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REVIEW

Nutritional and Probiotic Supplementation in Colitis Models

Radu M. Nanau · Manuela G. Neuman

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Abstract In vitro and animals models have long been used to study human diseases and identify novel therapeutic approaches that can be applied to combat these conditions. Ulcerative colitis and Crohn's disease are the two main entities of inflammatory bowel disease (IBD). There is an intricate relationship between IBD features in human patients, in vitro and animal colitis models, mechanisms and possible therapeutic approaches in these models, and strategies that can be extrapolated and applied in humans. Malnutrition, particularly protein-energy malnutrition and vitamin and micronutrient deficiencies, as well as dysregulation of the intestinal microbiota, are common features of IBD. Based on these observations, dietary supplementation with essential nutrients known to be in short supply in the diet in IBD patients and with other molecules believed to provide beneficial anti-inflammatory effects, as well as with probiotic organisms that stimulate immune functions and resistance to infection has been tested in colitis models. Here we review current knowledge on nutritional and probiotic supplementation in in vitro and animal colitis models. While some of these strategies require further fine-tuning before they can be applied in human IBD patients, their intended purpose is to prevent, delay or treat disease symptoms in a non-pharmaceutical manner.

Keywords Inflammatory bowel disease · Colitis models · Nutrition supplementation · Probiotic supplementation

R. M. Nanau · M. G. Neuman
Department of Pharmacology and Toxicology, Institute of Drug Research, Faculty of Medicine, University of Toronto, Toronto, ON, Canada

R. M. Nanau · M. G. Neuman (✉)
In Vitro Drug Safety and Biotechnology, MaRS Discovery District, 101 College Street, Suite 300, Lab 351, Toronto, ON M5G 1L7, Canada
e-mail: manuela.neuman@utoronto.ca
URL: http://www.marsdd.com/tenantdirectory/invitro.html

Introduction

Inflammatory bowel disease (IBD) is an idiopathic chronic condition of the gastrointestinal (GI) tract characterized by intermittent periods of inflammation and remission [1–3]. The identification of timely treatment and the possible prevention of complications are critical for patients undergoing early clinical relapse. There are currently no sub-clinical markers that predict relapse during states of remission [4]. Genetic approaches have the potential to be greatly effective against ulcerative colitis (UC) and Crohn's disease (CD), the most common entities of IBD [5, 6]. Single nucleotide polymorphisms in IBD-related genes (reviewed by Neuman and Nanau [7]), as well as a number of biomarkers (reviewed by Nanau and Neuman [3]), can be used to predict both early disease development and complications in terms of disease phenotype and behavior. For example, CD patients with nucleotide-binding oligomerization domain-containing protein 2 (NOD2) mutations exhibit early onset of disease, with mainly ileal involvement and increased risk of surgical intervention after developing complications such as strictures, fistulas and stenosis [8]. Disease phenotype and location can predict a disabling syndrome. Young age, smoking habits, perianal lesions and severe ulcerations are clinical risk factors for disease progression [9].

However, a more desirable strategy would be to prevent disease symptoms altogether or at least treat them in a

non-pharmaceutical manner. Cell cultures and animal models are frequently used to better understand the mechanisms that underlie the pathogenesis of human diseases. Here, we review recent knowledge on nutritional and probiotic supplementation in cell cultures and animal models of colitis.

Our group has recently reviewed nutritional and probiotic supplementation currently used in humans [10]. In vitro and in vivo animal studies can uncover novel mechanisms of disease progression, revealing molecular targets that can be exploited by novel therapeutic approaches. These approaches allow the use of biotherapeutics in the treatment of acute and chronic GI disorders [11].

Human IBD

Colitis models attempt to replicate common clinical, pathological and laboratory features of IBD observed in human patients. Weight loss, diarrhea accompanied by blood and abdominal pain are common symptoms of IBD [12]. Inflammation is brought about by an uncontrolled immune response in the intestinal lumen [13]. UC is characterized by localized inflammation of the superficial layer of the colon mucosa and defective regulation of tight junctions, whereas CD is characterized by discontinuous, transmural lesions of the gut wall, particularly in the lower ileum and the colon [3, 12, 13]. Goblet cells control mucus production, while Paneth cells control the secretion of defensins, and both are deregulated in the course of IBD [10]. The intestinal lamina propria contains immune cells that balance immune tolerance to the normal intestinal microbiota with defenses against microbial pathogens [10]. IBD is further characterized by inflammatory infiltrates of mast cells, lymphocytes, macrophages and activated neutrophils [13]. A strong T helper (Th) 1 response mediated by pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ), is characteristic of CD, while UC is associated with a Th2 response with high levels of Th17 cells [3, 10]. Most IBD-related genes are involved in immune responses, transport or bacterial recognition [7, 10].

Efforts have been made to predict disease behavior over time and the response to treatment in CD. There are multiple pharmaceuticals and biological therapies in use. The current paradigm for colitis treatment is either to combine several monotargeted drugs or to design drugs that modulate multiple targets. As a result, pharmaceutical companies have been increasingly interested in developing multitargeted therapies. Many dietary agents and probiotics have multitargeting properties. In addition, these products are less expensive, safer and more readily available than synthetic agents.

The aim of this review is to investigate the therapeutic potential of specific nutrient supplements and probiotics in in vitro cell cultures and in vivo animal colitis models.

Colitis Models

A wide range of nutrients [14–39] and probiotics [40–77] that can be supplemented into the diet are presented in Tables 1 and 2, respectively. Before being introduced in humans, the safety profile and therapeutic potential of these food components are tested in animal colitis models.

In order to fully understand the potential benefits of nutritional and probiotic supplements, general features of the colitis models used in these experiments must be presented first. This section describes in vitro cell cultures and animal models employed to mimic the mechanism of colitis.

Some of the cell lines used to test potential IBD treatments include the murine macrophage-like cell line RAW 264.7, the human T cell line Kit 225, the human epithelial cell line Caco-2 and the human colon cell lines HT-29 and T84. In terms of animal models, several mouse, rat and piglet colitis models are used (Tables 1 and 2).

One of the most commonly used animal colitis models is the chemically induced dextran sodium sulfate (DSS) model. DSS colitis is characterized by growth retardation and reduced colon length [18, 24, 25], as well as by inflamed colonic mucosa, with massive leukocyte infiltration [72]. Increased myeloperoxidase (MPO) activity is an indicator of granulocytic infiltration in DSS colitis [34, 52]. Some common histological changes include ulcers and areas of severe tissue erosion, primarily in the distal colon and rectal mucosa, focal erosions, crypt dilatation and heavy inflammatory cell infiltration of the mucosa [18, 23]. DSS treatment is further characterized by a Th1 response, with increases in the mRNA expression of the pro-inflammatory cytokines TNF-α, interleukin (IL)-1β, IL-2, IL-6, IL-12, IFN-γ and osteopontin (OPN), the chemokines macrophage inflammatory protein (MIP)-2 and monocyte chemotactic protein (MCP)-1, the cell adhesion molecule (CAM) intercellular adhesion molecule (ICAM)-1 and the enzymes cyclooxygenase (COX)-2, inducible nitric oxide synthase (iNOS), serum amyloid A and matrix metalloproteinase-9, as well as by decreased expression of some biochemical markers of the epithelial integrity, including mucin (MUC)3 and zonula occludens (ZO) 1 [18, 37, 43, 58]. On the other hand, DSS colitis is associated with enhanced MUC1 expression [39]. DSS treatment increases mucosal epithelial permeability by disrupting epithelial tight junctions [15]. Furthermore, DSS colitis is associated with depletion of hepatic glutathione levels [18], while plasma leukotriene B4 levels are enhanced [25].

