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Inflammatory bowel disease and immunonutrition: novel therapeutic approaches through modulation of diet and the gut microbiome

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Summary

Inflammatory bowel disease (IBD) is a chronic inflammatory condition of the gastrointestinal tract, thought to at least in part reflect an aberrant immune response to gut bacteria. IBD is increasing in incidence, particularly in populations that have recently immigrated to western countries. This suggests that environmental factors are involved in its pathogenesis. We hypothesize that the increase in IBD rates might reflect the consumption of an unhealthy Western diet, containing excess calories and lacking in key nutritional factors, such as fibre and vitamin D. Several recent studies have determined that dietary factors can dramatically influence the activation of immune cells and the mediators they release through a process called immunonutrition. Moreover, dietary changes can profoundly affect the balance of beneficial versus pathogenic bacteria in the gut. This microbial imbalance can alter levels of microbiota-derived metabolites that in turn can influence innate and adaptive intestinal immune responses. If the diet–gut microbiome disease axis does indeed underpin much of the 'western' influence on the onset and progression of IBD, then tremendous opportunity exists for therapeutic changes in lifestyle, to modulate the gut microbiome and to correct immune imbalances in individuals with IBD. This review highlights four such therapeutic strategies – probiotics, prebiotics, vitamin D and caloric restriction – that have the potential to improve and add to current IBD treatment regimens.

Keywords: fasting; gut; immunonutrition; microbiome; prebiotics; probiotics; vitamin D.

Introduction to inflammatory bowel disease

Inflammatory bowel disease (IBD) is an immune-mediated disease characterized by inflammation of the gastrointestinal (GI) tract. IBD encompasses both Crohn's disease and ulcerative colitis; conditions that are chronic and often progressive, but are most commonly associated with intermittent disease flares. The exact aetiology of IBD remains elusive. However, it is well accepted that IBD occurs at the intersection between genetic, immune and environmental factors. Genetically, IBD is one of the best-described complex diseases with > 200 gene variants associated with disease.[1] Many of these risk variants, for genes such as *NOD2* and *ATG16L1*, are predicted to cause defects in epithelial barrier function and/or bacterial recognition and clearance,[2] thereby implicating gut microbes as drivers of IBD. On their own the currently identified gene risk variants only predispose individuals to disease. Clearly, modifiable factors such as gut microbes and diet play an important role in disease development, considering that IBD incidence has dramatically increased in developed countries over the second half of the twentieth century, pointing to a role for the 'western' lifestyle. Over the past decade, studies have repeatedly identified an imbalance in the gut microbiota, referred to as dysbiosis, in patients with IBD.[3] Characterized by reduced microbial diversity and increased numbers of potential pathobionts, such as adherent/invasive *Escherichia coli*, it remains unclear whether these shifts in microbial communities are a result of inflammation, or instead reflect the high-sugar, high-fat, low-fibre diets common in developed countries. In either case, the

microbial dysbiosis seen in patients with IBD leads to altered levels of microbial metabolites within the gut, thereby, along with diet, inducing changes in host metabolism and function. Hence, although the current paradigm is that IBD occurs in genetically susceptible individuals due to an inappropriate immune response to enteric commensal microbes, an alternative hypothesis is that diet/aberrant microbiota initiates and/or promotes the damaging immune responses seen in IBD. Through this review, we will address how immunonutrition, referring to the effect of nutrients and metabolites on the immune system, is altered in IBD, and how manipulation of diet and the gut microbiome may offer new therapeutic strategies to treat IBD.

Current therapies for IBD

At present, treatments for individuals with IBD predominantly target their pathological immune responses rather than any potential causal factors. The traditional step-up approach to therapy moves sequentially through immunosuppressives (5-aminosalicylic acid), immunomodulators (azathioprine and methotrexate), and finally to biologics.[4,5] Biologics used to treat IBD include antibodies that block inflammation by targeting the pro-inflammatory cytokines tumour necrosis factor-α (TNF-α) or interleukin-12 (IL-12)/23p40, or by preventing immune cell recruitment to the gut. The anti-TNF-α antibody (infliximab) revolutionized the treatment for Crohn's disease upon its introduction in 1998,[6,7] and was subsequently found to be effective for the treatment of ulcerative colitis.[8] A fully humanized version of the same antibody (adalimumab) is also used to treat both Crohn's disease[9] and ulcerative colitis[10] with similar efficacy to infliximab treatment.[11] Other newer biologics include antibodies that block $\alpha_4\beta_7$ (vedolizumab) or $\alpha_e\beta_7$ (etrolizumab) integrin-mediated immune cell recruitment into the gut,[12–14] or antibodies that target the pro-inflammatory effects of IL-12/23p40 (ustekinumab).[15] Additional biological therapies including anti-cytokine antibodies, anti-immune cell trafficking therapies, inhibitors of the Janus kinase pathway, and antisense oligonucleotides are all in various stages of clinical trials (reviewed in ref. 16).

Although representing the most widely accepted IBD therapies, these approaches have important limitations. For instance, most biologics only work in subsets of patients,[17] and although they can prove highly effective for that subset, they can also induce substantial adverse effects.[17] For example, steroids or biologics increase susceptibility to infections and cancer,[18,19] and evidence suggests that their efficacy decreases over time in a substantial number of patients.[20] Moreover, it can be argued that steroids[21] are not specifically targeting the underlying, causal abnormalities in immune cells or in the gut microbiome. Hence, we propose that it is worthwhile examining non-genetic, modifiable contributors to IBD. As our understanding of the mechanisms underlying IBD expands, rational alternative approaches targeting the gut microbiome and immune system through dietary modulation are receiving increased attention.

Inflammatory bowel disease and the dysbiotic gut microbiome

The human GI tract harbours a vast array of microorganisms that provide many benefits to the health and well-being of the host.[22] These microbes include bacteria, viruses, fungi and eukaryotes; collectively referred to as the multibiome (reviewed in ref. 23–26). Development of the multibiome begins in early infancy and is influenced by several factors including route of delivery (vaginal versus caesarean section), environment, breast feeding, diet, genetics, infections, antibiotic use, age and hygiene.[27,28] Humans and their gut microbiota have co-evolved to possess a symbiotic relationship, wherein the microbes obtain a place to dwell, as well as nutrition, while they help their hosts by aiding in food digestion, out-competing potential pathogens, and releasing beneficial metabolites such as short-chain fatty acids (SCFA), notably acetate, propionate and butyrate. In contrast, when an imbalance in bacterial composition and a lack of bacterial diversity (termed dysbiosis) occurs, microbial functionality may become perturbed. Dysbiotic microbiota have been implicated in a number of diseases including colon cancer, obesity and type 2 diabetes.[29–32] The gut microbiota of individuals with IBD is characterized by low microbial diversity,[33,34] a reduced abundance of *Bifidobacterium* spp.,[33,35] *Lactobacillus* spp.[34] and *Faecalibacterium prausnitzii*,[33,35,36] and a higher abundance of pathobionts such as adherent/invasive *E. coli*[37,38] and *Clostridium difficile*,[39] resulting in lower SCFA concentrations[40] compared with healthy individuals (Fig. 1).

