gut ecosystem. Germ-free C57BL/6J mice were transplanted with fresh or frozen fecal microbial communities from adult humans. The authors followed the patterns of bacterial colonization using culture-independent metagenomic analyses, to confirm that these humanized mice were stably and heritably colonized, with representative bacteria. They were also able to demonstrate the effects of moving from a low-fat, high plant polysaccharide diet to a high-fat, high-sugar "Western-style" diet. Within a single day, such a diet had shifted the composition of the microbiota, shifted various metabolic pathways in the microbiome, and altered gene expression. Although the nature of the donor affects the initial structure of the microbial community, the composition of this community could be rapidly shifted by diet.

Such models permit a detailed study of the complex interactions between the gut microbiome and immune system, and the way in which they contribute to IBD. For example, certain filamentous bacteria have been shown to promote the expansion of IL-17 cells, administration of the potential probiotic, *Bacteroides fragilis,* reduced the symptoms of colitis in animal models, while certain *Clostridium* strains promoted the expansion of regulatory T-cells [99]. Such studies would, at present, be very difficult to move to humans with IBD.

Young et al. [100] comment on the necessary next steps for the study of IBD in humans, that integrate work on human and microbe genes, diet, bacteria, smoking, medication, and other environmental factors, possibly including viruses. For example, infection by *Clostridium difficile* and *Salmonella* sp. can be enhanced by antibiotic treatment. Furthermore, the gut microbiome may play an important role in viral infection. Peterson and Turnbaugh [101] have taken this further, to suggest the existence of a viral "key" that irreversibly renders a genetically susceptible model of CD immune to the pathological changes usually associated with the disease. While thus far only shown in mice, such studies may have major human implications.

A detailed time series analysis of luminal and mucosa-associated microbial communities may be required. This information would be used in designing the most appropriate trials to distinguish relevant from confounding variables in human clinical studies. There is also a need for new technical developments, including better timed and more systematic patient sampling methodologies, integrative use of the most appropriate animal models, and the most appropriate DNA and RNA sequencing methods. In their opinion [100], a considerable resource needs to be put into the most appropriate bioinformatic methods for concomitant analysis of DNA, RNA, proteins, metabolites, and spatial organization.

6 Conclusions

Nutrition plays a complex role in the etiology of IBD. At present, however, it is difficult to establish the relative roles of nutrients, gut microbes, various environmental factors, host and bacterial genetics, and their interactions, in risk of the disease. The original observations have been based upon case–control studies, which depend upon adequate remem-

brance of predisease diet, and may be inaccurate. However, data from cohort studies are now becoming available.

For those patients with IBD, nutrition will profoundly affect both the control of disease symptoms, and rate of disease progression. Each IBD patient has a distinctive genetic profile and nature of disease presentation. It will become increasingly important to recognize a need for personalized dietary regimes for disease prevention and for symptom control. Dietary factors that may reduce symptoms are few in nature, while there is an extensive list of potentially adverse items. In consequence, malnutrition is common in this group, and may be part of the reason for decreased general health and strength. There is a strong need for better co-ordinated studies that combine groups internationally, with similar protocols, in order to strengthen the currently available databases on these important topics.

The role of the microbiota is becoming increasingly recognized as important. Dysbiosis is particularly important in CD, but whether it is cause or effect of the disease, and whether focusing treatment effects on shifting the microbiota would be productive, is not yet certain. It will become increasingly important to integrate work on human and microbe genomes, diet, bacteria, smoking, medication such as antibiotic use, and other environmental factors.

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