Table 1 Nutritional supplementation

Nutrient supplemented	Colitis model	Clinical and pathological findings	Laboratory findings	Reference no.
Dietary calcium	HLA-B27/β2-microglobulin rat	Protects against diarrhea Lower intestinal permeability Lower levels of circulation luminal antigens, particularly LPS	Lower inflammation scores and colonic mucosal expression of IL-1β No effect on colonic MPO expression Lower expression of genes involved in ECM remodeling, the immune system and COX-2 Up-regulates genes important for the mucosal barrier, such as MUC2 and trefoil factors 1 and 3 in colonic mucosa Down-regulates mRNA expression of MMP- 9, 10 and 13, procollagen type V α 2 and fibronectin 1	[14]
1,25-dihydroxyvitamin D3	VRD −/− DSS mouse		Increases levels of tight junction proteins ZO-1, claudin-1, claudin-2 Increases levels of adherens junction protein E-cadherin Little effect on claudin-5 and occludin	[15]
Iron	TNFΔARE/WT mouse		Up-regulates proteins involved in catabolic processes and ATP-binding Up-regulates proteins involved in oxidative stress such as superoxide dismutase or peroxiredoxin 1 Down-regulates antioxidative proteins such as catalase	[16]
Calcium and antioxidants	HLA-B27/β2-microglobulin rat		Lower MPO activity and IL-1β concentration	[17]
Cysteine prodrugs 2-(RS)-*n*-propylthiazolidine-4(R)-carboxylic-acid and D-ribose-L-cysteine L-cysteine-glutathione mixed sulfide	DSS mouse	Reduces colon shortening Improves colonic lesions	Normalizes hepatic glutathione levels Attenuates CD68, COX-2, IL-12, TNF-α and OPN up-regulation Attenuates serum amyloid A expression	[18]
L-cysteine	DSS piglet	Increases weight gain Restores epithelial barrier integrity Reduces inflammatory cell infiltration and crypt damage	Lower MPO activity Lower IL-1β, IL-6, IL-8, TNF-α, IFN-γ, IL-12p40, MCP-1, MIP-1α and MIP-2 expression Up-regulates IL-10 expression Restores pro-apoptotic pathways	[19]
Milk fat globule-epidermal growth factor 8	DSS mouse	Reduces weight loss Decreases lamina propria infiltration by mononuclear cells Decreases crypt epithelial damage	Decreases IL-1β and TNF-α expression	[20]

Table 1 continued

Nutrient supplemented	Colitis model	Clinical and pathological findings	Laboratory findings	Reference no.
Full-length murine adiponectin gene Osmotin	DSS mouse	Milder signs of colitis Increases crypt proliferative activity consistent with normal epithelial turnover	Decreases TNF-α, IL-1β, MCP, OPN, angiotensin, angiotensin converting enzyme, angiotensin receptor 1, and cellular stress and apoptosis markers Up-regulates IL-10, alternative macrophage marker, arginase 1 and leukoprotease inhibitor	[21]
Arachidonic acid Eicosapentanoic acid	Caco-2 cells		Higher ICAM-1 levels, NF-κB activation, PGE2 production, and MCP-1 and angiogenin expression Higher IL-10, IL-6, MIP-1d and growth regulated protein expression	[22]
ω-3 PUFA	DSS rat	Ameliorates macroscopic colonic lesions and epithelial erosion Little change in colitis injury scores	Increases MPO expression and activity in the inflammatory cell infiltrate	[23]
Conjugated linoleic acid Fish oil	DSS piglet		Up-regulates colonic PPAR-γ, PPAR-γ-responsive gene PPAR-γ co-activator 1-α and keratinocyte growth factor mRNA Decreases TNF-α up-regulation Up-regulates colonic PPAR-δ-responsive gene UCP3 and keratinocyte growth factor mRNA	[24]
Fish oil and curcumin	DSS mouse	Increases injury scores Decreases inflammation	Decreases NF-κB expression	[25]
Polydextrose	TNBS rat	Reduces colon damage Reduces colon weight/length ratio	Maintains colonic glutathione Reduces MPO activity	[26]
Plant-derived polysaccharide supplements	DSS rat	Dose-dependent lower inflammation scores Reduces colon shortening No effect on body weight	Decreases monocyte count No effects on cholesterol and triglycerides	[27]
Di-D-fructose dianhydrides	TNBS rat	Lower colonic damage score Reduces colonic necrosis and inflammation Reduces colon weight/length ratio Recovers intestinal architecture Partially restores the epithelial cell layer Less severe goblet cell depletion, with replenished mucin content	Reduces colonic MPO activity Partial recovery of glutathione depletion Lower TNF-α and IL-1β up-regulation Lower iNOS expression Reverses lactobacilli and bifidiobacteria loss Reverses SCFA (acetate, propionate and butyrate) loss	[28]

Table 1 continued

Nutrient supplemented	Colitis model	Clinical and pathological findings	Laboratory findings	Reference no.
Fructooligosaccharides and resistant starch	TNBS rat	Lower colonic damage Lower colonic necrosis and inflammation	Higher trefoil factor-3 and MUC-2 levels Reduced MPO activity Decreased TNF-α and IL-1β production Lower iNOS expression Decreases MCP-1, CINC-1 and ICAM-1 up-regulation Increases the proportion of lactobacilli and bifidiobacteria	[29]
Low-digestible carbohydrate (4 % nutriose)	TNBS piglet	Prevents weight loss	Reduces circulating TNF-α, IL-12 and IL-1β levels Reduces colonic TNF-α, IL-12 and IL-1β up-regulation Reduces colonic MPO activity Increases colonic IL-10 levels	[30]
Enzyme-treated rice fiber (heat-resistant amylase, protease and hemicellulase)	DSS mouse DSS rat Adoptive transfer mouse	Reduces colon shortening Milder mucosal damage Reduces histological mucosal damage score and relative spleen weight	Reduces IL-6 level Decreases serum pro-inflammatory TNF-α, IFN-γ, IL-1β, IL-6 and IL-12p70 and anti-inflammatory IL-4 levels Increases SCFA (acetate and butyrate) Attenuates T cell activation and maturation Decreases mucosal IFN-γ, IL-6 and IL-4 No change in mucosal TNF-α	[31]
Fresh pineapple juice	IL-10 −/− mouse	Decreases histologic colon inflammation scores		[32]
Ethyl acetate extracts of kiwifruit	IL-10 −/− intestinal epithelial cells RAW 264.7 cells		Reduces nitric oxide, IL-6, TNF-α and IL-10 secretion in LPS-stimulated cells	[33]
Aloin, aloesin and aloe-gel	DSS rat	Reduces colon shortening Reduces erosive lesions, inflammatory cell infiltration and crypt shortening	Lower MPO activity Lower plasma TNF-α, PGE2 and LTB4 levels Lower colonic IL-1β and TNF-α mRNA levels	[34]
Enzymatic hydrolysate of corn gluten	TNBS rat	Decreases extent and severity of injury	Lower MPO activity	[35]
Abscisic acid	DSS mouse	Reduces colon inflammation, epithelial erosion, mucosal thickening and leukocyte infiltration	Decreases VCAM-1, MAdCAM-1, E-selectin, IL-6, iNOS and MMP-9 expression No effects on ICAM-1 and PECAM-1	[36]
Astaxanthin	DSS mouse	Dose- and time-dependent decrease in incidence of mucosal ulceration Dose-dependent decrease in colonic inflammation	Dose-dependent decrease in TNF-α, IL-1β, NF-κB, COX-2 and iNOS expression	[37]

Table 1 continued

Nutrient supplemented	Colitis model	Clinical and pathological findings	Laboratory findings	Reference no.
Resveratrol	DSS mouse	Reduces weight loss, diarrhea and rectal bleeding	Lower TNF-α and IL-1β up-regulation	[38]
			Increases IL-10 levels	
		Lower DAI	Reduces iNOS activity	
		Lower macroscopic inflammatory score	Lower p38 MAPK protein activation	
		Reduces histological signs of cell damage and regeneration of crypts		
Soy protein	DSS mouse	Reduces colon shortening	Decreases colonic MUC1 up-regulation	[39]
		Lower inflammation scores	Increases colonic MUC2 expression	
			No effect of colonic MUC3 and MUC4 expression	
			Decreases colonic TNF-α up-regulation	