At present, it remains unclear whether the dysbiotic microbiota found in many IBD patients truly plays a causative role or alternatively, is simply a reflection of the inflammatory and antimicrobial responses elicited during the course of disease. The truth probably lies somewhere in between. Several observations have indicated that intestinal dysbiosis might promote IBD pathogenesis, as inflammation is usually located in the distal ileum or colon, which are also the sites of highest bacterial abundance in the intestine. Moreover, studies using spontaneous and induced animal models of IBD have shown that animals develop little if any inflammation under germ-free (GF) conditions.[41–43] Nonetheless, inflammation on its own seems to favour the typical dysbiosis seen in patients with IBD, i.e. depletion of *Firmicutes* and the expansion of *Enterobacteriaceae*, especially *E. coli* strains.[44,45] This dysbiotic state is probably due to

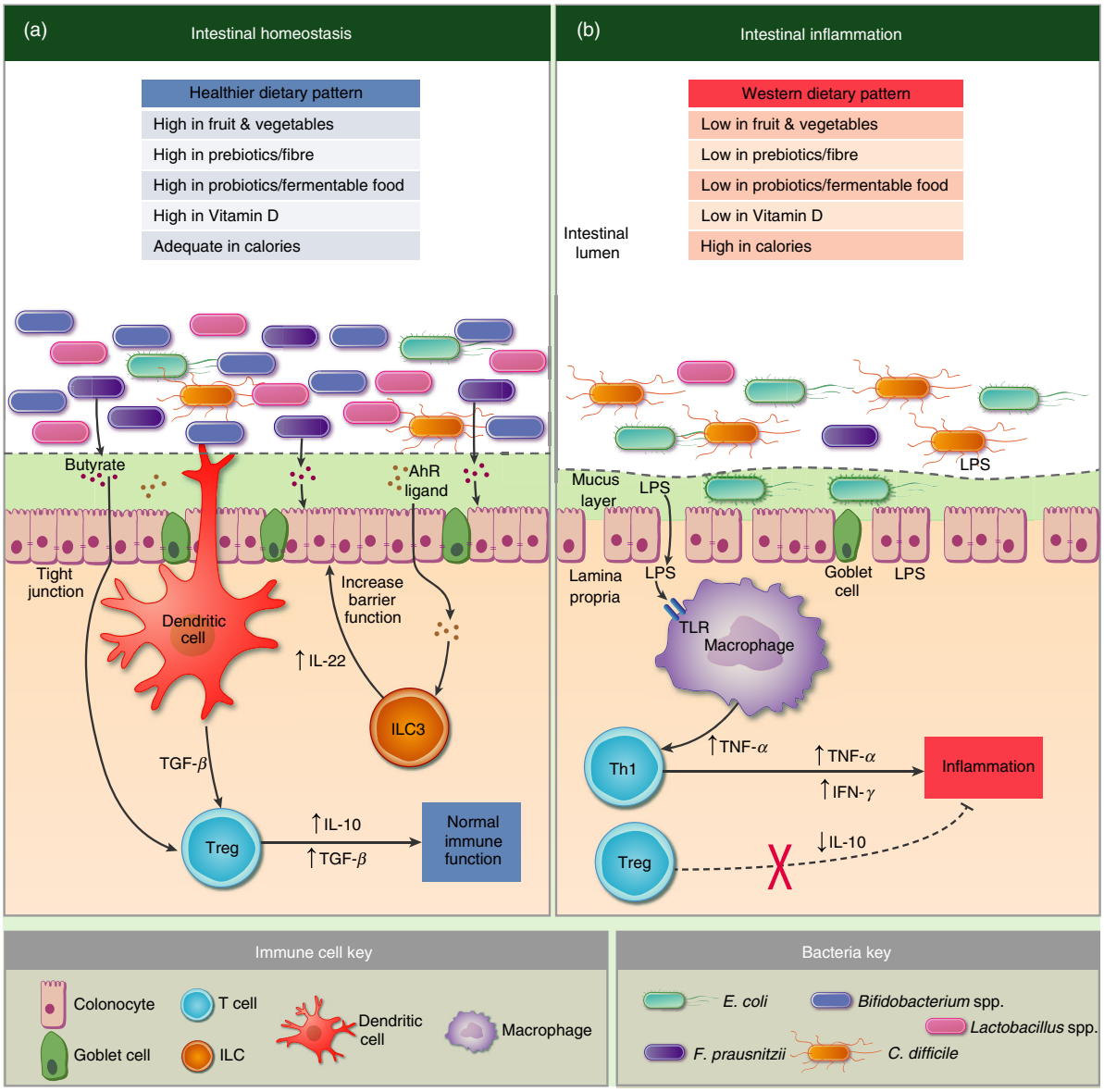

Figure 1. Complex interactions that exist between diet, gut microbiota, colonocytes and immune cells during intestinal homeostasis and inflammation. (a) Intestinal homeostasis is associated with a healthier dietary pattern, microbial diversity, higher abundance of beneficial bacteria such as *Bifidobacterium* spp., *Faecalibacterium prausnitzii* and *Lactobacillus* spp., and increased short-chain fatty acid production, particularly butyrate. Butyrate possesses powerful anti-inflammatory properties as it promotes regulatory T (Treg) cell proliferation and enhances intestinal barrier function. Dendritic cells within the lamina propria sample commensal microbiota antigens within the intestinal lumen. In response to these commensal antigens, dendritic cells release transforming growth factor-β (TGF-β) which activates Treg cells to release interleukin-10 (IL-10) and TGF-β, leading to a more tolerant immune phenotype. A thicker mucus layer is another characteristic of intestinal homeostasis, providing a protective barrier between luminal bacteria and epithelial cells. Lastly, aryl hydrocarbon receptor (AhR) ligands, derived from fruits and vegetables, induce innate lymphoid cell 3 (ILC3) to produce IL-22, which helps maintain intestinal barrier function. (b) Conversely, intestinal inflammation is associated with a Western dietary pattern and bacterial dysbiosis (i.e. lower microbial diversity, reduced short-chain fatty acid production, lower abundance of beneficial bacteria, and higher abundance of pathobionts such as *Clostridium difficile* and *Escherichia coli*). A thinner, more patchy mucus layer provides less of a protective barrier between the luminal bacteria and colonocytes. Along with a reduction in expression of cellular tight junctions this leads to impaired intestinal barrier function resulting in leakage of bacterial products, such as lipopolysaccharides (LPS), from the intestinal lumen into the lamina propria. LPS bind to toll-like receptors (TLR) activating macrophages to produce tumour necrosis factor-α (TNF-α) which promotes T helper cell type 1 (Th1) to proliferate and release pro-inflammatory cytokines [i.e. TNF-α and interferon-γ (IFN-γ)] leading to inflammation, which also compromises intestinal barrier function. Additionally, a reduction in IL-10-producing Treg cells further contributes to intestinal inflammation.

changes in oxygen levels and other environmental features of the gut including the development of new nutritional niches only suitable for this group of bacteria.[38,44,45] Considering the unique ability of *Enterobacteriaceae* to thrive proximal to inflamed tissues, these examples support the concept that microbial dysbiosis might be a consequence rather than a cause of inflammation in patients with IBD.

Inflammatory bowel disease and the Western diet: calorically dense, nutritionally scarce

Nutrition is a critical component of health that can impact the onset and progression of IBD.[46] Although healthy diets are thought to provide the body's caloric requirements from whole foods that are nutritiously diverse, modern (Western) dietary patterns consist of sugar- and fat-rich, high-calorie processed foods generally devoid of fibre. As such, Western diets typically contain few fruits, vegetables, legumes and whole grains.[47] Aside from lacking fibre, it is also notable that several foods present in the Western diet have been shown to have detrimental effects on the gut and the residing microbes or are associated with an increased risk of developing IBD. These foods include non-caloric artificial sweeteners, alcohol, preservatives and stabilizers, refined carbohydrates and high amounts of processed animal products, especially meat (as reviewed in ref. 48,49). Interestingly, most foodstuffs considered detrimental in the western style diet are also calorically dense foods, whereas beneficial high-fibre foods contain far fewer calories per gram. Western diets therefore lead to increased rates of obesity, in concert with a rise in the occurrence of colon cancer and inflammatory conditions such as type 2 diabetes and IBD.[50] Indeed, it is well documented that excess body fat leads to a state of systemic low-grade inflammation in humans,[51] as excess nutrients can activate and fuel immune cells to drive inflammation[52,53] as well as promote endoplasmic reticulum stress. Consistent with that, recent research suggests that limiting nutrients or even fasting can have the opposite effect on immune cells and thereby dampen inflammation.[54,55]

Interestingly, the clinical course of IBD itself can impair nutrient uptake, as chronic gut inflammation, intestinal surgery and the adverse effects of IBD-specific medications can predispose individuals to deficiencies in certain micronutrients such as iron, calcium, zinc, magnesium, folic acid, vitamin B12, vitamin A and vitamin D.[56] Other dietary factors of importance and at risk of deficiency in some individuals with IBD are natural plant flavonoids and indoles.[57] Recent reviews provide additional insight into the other IBD-related micronutrient deficiencies mentioned above.[58,59] However, among these deficiencies, the role of vitamin D (also known as the sunshine vitamin) in gut immunity is one of the best-characterized relationships in the literature, and is described later in this review.