CINC Cytokine-induced neutrophil chemoattractant, *COX* cyclooxygenase, *DAI* disease activity index, *DSS* dextran sodium sulfate, *ECM* extracellular matrix, *ICAM* intercellular adhesion molecule, *IFN* interferon, *IL* interleukin, *iNOS* inducible nitric oxide synthase, *LPS* lipopolysaccharide, LTB_4 leukotriene B4, *MAdCAM* mucosal vascular addressin cell adhesion molecule, *MAPK* mitogen-activated protein kinase, *MCP* monocyte chemotactic protein, *MIP* macrophage inflammatory protein, *MMP* matrix metalloproteinase, *MPO* myeloperoxidase, *MUC* mucin, *NF* nuclear factor, *OPN* osteopontin, *PECAM* platelet endothelial cell adhesion molecule, PGE_2 prostaglandin E2, *PPAR* peroxisome proliferator-activated receptor, *PUFA* polyunsaturated fatty acid, *SCFA* short-chain fatty acid, *TNBS* trinitrobenzene sulfonic acid, *TNF* tumor necrosis factor, *VCAM* vascular cell adhesion molecule, *VDR* vitamin D receptor, *ZO* zonula occludens

Certain variations of this model are possible using gene deletion or knockout (-/-) animals rather than wild-type animals. The peroxisome proliferator-activated receptors (PPAR) are nuclear receptors expressed in the colon. The PPAR-γ deletion model is particularly sensitive to low doses of DSS. In this model, PPAR-γ fl/fl; CD4-Cre$^+$ animals or PPAR-γ flfl; Villin-Cre$^+$ animals express a transgenic recombinase under the control of a tissue-specific CD4-Cre and Villin-Cre promoter, respectively, in which the PPAR-γ gene is deleted from both CD4$^+$ and CD8$^+$ T cells or intestinal epithelial cells, respectively [78, 79].

DSS colitis is more severe in animals with targeted disruptions in the immunoglobulin A (IgA) gene (IgA −/−) or the polymeric immunoglobulin receptor (pIgR) gene (pIgR −/−). Both IgA and pIgR are important in maintaining epithelial integrity and mucosal homeostasis [80].

Another chemically induced colitis model uses trinitrobenzene sulfonic acid (TNBS). TNBS colitis is characterized by weight loss, severe diarrhea, rectal bleeding, severe mucosal necrosis, macroscopic inflammation and histological and biochemical intestinal changes, including increased MPO activity [26, 35, 48, 73]. Histological lesions are prominent in TNBS-treated animals, with epithelial erosions and ulcerations of the submucosa and with areas of necrosis extending to the muscular layers [74]. TNBS-induced colonic damage is proportional to the colitis-associated weight loss and the degree of colonic inflammation [57]. Severe transmural disruption of the normal architecture of the colon and extensive ulceration and inflammation involving all intestinal layers of the colon are observed in TNBS colitis, with crypt hyperplasia and dilation, and moderate to severe goblet cell depletion [57]. TNBS colitis is also characterized by enhanced TNF-α and IL-1β production, high iNOS expression and IL-17 up-regulation, as well as increased expression of chemokines MCP-1, cytokine-induced neutrophil chemoattractant-1 (CINC-1) and ICAM-1 [28, 29]. Impaired trefoil factor-3 expression is also common. A decreased ratio between beneficial bacteria (lactobacilli and bifidobacteria) and potential pathogens (total aerobic bacteria and enterobacteria) has been observed, along with reduced richness and biodiversity of the luminal microbiota [28, 29, 81]. Alternatively, dinitrobenzene sulfonic acid (DNBS) has been used, with similar effects [42]. It is useful to note that dietary supplementation needs to be provided prior to TNBS treatment in order to attain protection from colitis [48, 81, 82].

Other chemically induced colitis models include iodoacetamide [44] and acetic acid [55]. The acetic acid colitis model is associated with oxidative damage and reduced antioxidant enzyme activities, while high levels of malondialdehyde indicate lipid peroxidation [55].

Table 2 Probiotic supplementation

Nutrient supplemented	Colitis model	Clinical and pathological findings	Laboratory findings	Reference number
Escherichia coli Nissle 1917	*Escherichia coli* E2348/68-infected polarized T84 cells	Enhances transepithelial resistance	Recovers baseline ZO-2 mRNA expression	[40]
Escherichia coli Nissle 1917	DSS mouse	Lower DAI Reduces mucosal damage Facilitates mucosal repair	Reduces MPO activity Decreases TNF-α, IFN-γ, MCP-1 and IL-10 levels	[41]
Escherichia coli Nissle 1917	DSS mouse	Reduces weight loss Reduces colonic epithelial permeability	Increases ZO-2 mRNA expression	[42]
Escherichia coli Nissle 1917	DSS mouse	Reduces weight loss Reduces colon shortening Improves recovery of colonic tissue Recovers mucin production	Reduces IL-1β, IL-2, MIP-2, MCP-1, ICAM-1 and iNOS up-regulation Recovers ZO-1 expression No change in MMP-9 expression Increases beneficial/potential pathogen bacteria ratio	[43]
VSL#3 *Lactobacillus* GG	Iodoacetamide rat	Reduces colonic lesions Preserves mucosa without ulceration Minimal inflammatory infiltrate in submucosa	Reduces MPO and iNOS activity Reduces PGE2 generation	[44]
VSL#3	Enriched blood dendritic cells		Up-regulates IL-10 Down-regulates IL-12 Decreases Th1 response, with lower IFN-γ production	[45]
VSL#3	DSS mouse	Time-dependent lower DAI Reduces colon shortening Reduces inflammation No effect on crypt damage score Reduces colonic epithelial permeability	Prevented occludin, ZO-1, claudin-1, claudin-3 and claudin-5 loss from tight junctions Lower apoptosis of colonic epithelial cells Prevents increases in *Enterococcus* species Increases incidence of streptococci and lactobacilli	[46]

Table 2 continued

Nutrient supplemented	Colitis model	Clinical and pathological findings	Laboratory findings	Reference number
VSL#3	IL-10 −/− mouse	Reduces cecal inflammation	Up-regulates expression of PPAR-α and the transcription cofactor PPARGC1-α, important in NF-κB control Up-regulates pathways associated with lipid, nitrogen, amino acid, and xenobiotic metabolism Down-regulates T cell, B cell and TLR signaling Down-regulates expression of Th1 transcription factors (TNF-α and TNF-α-induced chemokines such as CCL5, CXCL9 and CXCL10)	[47]
VSL#3	TNBS mouse	Protects against weight loss and diarrhea Reduces macroscopic scores of colitis Attenuates colon neutrophils infiltration	Attenuates colonic expression of inflammatory and immune mediators including IL-6, TNF-α, IL-1β and IFN-γ No change in mRNA levels of IL-10 and TGF-β Reverses down-regulation of nuclear receptors PPAR-γ, pregnane X receptor and farnesoid X receptor Induces expression of leptin and adiponectin mRNAs in mesenteric fat	[48]
Lactobacillus acidophilus A4	HT-29 cells (MUC2 expression suppressed)		Increases MUC2 secretion Induces TNF-α, IL-8 and IL-1β mRNA levels Lower attachment capabilities of *Escherichia coli* O157:H7	[49]
Heat-killed *Lactobacillus brevis* SBC8803	DSS mouse	Inhibits small intestinal mucosal permeability caused by oxidant exposure Time-dependent reduction in weight loss and intestinal shortening Decreases histological severity of intestinal inflammation	Induces intestinal Hsp 27/25, Hsp 70 and p38 MAPK activation Decreases TNF-α, IL-1β and IL-12 mRNA No effect on IL-4, IL-6, IL-10 and IL-17	[50]
Lactobacillus bulgaricus OLL1181	DSS mouse		Reduces colonic TNF-α and MPO up-regulation	[51]

Table 2 continued

Nutrient supplemented	Colitis model	Clinical and pathological findings	Laboratory findings	Reference number
Recombinant *Lactobacillus casei* secreting α-melanocyte-stimulating hormone	DSS mouse	Protects against weight loss Increases survival	Reduces MPO activity Reduces IL-1β, IL-6 and TNF-α up-regulation Reduces IκB-α activation Increases IL-10 and IL-4 expression	[52]
Lactobacillus casei GG	Caco-2 cells	Dose-dependent decrease in *Escherichia coli* C25 translocation	Increases MUC2 mRNA expression	[53]
Lactobacillus casei Shirota	DSS mouse	Represses inflammatory cells infiltration		[54]
Lactobacillus delbrueckii subsp. *bulgaricus* B3 strain (high exopolysaccharide) *Lactobacillus delbrueckii* subsp. *bulgaricus* A13 strain (low exopolysaccharide)	Acetic acid rat	Increases antioxidant activities		[55]
Lactobacillus fermentum CECT 5716	TNBS mouse	Reduces weight loss Recovers body weight earlier Reduces colon weight/length ratio Lower histological damage score Lower neutrophil and mononuclear cell infiltrate Less severe mucosal ulceration	Reduces MPO activity Decreases LTB4 production	[56]
Lactobacillus fermentum 5716	TNBS rat	Lower colonic damage score Reduces colon weight/length ratio Recovers intestinal architecture Restores the epithelial cell layer Recovers goblet cells, with replenished mucus content Reduces inflammatory infiltrate Reduces colonic MPO activity	Increases colonic glutathione levels Reduces colonic TNF-α levels and iNOS expression No change in colonic LTB4 levels Increases counts of lactobacilli species No change in *Bifidobacteria* counts Increases SCFA (acetate, butyrate and propionate) production	[57]
Lactococcus lactis subsp. *cremoris* FC	DSS mouse	No effect on weight loss Reduces colon shortening Lower histological scores for inflammation severity, thickness of inflammatory cell infiltration and extent of lesions	Suppresses TNF-α and IFN-γ mRNA expression	[58]

Table 2 continued

Nutrient supplemented	Colitis model	Clinical and pathological findings	Laboratory findings	Reference number
Lactobacillus paracasei	Adoptive transfer mouse	Protects against weight loss Lower neutrophil and mononuclear cell infiltrates	Lower colonic MPO, IL-1β, IL-6, IL-12, IL23, IFN-γ and TNF-α levels	[59]
Lactobacillus plantarum	IL-10 −/− mouse	Reduces mucosal ulceration, epithelial hyperplasia and mononuclear and neutrophilic infiltrate into the lamina propria	Reduces submucosal and lamina propria MAdCAM-2 and ICAM-1 expression No changes in IFN-γ and TNF-α levels Lower CD3, $\alpha 4\beta 7$, ICAM-1 and MAdCAM-1 mRNA levels Reduces colonic CD3, ICAM-1 and MAdCAM-1 up-regulation	[60]
Lactobacillus reuteri	DSS rat	Lower DAI Lower mucosal damage score	Prevents P-selectin up-regulation in mucosa and submucosa/muscularis Prevents the increase in leukocyte rolling and adherence in mucosal and submucosal venules Prevents the increase in platelet-endothelial interactions in both the mucosal and submucosal colonic venules	[61]
Lactobacillus rhamnosus Ls32	TNBS mouse	Reduces weight loss Improves clinical parameters Reduces macroscopic inflammation scores Reduced colonic inflammation	Reduces TNF-α, IL-6, IL-1β, IL-12, IL-23, IL-17, COX-2, IL-10 and IFN-β up-regulation Decreases MPO activity	[62]
Lactobacillus rhamnosus OLL2838	DSS mouse	Reduces weight loss Reduces colon shortening Reverses increases in mucosal permeability	Increases ZO-1 and myosin light-chain kinase expression	[63]
Lactobacillus salivarius Ls33	TNBS mouse	Reduces weight loss	Reduces colonic MPO activity Reduces IL-1β, IL-6 and TNF-α up-regulation Increased IL-10 production	[64]

Table 2 continued

Nutrient supplemented	Colitis model	Clinical and pathological findings	Laboratory findings	Reference number
Lactobacillus plantarum AK8-4 and *Bifidobacterium longum* HY8004	TNBS mouse	Reduces colon shortening Less severe inflammation	Reduces MPO activity Reduces INF-γ and TNF-α up-regulation Inhibits NF-κB activation and TLR-4 expression Reduces the degree of chondroitin sulfate and hyaluronic acid degradation Reduces tryptophanase and β-glucuronidase activities	[65]
Lactobacillus acidophilus and *Bifidobacterium longum*	TNBS mouse	Reduces weight loss Prevents morphologic alterations Decreases CD4+ subpopulation Increases T cell receptor $\gamma\delta$ + subpopulation Decreases $\gamma\delta$ T cells percentage	Decreases IL-12, IFN-γ, TNF-α and MCP-1 up-regulation No effect on IL-6 Increases IL-10 levels	[66]
Lactobacillus plantarum, *Streptococcus thermophilus* and *Bifidobacterium animalis* subsp. *lactis*	TNBS mouse	Reduces weight loss Prevent morphologic alterations	Decreases TNF-α and MCP-1 up-regulation No effect on IL-6 Increases IL-10 levels	
Bifidobacterium bifidum BGN4	Adoptive transfer mouse	Protects against weight loss Normal intestinal gross architecture Lower total number of isolated lymphocytes in the spleen and large intestine Ameliorates goblet cells loss	Reduces levels of Th1 cytokines TNF-α and INF-γ No change in Th2 cytokines IL-4, IL-5 and IL-10	[67]
Bifidobacterium bifidum S17	Adoptive transfer mouse TNBS mouse	Protects against weight loss Normal colon wall appearance Normal colon weight/length ratio Reduces inflammation Improves inflammatory score		[68]
Bifidobacterium bifidum S17	TNBS mouse	Partially inhibits weight loss Improves histological scores	Decreases TNF-α, IL-1β, IL-6, IL-12 and IL-10 up-regulation Decreases keratinocyte-derived chemokine levels Decreases COX-2 and MPO activity Impairs recruitment and activation of neutrophils	[69]

Table 2 continued

Nutrient supplemented	Colitis model	Clinical and pathological findings	Laboratory findings	Reference number
Bifidobacterium lactis	Adoptive transfer mouse	Delays and reduces weight loss Lower colitis scores (attenuates mucosal thickening and reduces epithelial hyper-proliferation)	Reduces colonic INF-γ, TNF-α and IL-6 up-regulation Reduces STAT-3 and p38 phosphorylation Reduces CD40L and OX40/OX40L induction	[70]
Bifidobacterium lactis	Caco-2 cells	Dose-dependent protection against increased epithelial cell permeability Improves tight junctions appearance		[71]
Bacillus polyfermenticus	DSS mouse TNBS mouse	Increases survival rate Protects against weight loss Reduces leukocyte infiltration in the colon	Suppresses CXCL-1, TNF-α and ICAM-1 up-regulation Enhances IL-10 expression Reduced number of apoptosis-positive cells	[72]
Bacillus subtilis PB6	TNBS rat	Reduces diarrhea and weight loss Improves colonic macroscopic scores, with no visible inflammation and mucosal injury	Lower plasma TNF-α, IL-1β, IL-6 and INF-γ levels Increases IL-10 levels No change in TGF-β levels	[73]
Oenococcus oeni IOEB 9115	TNBS mouse	Reduces colon shortening Reduces macroscopic inflammation Lower colonic damage Decreases depletion of goblet cells Decreases loss of mucosal architecture	Reduces MPO activity	[74]
Clostridium tyrobutyricum (butyrate)	DSS mouse	Lower histological damage Prevents colon shortening in SCID mice only Prevents infiltration of inflammatory cells in lamina propria	Reduces intracolonic IL-18 content in immunocompetent mice Enhances colonic IL-18 expression in SCID mice Decreased colonic TNF-α up-regulation in SCID mice Attenuates loss of colonic mucin production, with MUC2 production unaltered vs. unsupplemented conditions in the absence of DSS exposure Preserves ZO-1 production	[75]