Immunonutrition and its potential impact on IBD

As described above, immunonutrition refers to the effects that dietary factors can have on different aspects of the immune system as well as the microbiome. Within the GI tract, nutrients are likely to affect mucosal barrier function and cellular defence, as well as modulate local inflammation. For example, intestinal homeostasis in individuals consuming a healthy, balanced diet is normally maintained through the release of a variety of microbial metabolites, including SCFA (e.g. acetate, propionate and butyrate), which improve intestinal barrier function by providing energy for colonic epithelial cells (colonocytes), and by promoting regulatory T cell (Treg) function. SCFA are rapidly used by colonocytes or absorbed into the systemic circulation where they bind to the G protein-coupled receptors GPR41 and GPR43, mediating protective immunity by promoting epithelial cell production of cytokines and chemokines.[60] Butyrate is the best studied of the SCFA, acting as a key source of energy for colonocytes as well as possessing powerful anti-inflammatory properties. Anti-inflammatory activities include altering the maturation of dendritic cells (DC), increasing the number of Treg cells and levels of the anti-inflammatory cytokine IL-10, while inhibiting production of pro-inflammatory cytokines such as interferon-γ and IL-2.[61–63] A healthy gut microbiome can also direct the production of the anti-inflammatory mediators transforming growth factor-β, retinoic acid and thymic stromal lymphopoietin, all of which help to maintain normal mucosal immune function and intestinal homeostasis. The intestinal immune system can also be directly modulated by products such as aryl hydrocarbon receptor (AhR) ligands, derived from dietary tryptophan as well as flavonoids and indoles from fruits and vegetables. The AhR ligands induce immune cells [innate lymphoid cells 3 and T helper type 17 (Th17) cells] to produce mediators such as IL-22 that help to maintain the epithelial barrier (Fig. 1).

In contrast, the typical western style diet that is high in sugar and saturated fats and low in fibre can lead to systemic low-grade inflammation, as a well-characterized consequence of obesity.[64] Although inflammation in adipose tissues and in the liver is a more established feature of obesity, the intestine is emerging as a key site for immunological changes that affect whole-body metabolism.[65] Specifically, microbial and dietary factors influence underlying innate and adaptive responses of the intestinal immune system. These responses can lead to disrupted intestinal barrier function, system inflammation, impaired glucose metabolism and can promote bacterial dysbiosis (as reviewed in ref. 66). These changes in the gut microbiota are sufficient to promote a state of low-grade chronic mucosal inflammation, thereby disrupting intestinal homeostasis. A lack of fruit and vegetables reduces

the levels of AhR ligands, reducing IL-22 production by innate lymphoid cells 3 and Th17 cells, and thereby weakening intestinal barrier integrity.[67] Microbial dysbiosis can also lead to reduced levels of IL-10, coupled with increased local levels of TNF-α, thereby promoting inflammation.[68] Impaired gut barrier function results in the leakage of luminal bacterial products such as lipopolysaccharides out of the intestine, which drives a shift in T-cell polarity towards interferon-γ-releasing Th1 cells.[69] The ensuing low-grade inflammation further compromises intestinal barrier function,[70] resulting in the characteristic leaky gut associated with diet-induced obesity (Fig. 1).

Although metabolic disorders and IBD are distinct conditions, it is notable that many of the pathological features seen in the intestine in response to high-fat diets (baseline inflammation, barrier dysfunction) are similar to those seen in, or that predispose to, IBD. For example, although nucleotide-binding oligomerization domain sensors are best known as innate receptors involved in recognizing intracellular bacteria, they also respond to endoplasmic reticulum stress,[71] highlighting the potential for alterations in immunonutrition to play a role in IBD development. A reduction in vitamin D levels as well as AhR-activating compounds are also seen in patients with IBD, in concert with impaired mucosal healing.[72]

Aside from immune cells, recent studies have found a key role for epithelial cell metabolism in controlling the gut microbiota, and specifically the overgrowth of *E. coli* pathobionts. As noted above, under healthy conditions, colonocytes use butyrate as a key energy source. Metabolizing butyrate consumes oxygen, thereby rendering surface colonocytes hypoxic and promoting the luminal growth of strict anaerobic bacteria such as *Firmicutes*.[73,74] In contrast, intestinal inflammation typically results in a loss of *Firmicutes* (i.e. *F. prausnitzii*), leading to a reduction in butyrate production. The absence of butyrate forces colonocytes to obtain energy through the fermentation of glucose to lactate (fermentative metabolism), a process that does not consume oxygen and so increases oxygen levels in and near these cells to 3–10%. This increase in oxygen dramatically affects the make-up of local microbes, depleting strict anaerobes while permitting a bloom of facultative anaerobic bacteria such as the *E. coli* pathobionts[73,74] that have been implicated in IBD. Taken together, the similarity between the intestinal pathophysiology seen in metabolic disorders, and those seen in IBD raises the possibility that some IBD patients develop their disease because of heightened susceptibility to environmentally driven dysregulated intestinal immunity, such as that caused by western-style diet or other factors that promote microbial dysbiosis in the GI tract. As counter-regulatory measures, we propose and discuss dietary immunomodulation – or immunonutrition strategies that could help to alleviate and re-balance an inflamed gut (summarized in Fig. 2).

Changing the gut microbiome – probiotics as therapy

One approach to overcome the microbial dysbiosis seen in patients with IBD is through the oral or per enema delivery of beneficial gut microbes (probiotics) known to be lacking in IBD patients. As defined by the World Health Organization, probiotics are microorganisms that confer a health benefit to the host when administered in adequate amounts.[75,76] Probiotics can be easily incorporated into the diet through the consumption of fermented foods (i.e. yoghurt, kefir, kimchi, sauerkraut), or consumed on a daily basis as a probiotic supplement. Studies attribute several health benefits to probiotics including direct effects such as producing SCFA (e.g. butyrate) and excluding pathogens from the gut by competition for space and nutrients, as well as indirect effects such as enhancing epithelial barrier function and promoting antimicrobial peptide production and secretory IgA. Moreover, probiotics have been shown to increase mucin secretion from intestinal goblet cells and to beneficially modulate the host immune system through the stimulation of anti-inflammatory cytokines such as IL-10 and transforming growth factor-β as well as stimulate the induction of Treg cells.[77,78] The exact mechanism(s) by which probiotics exert these positive effects are unclear; however, it is clear that the efficacy of probiotics varies depending on the microbial strain used and the dose administered.