Table 2 continued

Nutrient supplemented	Colitis model	Clinical and pathological findings	Laboratory findings	Reference number
Saccharomyces boulardii LV01/ CNCM I-3799	TNBS mouse	Attenuates weight loss	Anti-inflammatory effects	[76]
		Reduces microscopic scores of inflammation	Reduces IL-6 and serum amyloid A protein up-regulation	
Saccharomyces cerevisiae LV02/ CNCM I-3856			Reduces MPO activity	
Saccharomyces cerevisiae LV09				
Saccharomyces boulardii	TNBS rat	No significant differences		[77]

CCL chemokine (C–C motif) ligand, *CD40L* CD40 ligand, *COX* cyclooxygenase, *CXCL* chemokine (C-X-C motif) ligand, *DAI* disease activity index, *DSS* dextran sodium sulfate, *Hsp* heat shock protein, *ICAM* intercellular adhesion molecule, *IFN* interferon, *IL* interleukin, *iNOS* inducible nitric oxide synthase, LTB_4 leukotriene B4, *MAdCAM* mucosal vascular addressin cell adhesion molecule, *MAPK* mitogen-activated protein kinase, *MCP* monocyte chemotactic protein, *MIP* macrophage inflammatory protein, *MMP* matrix metalloproteinase, *MPO* myeloperoxidase, *MUC* mucin, *NF* nuclear factor, PGE_2 prostaglandin E2, *PPAR* peroxisome proliferator-activated receptor, *SCFA* short chain fatty acid, *SCID* severe combined immunodeficiency, *STAT* signal transducer and activator of transcription, *TGF* transforming growth factor, *Th* T helper, *TLR* toll-like receptor, *TNBS* trinitrobenzene sulfonic acid, *TNF* tumor necrosis factor, *ZO* zonula occludens

The $TNF^{\Delta ARE/WT}$ model is an ileitis model resembling human CD. Energy deficiency and an inability to maintain anti-oxidative defenses are associated with chronic inflammation observed in this model [16].

Another widely used animal model is the adoptive $CD4^+$ T cell transfer model, in which purified splenic $CD4^+$ CD45RBhigh T cells are transferred by intraperitoneal injection into recipient animals, a model chosen for its Th1-type cytokine-derived hyper-response [31, 67, 70]. This model is associated with weight loss [70].

The transgenic HLA-B27/β2-microglobulin colitis model is characterized by time-dependent worsening of intestinal permeability and inflammation marked by increased mucosal IL-1β levels and MPO activity [14]. The HLA-B27 microglobulin gene is associated with inflammatory disorders, such as IBD in humans, while its overexpression in transgenic animals leads to the development of spontaneous colitis, particularly in the colon, through heightened Toll-like receptor (TLR)-mediated bacterial recognition [14].

The IL-10 −/− colitis model is associated with spontaneous intestinal inflammation in the colon and cecum and the up-regulation of many transcription factors involved in IBD pathogenesis—unless animals are raised in germ-free conditions [47, 88]. Colonic inflammation is characterized by multifocal mild or severe inflammatory cell infiltrates composed primarily of mononuclear cells with few neutrophils in the lamina propria. IL-10 −/− animals exhibit TNF-α, IFN-γ, ICAM-1 and mucosal vascular addressin cell adhesion molecule (MAdCAM)-1 up-regulation [60]. Genes involved in TNF-α-induced chemokines and chemokine receptors, major histocompatibility complex (MHC) type II proteins, integrins, C-type lectins and TLRs involved in the recognition of commensal bacteria and the innate immune response are up-regulated in IL-10 −/− animals, while genes involved in fatty acid metabolism are down-regulated [47]. Another spontaneous colitis model is the vitamin D receptor (VDR) knockout model (VDR-/-), in which experimental colitis develops through T cell-dependent overexpression of IL-17 and IFN-γ [15]. There is also severe diarrhea, rectal bleeding and body weight loss, with a high mortality rate. DSS increases mucosal epithelial permeability by disrupting epithelial tight junctions [15].

Features of experimental colitis outlined in the previous section show a high degree of similarity with clinical, pathological and laboratory characteristics of IBD. Experimental colitis is characterized by common features of IBD, including weight loss, diarrhea and colon shortening, inflammation, disruption of tight junctions, decreased mucus secretion, up-regulated expression of pro-inflammatory cytokines and a decrease in the beneficial/potential pathogen bacteria ratio.

Nutritional Supplementation

Nutritional supplementation can range from essential macronutrients, such as calcium [14], vitamin D [15] and iron [16], whose dietary intake in known to be low in IBD patients, to antioxidants, including prodrugs capable of being biotransformed into the antioxidant tripeptide glutathione [17–19]. The purpose of the glycoprotein milk fat globule-epidermal growth factor 8 (MFG-E8) [20] and adiponectin [21] is to prevent wasting disease and the development of macroscopic and histological lesions. Several different polyunsaturated fatty acids (PUFA) [22–25] have been used, with varying degrees of success. Polysaccharides [26–29] and low-digestible fibrous plant components [29–31] can also alleviate colitis symptoms. Promising biological anti-inflammatory macromolecules include plant-derived compounds, such as fresh pineapple juice [32], ethyl acetate extracts of kiwifruit [33], the aloe components aloin, aloesin and aloe-gel [34] and enzymatic hydrolysate of corn gluten [35], as well as the phytohormone abscisic acid [36], the carotenoid pigment astaxanthin [37] and the polyphenolic compound resveratrol [38]. Soy protein has received recent attention for its role as a prebiotic that promotes the growth of probiotic microorganisms [39]. Actually, these molecules are intended to restore mucosal immunological balance by promoting the inactivation of lamina propria T cells, seemingly beneficial in CD models. Mechanisms of immunological homeostasis will be discussed in this review.

Probiotic Supplementation

Several nutritional supplements have prebiotic properties, with the primary goal of increasing the ratio between beneficial microorganisms and potentially pathogenic microbes. In addition, the direct administration of live probiotic bacteria and fungi, or those parts of their bodies with anti-inflammatory properties, has also been tested in colitis models. A few examples of these microorganisms currently being tested include *Escherichia coli* Nissle 1917 [40–43], the probiotic mixture VSL#3, consisting of eight lactic acid bacteria (*Lactobacillus acidophilus*, *L. bulgaricus*, *L. casei*, *L. plantarum*, *Streptococcus thermophilus*, *Bifidobacterium breve*, *B. infantis* and *B. longum*) [44–48], various individual *Lactobacillus* [49–66] and *Bifidobacterium* species [45, 65–71, 76–80], as well as *Streptococcus thermophiles* [66], *Bacillus polyfermenticus* [72], *Bacillus subtilis* [73], *Oenococcus oeni* [74] and *Clostridium tyrobutyricum* [75]. In addition, a couple of *Saccharomyces* strains have been tested for their ability to potentially alter disease treatment [76, 77].

Nutritional and Probiotic Supplementation in Colitis Models

Colitis models enable researchers to better understand the mechanisms of disease development and progression while uncovering new pathways that can be targeted with therapeutics. Based on the similarity between human IBD and experimental colitis, it is expected that toxic and beneficial effects observed in in vitro models would also be observed in vivo in animal models and, subsequently, in human patients. The main purposes of therapy in IBD patients are to reduce inflammation and to block the signal to the immune system that might lead to immune imbalance. Nutritional and probiotic supplementation is thus aimed largely towards finding non-invasive, non-medication ways to reduce inflammation.

Beneficial effects identified in colitis models depend largely on the parameters measured in each study. Oftentimes, these include lowering the symptoms of colitis, reducing inflammation and the levels of pro-inflammatory cytokines as well as increasing the levels of anti-inflammatory cytokines, lowering the levels of CAMs, protecting tight junctions and lowering epithelial permeability, aiding the recovery of mucin production and decreasing the attachment capabilities of pathogenic microorganisms and increasing levels of short-chain fatty acids (SCFA) and the proportion of beneficial bacteria. Tables 1 and 2 clearly describe mechanisms by which nutrients and probiotics modulate clinical, pathological and laboratory parameters in several colitis models.

Discussion

Compromised nutritional intake and malabsorption are common among human IBD patients [84]. Malnutrition, especially protein-energy malnutrition, as well as vitamin and micronutrient deficiencies are particularly prominent in CD patients [10, 85, 86].

Inadequate calcium levels are observed with a relatively high incidence in IBD patients, with calcium supplementation often recommended in this human population [86, 87]. Calcium supplementation protects tight junctions and up-regulates genes important for the mucosal barrier in HLA-B27/β2-microglobulin transgenic rats. Lower intestinal permeability is associated with lower levels of luminal antigens entering the circulation [14]. Schepens et al. show that calcium supplementation can decrease intestinal inflammation [14], while not discussing the influence of calcium supplementation on bone health, a topic showing very little promise in human IBD patients [88, 89].

1,25-Dihydroxyvitamin D_3 (also known as 1,25-dihydroxycholecalciferol), the bioactive component of vitamin

D_3, is important in calcium and phosphorus homeostasis, bone formation and mineralization, as well as immune system homeostasis [90]. This molecule acts upon the innate and the adaptive immune systems, leading to immune tolerance. The VDR is another important regulator of the immune system, with vitamin D or VDR deficiencies being involved in the etiology of autoimmune conditions, including IBD [90, 91]. Dietary supplementation with 1,25-dihydroxycholecalciferol is associated with blocked progression and the amelioration of symptoms in a DSS mouse colitis model [91]. Vitamin D protects tight junctions by increasing the expression of specific junction proteins. A functional VDR is required for this effect, as no improvement is observed in VDR −/− mice exposed to vitamin D supplementation [15]. Vitamin D supplementation in the presence of a functional VDR can reverse some of the IBD symptoms associated with deregulated immune responses [92]. Administration of the probiotic formulation VSL#3 led to increased VDR expression in a TNBS rat colitis model [82].

1α,25(OH)(2)-16-ene-20-Cyclopropyl-vitamin D_3, a potent VDR agonist, shows promising results in peripheral blood mononuclear cells from IBD patients and a DSS mouse colitis model [93]. While vitamin D deficiency is widely spread in human IBD patients [94], hypercalcemia is a dose-limiting adverse reaction of vitamin D supplementation in humans. The development of less toxic VDR agonists shows the importance of in vitro and animal studies.

Iron deficiency is common among IBD patients, especially those with active disease [87, 95–97]. However, iron may also act as a pro-oxidative mediator affecting inflammation, such that iron supplementation may be problematic in this population [16].

Excess free iron is toxic in mammals [98]. The acute-phase is a state characterized by a loss of organ homeostasis [99]. The expression profiles of several cytokines, including the acute-phase cytokine IL-6, were up-regulated in a turpentine oil-induced sterile abscess rat model characterized by increased iron levels in the brain and liver [99]. The mRNA levels of transferrin receptor-1, erythropoietin, hepcidin, ferritin-H, iron-regulatory protein-1 and heme oxygenase-1 were up-regulated, while hemojuvelin, ferroportin-1 and the hemochromatosis gene were down-regulated [99, 100]. IL-1β and TNF-α were up-regulated in a Kupffer cell phagocytosis rat model. Kupffer cells in the liver are important in iron metabolism [98]. The cytokine profiles of peripheral blood leukocytes isolated from human IBD patients, characterized by elevated IL-6, TNF-α, IL-1β and IFN-γ levels, were found to be similar to those reported in excess iron-associated acute-phase response proteins. The levels of IL-6 and IFN-γ were reduced in vitro by exposure to the common IBD treatment infliximab [101].

The administration of iron nitrilotriacetic acid (500 μM for 24 h) led to activation of nuclear factor (NF)-κB subunit phospho-RelA and up-regulation of hypoxia inducible factor 1R in distal ileum cells, suggesting that iron has pro-oxidative and pro-inflammatory properties in the intestine [16]. The $TNF^{\Delta ARE/WT}$ mouse colitis model possesses balanced antioxidative cell functions, with the up-regulation of genes involved in energy homeostasis and oxidative stress responses, such as glutathione-S-transferase, superoxide dismutase, catalase and peroxiredoxin 1, in the presence of low iron. On the other hand, adequate iron is associated with oxidative stress in this model [16]. Based on these findings, Schepens et al. hypothesized that antioxidant therapy would have a protective effect at the mucosal level, in addition to its protective effect of calcium in the gut lumen [17]. Interestingly, antioxidant supplementation alone was ineffective, and antioxidants did not enhance the anti-inflammatory benefits of calcium in HLA-B27 transgenic rats. Dietary antioxidants were unable to raise glutathione levels and improve intestinal inflammation. Reactive oxygen species production was compromised in HLA-B27 transgenic rats, suggesting that inflammation is not mediated by oxidative stress in this colitis model, leading to inconclusive findings [17]. While oral iron is poorly tolerated in IBD patients, intravenous iron shows promising results in treating iron deficiency [96, 97, 102].

Nonetheless, oxidative-mediated injury and consequent glutathione deficiency is involved in IBD pathophysiology [18]. Since glutathione is not taken up by cells, antioxidant supplementation should be presented in the form of cysteine, glutamate and glycine, the molecule's three amino acids components [18]. Dietary supplementation with cysteine prodrugs normalized hepatic glutathione levels and attenuated inflammatory responses [18, 19]. Antioxidant-mediated intestinal inflammation decrease is associated with restored immune homeostasis and restored apoptosis of activated immune cells [19]. Therefore, cysteine supplementation presents a possible treatment in human IBD [19].

Plasma OPN levels are elevated in human IBD patients, particularly CD patients, with a direct correlation between OPN expression, inflammation and disease activity [103–105]. OPN appears to be integral for the development of DSS colitis, with milder symptoms observed in OPN −/− mice, brought about by a reduced Th1 response with lower pro-inflammatory cytokines production and macrophage chemotaxis [106, 107].

Recombinant MFG-E8 protein administered intravenously reduced OPN-dependent $\alpha v\beta 3$ integrin signaling in a DSS mouse colitis model [20]. In this study, DSS treatment down-regulated MFG-E8 expression, with a direct correlation between the DSS dose used, the level of

MFG-E8 down-regulation and the degree of inflammation. MFG-E8 treatment improved colitis symptoms, while acting as a strong inhibitor for the binding site of OPN on $\alpha v\beta 3$ integrin and the consequent integrin-dependent NF-κB signaling [20]. CAMs, such as ICAM-1, MAdCAM-1, vascular CAM (VCAM)-1 and platelet endothelial CAM (PECAM)-1 bind to integrins. Therefore, possible therapies that interfere with this binding would limit leukocyte trafficking and inflammation. OPN also contains a recognition domain for $\alpha 4$ integrin [108]. Monoclonal antibodies directed against $\alpha 4$ integrin led to colitis amelioration in a spontaneous cotton-top tamarin colitis model [109, 110].

Hypoadiponectinemia is associated with chronic inflammation in humans. Therefore, supplementation of the protein hormone adiponectin could be beneficial in IBD patients. Low adiponectin levels in IBD patients correlate with hyperinsulinemia, which is a predictor of a longer remission period [111]. While interesting, this observation emphasizes the intricate relationship between inflammation and metabolism in IBD. In comparison, one study found elevated adiponectin levels in IBD patients [112], whereas a separate report shows elevated adiponectin levels in UC patients compared to controls, and lower levels in CD patients compared to UC patients [113]. Osmotin, an adiponectin plant-derived homolog, was associated with milder signs of colitis in a DSS mouse model [21].