Microorganisms from the genera *Lactobacillus* and *Bifidobacterium* as well as the yeast *Saccharomyces boulardii* are among the most common probiotic candidates (Tables 1 and 2). Extensive *in vitro* research has shown that several *Lactobacillus* spp. exhibit anti-inflammatory effects, as primarily assessed by Toll-like receptor (TLR) activation.[79] For example, *Lactobacillus casei* Shirota treatment restores the normal stimulatory capacity of DC from patients with ulcerative colitis by reducing TLR2 and TLR4 expression.[80,81] *Lactobacillus plantarum* CGMCC1258 increases tight junction protein levels and decreases permeability in the intestinal epithelial cell line, IPEC-J2. Moreover, this probiotic reduces IL-8 and TNF-α expression in intestinal porcine epithelial cells challenged by *E. coli* K88, possibly through a decrease in TLR expression, nuclear factor-κB activation, and mitogen-activated protein kinase pathways.[82]

In mouse models of intestinal inflammation, *Lactobacillus acidophilus* Bar 13 and *Bifidobacterium longum* Bar 33 promote the expansion of Treg cells and reduce the number of intraepithelial lymphocytes in 2,4,6-trinitrobenzene sulphonic acid-induced colitis.[83] In a similar model of murine colitis (2,4-dinitrobenzene sulphonic acid),

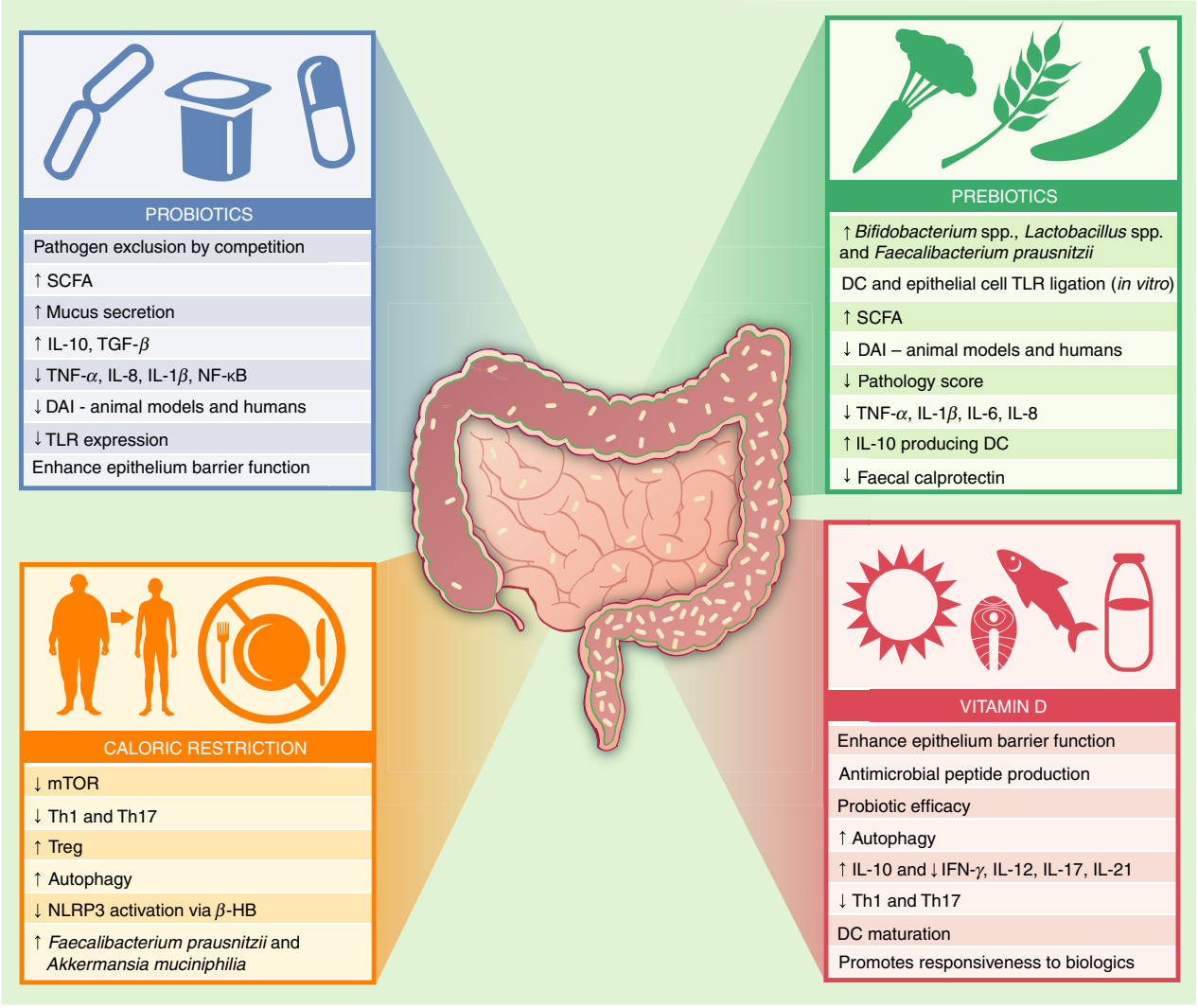

Figure 2. Summary of immunonutrition strategies that modulate the gut microbiota and help alleviate and re-balance an inflamed gut. DAI, disease activity index; DC, dendritic cells; HB, hydroxybutyrate; IFN, interferon; IL, interleukin; mTOR, mechanistic target of rapamycin; NF, nuclear factor; SCFA, short-chain fatty acids; Th, T helper cell; TLR, toll-like receptors; TNF, tumour necrosis factor; Treg, regulatory T cell.

L. casei DN-114 001 ameliorates disease severity through the induction and expansion of colonic $CD4^+$ $FoxP3^+$ Treg cells.[84] Other studies using mice with dextran sulphate sodium (DSS) colitis show that a combination of eight different probiotic strains (VSL#3) effectively reduces disease activity and colon inflammation including a significant reduction in inflammatory markers such as IL-1β, nuclear factor-κB and the neutrophil marker, myeloperoxidase.[85–87] Similarly, administration of *L. plantarum* 299V prevents spontaneous colitis development in IL-10-deficient ($Il10^{-/-}$) mice,[88] and treatment with VSL#3 ameliorates colitis and overall disease activity in $Il10^{-/-}$ mice.

Curiously, despite the broad success of probiotics in animal models of colitis, their effects in clinical IBD trials have been less successful, with only small subsets of treated patients showing beneficial effects (reviewed in ref. 89–91). One reason behind this limited effect in patients with IBD may stem from the 'one size fits all' approach that has been commonly employed with probiotics. It is strongly believed that as an infant's immune system matures, it develops a mutualistic relationship with the resident microbes in the intestine. This ensures that these resident gut microbes establish an environmental niche, as well as an immunological niche, that is recognized by the immune system as a long-term part of the host. In contrast, new microbes encountered after this relationship has developed are typically seen as foreign and are expelled. Hence, giving exogenous probiotic microbes to patients without defining whether there are environmental/immunological niches for those gut microbes may mean that the probiotics will be seen as

Table 1. Effect of prebiotic and/or probiotic interventions on gut immunity and microbiota in animal models of colitis