As colonic nuclear receptors, PPARs modulate macrophage- and T cell-mediated inflammation. PPAR-γ heterodimerizes with the retinoid X receptor-α and binds to specific DNA sequence elements known as peroxisome proliferator response elements [114]. This heterodimer mediates inflammation, including bacterial inflammation, and regulates cell proliferation through kinases and transcription factors such as NF-κB, c-Jun, c-Fos, and nuclear factor of activated T cell. In addition, it inhibits IL-1β, TNF-α and chemokines, as well as the proliferation of inflammatory cells and some CAMs [22, 114]. Under normal conditions, colonic epithelial cells express high levels of PPAR-γ. Impaired PPAR-γ expression is observed in human IBD patients and colitis models. Decreased PPAR-γ levels at both mRNA and protein levels are present in both healthy and inflamed sections of the colon in IBD patients [114].

PPAR-γ deletion in PPAR-γ fl/fl; CD4-Cre$^+$ mice and PPAR-γ flfl; Villin Cre$^+$ mice was associated with increased colonic epithelial mucosal erosion and leukocyte infiltration, compared to control animals [78, 79]. Genes characterized as leukocyte extravasation markers were significantly enhanced in both CD4Cre$^+$ mice and wild-type animals, with the highest values observed in the CD4Cre$^+$ group at day 7. CD4Cre$^+$ mice also showed higher levels of integrin αV, integrin $\beta 2$, ICAM-1, VCAM-1 and P-selectin than wild-type mice, as well as higher levels of IL-6 and IL-1β. Cytokine signaling 3, an inhibitory protein shown to increase in response to IL-6, was up-regulated, similar to observations from human IBD patients [78].

In humans, a loss-of-function mutation in the PPAR-γ gene (Pro12Ala, a CCA-to-GCA missense mutation in codon 12 of exon B) was associated with decreased PPAR-γ mRNA expression in diseased mucosa, which in turn was associated with a higher incidence of UC [115]. This result is, however, controversial as other studies have not found such an association, although this mutation has been found to be associated with more severe symptoms [116, 117].

Fatty acids are PPAR ligands, with arachidonic acid showing pro-inflammatory properties, while eicosapentanoic acid was anti-inflammatory in Caco-2 cells [22].

Long-chain PUFAs are precursors of prostaglandins and leukotrienes, which are important mediators of inflammation [10]. PUFAs possibly represent the most controversial class of compounds with respect to colitis management. For example, ω-3 PUFA decreased symptoms in a DSS rat colitis model, while showing pro-inflammatory properties [23]. Dietary supplementation with either conjugated linoleic acid (ω-6 PUFA) or fish oil (eicosapentanoic and docosahexanoic ω-3 PUFA) led to epithelial regeneration in a DSS piglet colitis model [24]. In this study, conjugated linoleic acid delayed the onset of enteric disease symptoms and was associated with less pronounced growth retardation, compared to fish oil supplementation. Furthermore, ω-3 PUFA had antagonistic effects on the growth retardation reduction induced by conjugated linoleic acid, as no effects were seen when the two substances were administered together [24]. That being said, weight management is one of the intrinsic properties of conjugated linoleic acid.

Dietary supplementation with fish oil and the natural phenol curcumin, alone or in combination with one another, led to an increased mortality rate in the acute inflammatory phase of DSS exposure, suggesting that susceptibility to DSS is enhanced by these dietary supplements. The same combination of compounds led to decreased inflammation scores and NF-κB expression [25]. The long-chain ω-3 PUFA docosahexanoic acid was not protective in a SMAD3 −/− *Helicobacter hepaticus*-induced colitis mouse model when administered at doses ranging from 0.75 to 6.00 % [118].

Dietary supplementation with ω-6 PUFA up-regulates PPAR-γ, decreases inflammation and delays the onset of colitis, whereas ω-3 PUFA up-regulates PPAR-δ and accelerates recovery from colitis [24]. IBD patients, particularly CD patients, often consume low levels of ω-3 PUFA [10, 119, 120], while ω-3 PUFA supplementation is generally well tolerated and associated with a lower incidence of IBD, as well as IBD amelioration. In humans, ω-3 PUFA intake is normally considered to be beneficial, while high levels of ω-6 PUFA are viewed as being detrimental [10].

In general, the diets of IBD patients contain a high ω-6 to ω-3 PUFA ratio and low levels of fruits and vegetables [6, 10]. While the role of PUFAs is inconclusive, nutritional supplementation with various plant components shows more promising results in colitis models. For example, aloe components have anti-inflammatory properties [34]. Aloe has been previously shown to decrease the colitis activity index and induce clinical remission in a higher fraction of UC patients compared to placebo administration [121].

The natural plant phytohormone and PPAR-γ agonist abscisic acid is associated with the decreased expression of inflammatory cytokines and proteins, and CAMs [36]. Since abscisic acid functions through a PPAR-γ-dependent mechanism, its potential to ameliorate intestinal inflammation was lost in PPAR-γ fl/fl; CD4-Cre^{+} mice [122]. Abscisic acid is not presently used in human IBD patients, but it has been identified as a pro-inflammatory endogenous hormone in humans. It is produced by activated human granulocytes and stimulates phagocytosis, reactive oxygen species and nitric oxide production, as well as chemotaxis in these cells [123]. A synthetic abscisic acid analog was shown to block the inflammatory functions of granulocytes and monocytes in vitro by competing with the hormone for the binding site of lanthionine synthetase C-like protein (LANCL2), the human binding site for abscisic acid [123, 124]. Therefore, in theory, it is possible to synthesize xenobiotics with anti-inflammatory properties that would exert their therapeutic potential through the LANCL2 receptor, although the practicality of this approach is unknown, especially in IBD. It is interesting to note that abscisic acid signals through different receptors in humans and mice, with what appears to be opposite effects.

Plant-derived polysaccharides and low-digestible fibers have also been tested. For example, polydextrose, a polysaccharide constituted by 90 % nondigestible and nonabsorbable soluble fibers, was able to reverse signs of colitis [26]. Plant-derived polysaccharides are believed to lower colitis activity through their effect on the monocyte count [32]. Fresh pineapple juice, but not boiled pineapple juice devoid of active proteolytic enzymes, was associated with colitis relief [27]. Bromelain (5 mg/day), a mixture of cysteine proteinases derived from the stem of the pineapple plant, had anti-inflammatory effects similar to that of fresh pineapple juice in the short term, but lost its beneficial potential in the long term [32]. Interestingly, ethyl acetate extracts of kiwifruit had anti-inflammatory properties in intestinal epithelial cells isolated from IL-10 −/− mice in vitro [33], but not in IL-10 −/− mice in vivo [125]. One possible explanation for these discrepancies rests in the colitis model used, as IL-10 −/− mice were unable to metabolize the kiwifruit extracts [83].

The marine carotenoid pigment astaxanthin [37] and the polyphenolic compound resveratrol found in grapes and wine [38], are powerful biological antioxidants, anti-inflammatory and anti-cancer compounds with immunomodulatory activities.

Dietary fructooligosaccharides and resistant starch, and especially their combination, had anti-inflammatory properties and brought about changes in the intestinal microbiota in the cecum and colon in a TNBS rat colitis model [29]. Heat-resistant amylase, protease and hemicellulase reduced inflammation by modulating the colonic environment and regulating immune cell differentiation [31]. Di-D-fructose dianhydride-enriched caramels have prebiotic properties [28], while nutriose is able to stimulate butyrogenic bacteria strains [30]. Soy protein is another prebiotic nutritional supplement, while the probiotic microorganism *Lactobacillus rhamnosus* GG did not offer additional beneficial effects [39].

Probiotics are live microbial food supplements that benefit health by stimulating immune functions and resistance to infection. There is a lack of general consensus with respect to the role played by intestinal microbiota dysregulation in IBD development and progression, as well as the involvement of probiotic supplementation in preventing, delaying and treating the symptoms of IBD [126]. By and large, probiotic supplementation has been associated with the amelioration of symptoms, increased mucosal integrity, normalization of immune homeostasis and decreased inflammation, with minimal to no adverse effects.