Animal model	Prebiotic/probiotic	Dose and duration	Effect on gut immunity	Effect on gut microbiota	Ref
HLA-B27 transgenic rats	IN/FOS (Synergy 1)	5 g/kg in drinking water; 7 weeks	↓ Histological & gross caecal scores, & IL-1β. ↓ mucosal inflammation. ↑ TGF-β. ϕ IL-10	↑ *Bifidobacterium* spp. & *Lactobacillus* spp. ϕ SCFA conc.	107
	IN or FOS	8 g/kg; 12 weeks	↓ Histology score (particularly FOS) & IL-1β in caecal tissue	↓ Total bacteria & *Bacteroides* spp. (FOS & IN). ↓ *Clostridium* cluster XI & XIVa, *Enterobacteriaceae* & *Clostridium difficile* & ↑ *Bifidobacterium* spp. & *Clostridium* cluster I (FOS). ↓ *Lactobacillus* spp. & *Clostridium* cluster IV (IN). ↑ total SCFA conc. (FOS & IN)	108
	IN and *Lactobacillus acidophilus* La-5 and *Bifidobacterium lactis* Bb	Unspecified; 8 weeks	↓ Histological score	↑ Microbial diversity & *Bifidobacterium animalis*	109
DSS-induced colitis in rats	scFOS or RS (type 3)	63 (FOS) or 115 (RS) g/kg; 14 days during DSS	↓ Macroscopic & histological scores (RS)	ϕ SCFA conc.	113
	OF/Inulin (Synergy 1) + *Bifidobacterium infantis* or OF/inulin only	0·5 g/day via oral gavage; 7 days before DSS & 7 days during DSS	↓ DAI, MPO activity & colonic IL-1β levels (both groups)	↑ Caecal *Bifidobacterium* spp. & *Lactobacillus* spp. counts. ϕ in *Enterobacteriaceae*. ↑ succinate conc.	105
	Inulin	1% of total diet in drinking water; 9 days before DSS & 5 days during DSS	↓ Mucosal damage scores & MPO activity	↑ Counts of *Lactobacillus* spp. ϕ in *Bifidobacterium* spp.	112
	VSL#3 + wortmannin (PI3k/Akt inhibitor)	15 mg VSL#3 + 1·4 mg/kg wortmannin daily after DSS for 7 days	↓ DAI and MPO activity ↓ iNOS, Cox-2, NF-kB, TNF-α, IL-6, p-Akt ↑IL-10		85

Table 1 *(Continued)*

Animal model	Prebiotic/probiotic	Dose and duration	Effect on gut immunity	Effect on gut microbiota	Ref
DSS-induced colitis in mice	FOS	1·5 g/mL bd via oral gavage; 7 days before DSS & 7 days during DSS	↓ DAI & ↑ colon length		106
	Fibre mix- GOS, FOS, oligofructose, gum arabic, soy polysaccharides, cellulose & RS	1·5% of total diet; 14 days before DSS & 7 days during DSS	↑ Colon length/weight ratio. ↑ in colonic IL-10 levels. ↑ in Treg/Th2+ Treg/Th17 ratio		163
	β-glucan: glucan, zymosan & curdlan	25 mg/kg by oral gavage; 14 days before DSS	↑ Histological evidence of colitis (all β-glucan types). ↑ TNF-α, CCL-2 and IL-6 (curdlan and zymosan). ↑ IL-10 (glucan)		164
	β-glucan	500 mg/kg or 1000 mg/kg by oral gavage; 7 days before DSS & 7 days during DSS	↓ DAI, spleen weight & colon shortening. ↓ MPO, malondialdehyde & nitrate (both doses). ↓ TNF-α, IL-6, iNOS & IL-1β		165
	Yeast β-glucan or β-glucan	5% of total diet; 7 days during DSS	↓ DAI, colon shortening & histological evidence of colitis. ↓ apoptosis rate & serum IgA, IgG and IgM. ↓ infiltration of macrophages & neutrophils. ↓ TNF-α, IL-6, IL-8, iNOS & COX-2. ↓ EPO & MPO in colonic tissue. ↑ expression of ZO-1, occludin, claudin-1 & JAM-1		166
IL-10-deficient mice	NRS, SFD-t, SFD-c or SCF	4% of total diet; Weaning until end of experiment (12 weeks)	↓ IL-6 & CXCL1 (NRS). ↓ TNF-α, IL-1β & IL-23 (SFD-t). ↓ IL-12p70, IL-6 & CXCL1 ($P < 0·05$). ϕ cytokines (SCF)	↓ *Prevotellaceae*, *Incertae sedis* XIV & *Lachnospiraceae* (NRS). ↑ *Incertae sedis* XIV, *Lachnospiraceae* & *Ruminococcaceae*, & ↓ *Lactobacillaceae* (SFD-t). ↑ *Porphyromonadaceae* & *Prevotellaceae*, ↓ *Lactobacillaceae*, *Incertae sedis* XIV, *Lachnospiraceae* & *Ruminococcaceae* (SFD-c). ↑ *Porphyromonadaceae* & *Prevotellaceae* (SCF)	167

Table 1 *(Continued)*

Animal model	Prebiotic/probiotic	Dose and duration	Effect on gut immunity	Effect on gut microbiota	Ref
TNBS-induced colitis in rats	FOS	1 g bd via intragastric catheter; 2 days before TNBS & 7 or 14 days after TNBS	↓ Gross inflammation score & MPO activity (14 days)	↑ Butyrate (7 days only), lactate & total lactic acid bacteria	110
	scFOS (2 types: DP4 and DP8)	60 g/kg/day; 7 days before TNBS & 3 or 10 days after TNBS	↑ Cecal weight and contents. Significant ↓ in macroscopic damage score. ↓ MPO activity (DP8 only)	ϕ SCFA conc.	111
	GOS	4 g/kg/day by oral gavage; 10 days before TNBS & 3 days after TNBS	ϕ Colonic damage score, oedema or myeloperoxidase activity	↑ *Bifidobacterium* spp. & total bacteria conc.	168
TNBS-induced colitis in mice	Mix1 (*Lactobacillus acidophilus* Bar 13 + *Bifidobacterium longum* Bar 33), Mix2 (*L. plantarum* Bar 10, *Streptococcus thermophilus* Bar 20, and *B. animalis* subsp. *lactis* Bar 30	10^9 CFU/day; 3 weeks	Significant ↓ in histological damage. ↓ $CD4^+$ cells of IELs and LPLs (Mix1). ↑ Tregs and IL-10 (Mix1 and Mix2) ↓ TNF-α and (MCP)-1 (Mix1 and Mix2)		83
DNBS-induced colitis in mice	*Lactobacillus casei* DN – 114 001	200 μL of 10^8 CFU/mL by oral gavage; 14 days before DNBS & 5 days after DNBS	↑ $Foxp3^+CD4^+$ T cells		84

bd, twice daily; CCL, chemokine ligand; COX, cyclooxygenase; DAI, disease activity index; DNBS, 2,4-dinitrobenzene sulphonic acid; DSS, dextran sulphate sodium; EPO, eosinophil peroxidase; scFOS, short-chain fructo-oligosaccharides; G-CSF, granulocyte colony-stimulating factor; IELs, intraepithelial lymphocytes; IFN, interferon; Ig, immunoglobulin; IL, interleukin; IN, inulin; IP, interferon-γ-induced protein; KC, keratinocyte chemoattractant; LPLs, lamina propria lymphocytes; MCP-1, monocyte chemotactic protein 1; MPO, myeloperoxidase; NRS, Corn-derived hydroxypropylated new resistant starch; iNOS, inducible nitric oxide synthase; RS, resistant starch; SFD-c, soluble fibre dextrin from corn; SFD-t, soluble fibre dextrin from tapioca; SCF, soluble corn fibre; SCFA, short-chain fatty acids; TGF, transforming growth factor; TLR, toll-like receptor; TNBS, 2,4,6-trinitrobenzene sulphonic acid; TNF, tumour necrosis factor. ϕ, no change, ↓, decrease/lower, ↑, increase/higher.

Table 2. Effect of prebiotic and/or probiotic interventions on gut immunity and microbiota in individuals with inflammatory bowel disease

Disease	Participants and duration	Probiotic/Prebiotic and dose	Effect on gut immunity	Effect on gut microbiota	Ref
CD	10 adults; 3 weeks	FOS; 15 g/day	↓ HBI. ↑ IL-10 positive $CD11c^+$ intestinal DC. ϕ IL-6 or IL-12 positive $CD11c^+$ intestinal DC. ↑ DC expression TLR2 & TLR4	↑ Faecal but not mucosal *Bifidobacterium* spp. ϕ total bacteria, *Bacteroides-Prevotella* or *Clostridium coccoides-Eubacterium rectale*	114
	103 adults; 4 weeks	IN/FOS (Synergy 1); 7·5 g bd	↑ DC IL-10 staining of rectal tissue. ϕ IL-12p40 production by intestinal DC or IL-6 positive DC. ϕ CDAI	ϕ *Bifidobacterium* spp. or *Faecalibacterium prausnitzii*	121
	35 adults; 6 months	*Bifidobacterium longum* with IN/FOS (Synergy 1); 6 g bd	↓ CDAI, histological scores & TNF-α	↑ Mucosal *Bifidobacterium* spp.	115
	40 adults; 4 weeks	OF-IN; 10 g bd	Positive correlation between ↓ HBI & in ↑ *B. longum*	↓ *Ruminococcus gnavus*. ↑ *B. longum*. ϕ *Faecalibacterium prausnitzii*	116
	165 adults; 52 weeks	*Saccharomyces boulardii*; 1 g/day	ϕ CDAI, C-reactive protein, remission rate		169
	70 adults; 12 weeks	*Lactobacillus johnsonii (LA1);* 10^{10} CFU/day	ϕ Endocospic score, severe recurrence, CDAI		170
UC	19 adults; 2 weeks	IN/FOS (Synergy 1); 4 g tds	ϕ Rachmilewitz scores. ↓ faecal calprotectin		119
	18 adults; 4 weeks	IN and FOS with *B. longum*; 6 g bd	↓ Sigmoidoscopy score, mRNA levels for human β-defensins 2, 3 and 4, TNF-α & IL-1α. Biopsies showed ↓ inflammation & regeneration of epithelial tissue	↑ *Bifidobacterium* spp. in rectal mucosa	117
	16 adults; 8 weeks	Arabinogalactan/xilo-oligosaccharides with *Lactobacillus paracasei* (B21060); 6 g bd	↓ Serum IL-6 & IL-8. ϕ in general well-being, rectal bleeding & number of bowel motions		118
	39 adults; 12 months	GOS with *Bifidobacterium breve* fermented milk (Yakult); 5·5 g/day	↓ Endoscopic score & MPO levels	↓ *Bacteriodaceae*. ϕ *Bifidobacterium* spp. ↓ faecal pH levels	120
	42 adults; 8 weeks	VSL#3; 10^{10} CFU bd	↓ DC and TLR2 expression. ↑ IL-10 and ↓ IL-12p40		171
	30 adults; 8 weeks	BIFICO; 1·26 g/day	↓ NF-κB, TNF-α and IL-1β, ↑ IL-10	↑ *Lactobacillus* spp. and *Bifidobacterium* spp.	172
UC/CD	52 adults; 4 months	Lactulose; 10 g/day	ϕ Clinical activity indices, endoscopic score or immunohistochemical parameters. ↑ QoL index in UC		122

bd, twice daily; CD, Crohn's disease; CDAI, Crohn's disease activity index; DC, dendritic cells; FOS, fructo-oligosaccharide; GOS, galacto-oligosaccharide; HBI, Harvey–Bradshaw index; IL, interleukin; IN, inulin; MPO, myeloperoxidase; NF-κB, nuclear factor-κB; OF, oligofructose; QoL, quality of life; tds, three times a day; TLR, Toll-like receptor; TNF, tumour necrosis factor; UC, ulcerative colitis. ϕ, no change; ↓, decrease/lower; ↑, increase/higher.

foreign and will be unable to take up permanent residence in the GI tracts of those patients. Similarly, the inflamed intestines of patients with IBD are inhospitable to probiotic microbes because of the exaggerated inflammatory and antimicrobial responses seen during disease. These responses clear new microbes, including potentially beneficial bacteria, rapidly from the intestine, often before they have the opportunity to work.

Clearly, new approaches to designing probiotics, and promoting their survival will be key to the future success of this potential therapy. Additionally, engineered probiotics have been developed that produce and release the anti-inflammatory cytokine IL-10[92,93] or trefoil factor as strategies to locally suppress intestinal inflammation and promote healing.[94] Moreover, recent insights regarding the make-up of the human microbiome should allow us to identify potential next-generation probiotic species with improved potential for colonizing the human GI tract. Recently, Maldonado-Gómez *et al.*[95] demonstrated that the microbe *B. longum* AH1206 was able to persist in

the intestines of a subset of individuals for at least 6 months after the administration without causing adverse effects or overtly altering the resident microbiota composition. This microbe's extended colonization is attributed to its ability to establish a nutritional niche related to genes involved in carbohydrate use. This finding suggests that the establishment of new probiotic microbes will depend on an individual's baseline microbiota as well as on the availability of nutritional resources, supporting the critical role of dietary substrates such as fermentable carbohydrates and prebiotics in permitting the long-term persistence of a probiotic strain.

Prebiotics – creating new niches for beneficial gut microbes

One strategy that has shown promise in promoting a healthier gut microbiome is the use of prebiotics. A prebiotic is 'a substrate that is selectively used by host microorganisms conferring a health benefit'.[96] Established prebiotics include inulin-type fructans (i.e. fructo-oligosaccharides, inulin and oligofructose), galacto-oligosaccharides and lactulose. Other fermentable carbohydrates that have shown prebiotic potential include resistant starch, β-glucans, arabinoxylan oligosaccharides, xylo-oligosaccharides, soy bean oligosaccharides, isomalto-oligosaccharides and pectin. Prebiotics are found naturally in foods (i.e. inulin is found in breads and cereals, onions, garlic and artichokes), are added to foods to increase their fibre content (i.e. inulin-containing yoghurts), or can be added to the diet in the form of powdered supplements. Prebiotics have the potential to create a new nutritional niche within the human GI tract, providing microbes with sufficient nutrients to establish residence. For example, inulin-type fructan prebiotics have been shown to increase the numbers of beneficial bacteria such as *Bifidobacterium* spp., *F. prausnitzii* and *Lactobacillus* spp.;[97,98] while reducing pathobiont (*E. coli*) adherence to epithelial cells.[99] Prebiotic interventions can also lead to the increased production of SCFA that possess many beneficial properties as noted above.[100,101] Prebiotics also regulate gut immunity, largely due to the indirect effects they have on gut microbiota and the metabolites they produce. However, prebiotics have also been shown to have direct effects on immune function *in vitro*, largely through their activation of innate immune pathways within DC and epithelial cells. As DC are constantly sampling the contents of the gut lumen, it has been proposed that prebiotics ligate the TLR found on the surface of DC[102] leading to a cascade of immune responses. They could also bind to TLR on epithelial cells, thereby modulating barrier function,[103] and signalling to adjacent immune cells (i.e. DC and intraepithelial lymphocytes).[104] As data suggesting that prebiotics directly influence gut immunity have only been acquired from *in vitro* studies, this topic requires further investigation.

Prebiotic intervention studies using murine models of colitis have shown promise in modulating gut microbiota and host immune response (Table 1). The most frequently studied prebiotics in these studies are inulin-type fructans, which have been shown to significantly reduce disease activity index,[105,106] histological and macroscopic damage scores,[107–112] pro-inflammatory cytokine production (e.g. IL-1β)[105,107,108] and neutrophil myeloperoxidase levels,[105,110–112] while increasing colon length/weight ratios.[106,111] In some studies, the amelioration of gut inflammation was accompanied by changes in gut microbiota composition and SCFA production with increases in microbial diversity,[109] *Bifidobacterium* spp.,[105,107–109] *Lactobacillus* spp.,[105,107,112] total SCFA[108] and butyrate concentrations[110] being observed. Notably, the effects of providing these prebiotics have not always been consistent, with some studies showing no changes in *Bifidobacterium* spp. concentrations[112] or SCFA production.[107,111,113] The heterogeneity in study results may reflect differences in prebiotic type, dose, intervention length, the colitis model used, and the microbial environment of the respective animal facility. The immunomodulatory capacity of other prebiotics, such as galacto-oligosaccharides and β-glucans, has also been investigated using experimental models of colitis; the effects that these and other prebiotics have on gut immunity and microbiota are summarized in Table 1.

Compared with the number of prebiotic studies performed in mice, far fewer intervention studies have been conducted in patients with IBD (Table 2). However, studies have demonstrated that prebiotics facilitate reduced disease activity scores (Crohn's disease activity index and Harvey Bradshaw index),[114–116] inflammation on biopsy,[117] pro-inflammatory cytokine production (e.g. TNF-α, IL-6 and IL-8),[115,117,118] faecal calprotectin[119] and myeloperoxidase,[120] and an increase in IL-10-positive CD11c$^+$ DC.[114,121] Conversely, some studies have demonstrated no change in IL-6- and IL-12-producing CD11c$^+$ DC[114] or disease activity indices.[119,121,122] Additionally, inconsistencies in the gut microbiota modulating effects of prebiotics have been observed in patients with IBD. Some studies have shown that prebiotics increase *Bifidobacterium* spp. concentrations in patients with IBD,[114,115,117] whereas others showed no effect on *Bifidobacterium* spp.[120,121] or *F. prausnitzii*.[116,121] Marked differences in prebiotic type, dose and intervention length, as well as the participant's disease severity, type and location, and characteristics such as age, gender, habitual dietary intakes and baseline gut microbiota composition, are likely to have contributed to the different effects observed between studies.

Although prebiotic interventions generally do not lead to adverse outcomes in patients with IBD, the totality of evidence does not suggest that prebiotics consistently improve IBD outcomes. It is possible that a personalized

approach to prebiotic interventions is required as it is likely that a universal approach may be responsible, in part, for the inconsistencies in results. Prebiotic interventions during IBD relapse show great promise. However, additional, well-designed, randomized controlled trials are required before this alternative, complementary treatment option can be routinely recommended. Future research is necessary to define the ideal prebiotic type/s, dose and administration regimen (i.e. in combination with a habitual or exclusion diet, and/or probiotics) for use in patients with IBD.

Other dietary interventions – vitamin D

Vitamin D, best known as the sunshine vitamin, is naturally present in a limited number of foods, such as fatty fish and eggs. It is also often provided as a supplement within milk and other common food products. In part based on dietary choices, as well as limited sun exposure due to an indoor lifestyle, use of sunscreen and/or geographic location, it is estimated that one-third of the healthy population in northern latitudes is vitamin D insufficient (30–75 nmol/L) or deficient (<30 nmol/L). Moreover, low vitamin D levels are among the most important nutrient deficiencies associated with the onset and progression of IBD. Roughly 68% of the patients with IBD have insufficient serum vitamin D levels, more than half of these patients are vitamin D deficient.[123] Vitamin D deficiency can result in increased IBD disease activity, as well as a higher chance of relapses and an increased risk of hospitalization and surgery during the course of disease. Moreover, vitamin D supplementation studies suggest that increasing vitamin D levels reduces disease activity while increasing the quality of life of patients with IBD (reviewed in ref. 123).

The beneficial effects of vitamin D appear to work through multiple mechanisms including regulating host gene transcription and modulating the gut microbiome. Vitamin D regulates a number of genes involved in maintaining intestinal homeostasis, including barrier function and antimicrobial peptide production. Several studies have also shown that vitamin D signalling can be a major determinant of an individual's gut microbiota composition, especially with regards to diversity. Compared with wild-type mice, the gut microbiota of vitamin D receptor (VDR) deficient mice show a loss of *Lactobacillus* spp. and increases in *Proteobacteria* spp. and *Bacteroidetes* spp.,[124] a composition that is markedly dysbiotic and similar to that in patients with IBD.[125] It is therefore not surprising that mice lacking VDR signalling or that are vitamin-D-deficient are highly susceptible to chemically or bacterially induced intestinal inflammation such as DSS colitis,[126] *Citrobacter rodentium*-induced colitis,[127,128] *S.* Typhimurium infections[129] and inflammation-associated malignancies, such as colon cancer.[130]

An example of how vitamin D signalling is pivotal for healthy interactions between the gut microbiota and intestinal immunity involves the SCFA butyrate. Studies have shown that butyrate increases the expression of VDR in epithelial cells *in vitro*, and unexpectedly, butyrate is also thought to bind directly to the VDR, thereby inducing the differentiation of Caco2 epithelial cells,[131] and down-regulating TNF-α production in HT-29 cells.[132] This implies that bacterially derived butyrate could be used to restore VDR-dependent transcriptional regulation, which is severely lacking in patients with IBD. Similar results have been obtained following administration of the probiotic strains *Lactobacillus rhamnosus* GG ATCC 53103 and *L. plantarum*, with these microbes increasing VDR protein levels in both mouse and human intestinal epithelial cells. Moreover, the role of probiotics in regulating VDR signalling was assessed *in vivo* using the *Salmonella*-induced colitis model.[133] Probiotic treatment reduced colitis manifestations in infected wild-type mice, whereas probiotics had no effect on $Vdr^{-/-}$ mice[133] suggesting a role for the VDR in probiotic efficacy. Since patients with IBD are often vitamin D insufficient (or deficient), these findings may clarify why probiotics often do not show beneficial effects in clinical trials. The ability of probiotics to inhibit intestinal inflammation and bacterial infection may depend on the VDR signalling pathway, so future studies should evaluate if there is a link between VDR expression and probiotic efficacy in a clinical setting.

Besides its actions on the gut microbiota, vitamin D also impacts host immunity, including immune cell differentiation, migration and anti-inflammatory functions. The immunomodulatory effects of vitamin D supplementation in patients with IBD are reviewed by Mohammed *et al.*[134] Human monocytes and macrophages treated with vitamin D show enhanced antimicrobial ability through improved autophagy and increased production of antimicrobial peptides, while also exhibiting reduced TLR-mediated inflammatory responses upon recognition of microbial products.[135] Additionally, vitamin D is involved in DC differentiation and maturation, resulting in a tolerogenic phenotype.[136] Treatment of human DC with the active form of vitamin D ($1,25(OH_2)D_3$) inhibits the production of the cytokines IL-12 and IL-23, which are responsible for driving Th1/Th17 responses, respectively. On the other hand, immunosuppressive IL-10 production by DC is enhanced following stimulation with active vitamin D, which results in DC developing a tolerogenic phenotype and subsequent induction of tolerogenic Treg cell immunity (increase of FOXP3 Treg cells and IL-10 secretion). Vitamin D also impacts adaptive immunity by inhibiting the proliferation of naive $CD4^+$ T cells as well as altering their functionality by suppressing the transcription of pro-inflammatory Th1 and Th17 cytokines like interferon-γ, TNF-α, IL-17 and IL-21. Vitamin D also

displays synergistic effects with antibodies targeting TNF-α in patients with IBD. Adequate levels of vitamin D before the start of therapy were shown to drastically increase the chance of patients reaching remission,[137] not only reducing the risk of the biological therapy failing, but also limiting the formation of antibodies that can neutralize the drug.[138]

Taken together, it appears clear that deficiency in vitamin D, or defects in its receptor can predispose to overt inflammatory responses, dysbiosis, and a worsened clinical outcome in patients with IBD. Moreover, vitamin D appears indispensable for maintaining intestinal mucosal homeostasis through its effects promoting intestinal barrier function, mucosal immunity, and subsequently shaping the intestinal microbiota. Vitamin D is clearly an important player in the emerging field of immunonutrition with exciting potential to improve IBD therapies and patient quality of life. Taken together, this suggests that an increased emphasis on treating vitamin D deficiency could help to reduce the onset and severity of IBD.[139] Hence supplementation strategies based on the severity of vitamin D deficiency in the patient should be incorporated into the treatment plan of IBD patients.[140]

Calorie restriction – taking fuel from the fire

Specific nutrients may be important in controlling IBD, but another major issue already mentioned is that excessive caloric intake on a daily basis creates metabolic pressure that negatively impacts whole body energy homeostasis, resulting in systemic inflammation.[51] This can give rise to various pathological conditions including insulin resistance, type 2 diabetes and cardiovascular disease.[141] Energy homeostasis, however, is not only important on an organismal level, but also and especially on a cellular level.[142] Perpetual activation of intracellular nutrient sensors leads to the activation of intracellular pro-inflammatory pathways. For example, continuous activation of biosynthetic pathways congests the protein folding capacity of the endoplasmic reticulum, thereby promoting the unfolded protein response, which can in turn induce the pro-inflammatory transcription factor nuclear factor-κB.[143] These mechanisms are not confined to a specific cell type, but it is in immune cells where activation of pro-inflammatory pathways due to nutrient excess has the most serious implications. It is now becoming increasingly clear that immune cells respond directly to differences in nutrient availability by modulating their effector functions. All major cell types of the immune system have been shown to undergo a switch in their metabolism dependent on the type of inflammatory stimulus and the availability of nutrients (mainly glucose), in their environment (reviewed in ref. 144,145).

Intracellular energy sensors determine cell fate through the integration of nutrient signals. One of the central intracellular energy sensors is the mechanistic target of rapamycin (mTOR). Primarily as part of the mTORC1 protein complex, mTOR regulates cellular energy metabolism, autophagy and proliferation as well as differentiation and effector functions of immune cells. For example, strong and incessant mTOR activation in concert with strong T-cell receptor binding favours Th1 and Th17 cell differentiation whereas low or oscillatory mTOR activation with weak T-cell receptor binding leads to increased Treg cell differentiation. Hence, if caloric intake is excessive, overactive mTOR signalling leaves fewer opportunities for Treg cell differentiation.[146] This, together with many other immunomodulatory effects of a high calorie diet, can lead to an imbalance between pro-inflammatory and anti-inflammatory cell types with chronic inflammation as a result. This imbalance is characteristic of patients with IBD, who have been shown to have more circulating Th17 and fewer Treg cells compared with healthy controls.[147]

Based on what is known about the impact of cellular metabolism on immune function, targeting mTOR could be a promising strategy to re-establish immunological homeostasis. Several pharmacological mTOR inhibitors exist, i.e. rapamycin and its analogues or metformin.[148] In a DSS model of colitis, treatment with an mTOR inhibitor alleviated intestinal inflammation by reducing the number of Th1 and Th17 cells and increasing the number of Treg cells in the lamina propria.[149] Furthermore, rapamycin has been successfully used in a paediatric population with refractory IBD.[150] However, these drugs have severe adverse effects.[151] Therefore, it is worth noting that one of the most natural and potent suppressors of mTOR is nutrient scarcity. Indeed, periods of fasting or caloric restriction decrease mTOR activity, with the potential to create a more anti-inflammatory environment.[54,152]

Different types of caloric restriction exist, including intermittent or every-other-day fasting and time-restricted feeding. Yet, the most likely to prove beneficial is a prolonged fast with minimal caloric consumption or a calorically restricted diet containing nutrients that mimic fasting (also known as a fasting mimicking diet). During a prolonged fast, an organism undergoes a metabolic switch from mainly carbohydrate to predominantly (stored) fat utilization. This changes the metabolism of most cells, from using glucose as a central nutrient, to instead using ketone bodies, the most abundant of which is β-hydroxybutyrate (β-HB).[153] In an animal model of multiple sclerosis, three cycles of a 3-day fasting mimicking diet were found to not only induce the apoptosis of autoimmune Th1 and Th17 effector T cells, but also to promote the expansion of the Treg cell population as well as the oligodendrocyte precursor pool. This supports the concept that caloric restriction is able to shift the immune environment from pro- to anti-inflammatory cell types.[154] Interestingly, mTOR suppression is also one of

the mechanisms of action of the anti-inflammatory cytokine IL-10. Interleukin-10 achieves mTOR suppression via DNA damage inducible transcript 4 (DDIT4) activation. Through this axis, IL-10 can metabolically reprogramme inflammatory macrophages towards a more anti-inflammatory phenotype.[155] Intriguingly, prolonged fasting has also been shown to dramatically increase DDIT4 expression,[156] indicating another possible link for fasting-mediated immunomodulation.

Additionally, the fasting-induced ketone body β-HB exhibits a host of beneficial effects, including anti-inflammatory properties. For example, β-HB inhibits NLRP3 inflammasome activation in response to danger-associated molecular patterns.[157] This reduces the release of the pro-inflammatory cytokine IL-1β both *in vitro* and *in vivo*, which may in part be facilitated through SIRT3-mediated activation of superoxide dismutase 2.[158] β-HB also acts as a histone deacetylase inhibitor, so promoting histone hyperacetylation and the subsequent induction of stress response genes, such as the transcription factor forkhead box O3 (FOXO3).[159] Moreover, β-HB up-regulates autophagy, which can be defective in patients with IBD, through FOXO3-mediated regulation of important autophagy genes such as *ATG4*, *ATG5* or *LC3* but also by indirectly inhibiting mTOR.[160,161] Moreover, fasting is known to change the composition of the gut microbiome in mice and most likely in humans. Although it still needs to be determined on a larger scale how fasting modulates the intestinal microbiome, isolated studies have shown an increase in *F. prausnitzii* and *Akkermansia mucinophilia*, both of which have been characterized as reflective of an anti-inflammatory intestinal environment.[162]

Taken together, it seems likely that any substantial reduction in calories will dampen inflammation. Hence, interventions that reduce caloric intake could prove a beneficial complementary treatment for autoimmune and auto-inflammatory diseases such as IBD, where the immune system is chronically activated. As suppression of immunity could however impair protective responses to pathogens, more research is needed to establish if prolonged periods of fasting or caloric restriction have any negative effects on susceptibility or clearance of infectious organisms. It is also important to assess each patient's nutritional status before recommending a prolonged fast. Malnourished patients as well as children with intestinal diseases who are at risk of developing an inadequate nutritional status that would negatively impact their growth and development should not be fasted. Clinical studies should determine which types of caloric restriction are the safest and promise the best clinical outcomes.

Conclusions

Current IBD therapies focus on suppressing immune responses and restoring intestinal barriers, but we propose that nutritional and microbiological interventions that are able to protect the gut against damaging inflammation offer the next source of IBD therapies. Elucidating the impact of nutrient intake on gut immunity, whether through direct effects on host immune cells or indirect effects on the gut microbiome, will provide us with critical information essential to promote gut health. Moreover, this understudied and underexploited area of research provides a tractable system that can be used to reduce damaging intestinal inflammation. Herein, we describe current paradigms as well as recent advances demonstrating that nutritional interventions, including modification of the gut microbiome by prebiotics and probiotics, as well as caloric restriction and supplementation with vitamin D, can modulate the mucosal immune response and suppress the damaging inflammation that characterizes IBD.

Disclosures

The authors declare no competing interests.

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