Intestinal epithelial cells are in direct contact with the intestinal microbiota and process information sent from the luminal microbiota and immune system cells. Thus, these cells have the dual function of physical barrier and modulators of innate and adaptive immune responses [49, 127]. The activation status of immune cells is determined by the kind of bacteria that are present in the intestinal lumen. Using pattern recognition receptors, such as TLRs, NOD2, C-type lectins and integrins, or the cytosolic nuclear oligomerization receptors expressed on intestinal epithelial cells or effector cell TLRs, intestinal epithelial cells can sense bacterial components and express MHC type II and co-stimulatory proteins [127, 128]. Intestinal immune system dysregulation can lead to apoptosis of intestinal epithelial cells [127]. For example, *Lactobacillus salivarius* Ls33 has anti-inflammatory properties through a NOD2-dependent pathway [64]. The anti-inflammatory effects of lactobacilli are strain-specific, as no relief from colitis was observed using *Lactobacillus acidophilus* NCFM [64].

Dendritic cells are crucial in early bacterial recognition and the development of T cell response and immune tolerance as they act as antigen-presenting cells. Lactic acid bacteria are strong activators of dendritic cells [45, 62]. *Lactobacillus rhamnosus* Lr32- and *L. salivarius* Ls33-treated bone marrow dendritic cells ameliorate disease

symptoms in a TNBS mouse colitis model through TLR-2 and nuclear oligomerization receptor signaling [62]. The molecular cell surface characteristics of bacteria are crucial to their ability to act as probiotics [129]. Bacterial cell-wall components of *Escherichia coli* strain Nissle 1917 had probiotic properties in a DSS colitis mouse model, signaling particularly through TLR-2 and TLR-4. No amelioration of colitis symptoms was observed in TLR-2 −/− or TLR-4 −/− animals [41].

The probiotic mixture VSL#3 prevented DSS-associated loss of tight junction proteins, while not modifying the expression pattern of these proteins [46]. VSL#3 further reduced colonic epithelial cells apoptosis, another feature associated with compromised epithelial barrier function [46]. *Bifidobacterium lactis* increased the expression of the tight junction protein ZO-1, decreasing epithelial permeability and protecting tight junctions in Caco-2 cells [71]. *Escherichia coli* strain Nissle 1917 restored disrupted intestinal epithelium in polarized T84 cells infected with the enteropathogenic *E. coli* strain E2348/68 through up-regulation of ZO-2 and distinct protein kinase C isotypes [40]. In vivo, *E. coli* strain Nissle 1917 up-regulated ZO-1 expression and provided protection against intestinal barrier dysfunction in a DNBS mouse colitis model [42]. Treatment with *E. coli* Nissle 1917 following minocycline exposure protected tight junctions through the enhanced expression of tight junction proteins in a DSS mouse colitis model [43]. This treatment regimen also had superior anti-inflammatory effects to micocycline treatment alone. Similar findings were observed after animals were exposed to a second cycle of DSS, followed by subsequent exposure to minocycline and *E. coli* Nissle 1917 [43]. *E. coli* strain Nissle 1917 was able to prevent the recurrence of UC to some degree, with little to no effect in human CD patients [130]. An interesting use for *E. coli* strain Nissle 1917 in humans is that of carrier for gut-focused in situ synthesis of therapeutic molecules [131].

VSL#3 increased the MUC2 expression and mucin production in healthy rats, emphasizing the crucial role played by the microbiota in maintaining the GI epithelium, particularly lactobacilli [132]. *Lactobacillus acidophilus* A4, *L. casei* GG and *Escherichia coli* strain Nissle 1917 all increased mucosal integrity, with up-regulated MUC2 expression and recovered mucin production by goblet cells in experimental colitis [43, 49, 53].

Probiotics, particularly lactic acid bacteria, have the potential to decrease the attachment capabilities of pathogenic bacteria through acidification with lactic acid, secreted non-acidic products and direct interference with attachment to receptors [49]. MUC2 up-regulation is associated with lower attachment capabilities. Thus, mucus has protective properties against GI tract colonization by pathogenic bacteria. Interestingly, up-regulated expression of pro-inflammatory cytokines is also associated with protection against *Escherichia coli* O157:H7 attachment [49].

Bacillus polyfermenticus ameliorated the development and progress of DSS colitis and reduced mucosal damage in DSS and TNBS colitis. *Bacillus polyfermenticus* had protective effects in terms of lower rates of colonic mucosal cells apoptosis [72]. *Bacillus polyfermenticus* induced epithelial cell growth, survival and proliferation through Akt phosphorylation and phosphatidylinositol 3-kinases activation and the up-regulation of cytoprotective factors [72].

Lactobacillus GG was associated with the maintenance of the remission phase in UC patients [133]. Significant improvements in small bowel permeability were observed in vivo in human patients following short-term treatment with a mixture of active *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, *L. acidophilus* and *Bifidobacterium longu*m [134] and *Saccharomyces boulardii* [135], measured as a decrease in the lactulose/mannitol ratio. Three probiotic yeast species have been identified in a TNBS rat colitis model, with various cytoprotective effects and strain-dependent mechanisms [76].

Bifidobacterium bifidum BGN4 had preventive effects in a $CD4^+$ CD45RB high T cell transfer mouse colitis model [67]. A diet containing skim milk supplemented with 0.3 % (w/w) *Bifidobacterium bifidum* BGN4 was found to aid the normalization of intestinal homeostasis [67]. This bacterium is believed to modulate the intestinal immune response in terms of the Th1 response but not the Th2 response, with no significant changes in intestinal microbiota [67]. VSL#3 has also been found to have immunomodulatory properties, reducing lipopolysaccharide (LPS) antigen presentation, Th1 cell generation and Th1 pro-inflammatory cytokine expression in dendritic cells [45]. On the other hand, *Bifidobacterium longum/infantis* E18 had no such beneficial effects [68].

IgA is the most important secreted antibody that prevents the access of commensal or pathogenic microorganisms to the epithelial cell surface. A deficiency of IgA was associated with UC in humans [80]. Increased IgA secretion, induced by a preparation obtained from 8 lysed bacteria, decreased both clinical and histological manifestations in a DSS mouse colitis model [136].

Probiotic formulations have protective effects on the intestinal barrier, owing largely to their anti-inflammatory properties. As a result, probiotic formulations with anti-inflammatory properties are effective, particularly under conditions of chronic inflammation [137]. Probiotics also have the potential to correct inflammation-driven metabolic dysfunction [48].

In one study, VSL#3 led to a change in intestinal bacterial diversity, but the species contained in this probiotic

formulation did not become dominant [47]. VSL#3 increased the proportion of lactobacilli in another study [46], while other nutritional and probiotic supplements had similar effects on the ratio of beneficial/potential pathogen bacteria [28, 29, 43, 57]. Yogurt had anti-inflammatory properties in a TNBS mouse colitis model and promoted a longer remission phase. This effect was TLR-specific, with decreased TLR-4 expression and enhanced TLR-9 expression. Additionally, yogurt may also lead to IL-10 up-regulation and intestinal microbiota modifications [138].

Interestingly, VSL#3 reduced the richness and biodiversity of the luminal microbiota in a TNBS rat colitis model, with no effect on the mucosally adherent microbiota [81]. Although the richness and biodiversity of these two anatomical compartments were comparable, the individual species inhabiting them were distinct. TNBS colitis severity was found to correlate with changes in richness and biodiversity in the luminal microbiota [81].

Lactobacilli and bifidobacteria have been observed to act upon lamina propria lymphocytes [66]. *Lactobacillus casei* Shirota was effective against LPS-induced IL-6 up-regulation in lamina propria mononuclear cells, while *L. casei* ATCC 334 and *L. rhamnosus* ATCC 53103 were ineffective. This phenomenon also led to an inhibition of NF-κB and IκB phosphorylation following LPS stimulation. The protective effects of *L. casei* Shirota were mediated by increasing NOD2 concentrations, and this strain exhibited its response through components of the polysaccharide–peptidoglycan complex derived from the cell wall [54]. *Lactococcus lactis* subsp. *cremoris* FC was observed to suppress experimental colitis by modulating the mRNA expression of pro-inflammatory cytokines in the gut. This bacterial strain further reduced LPS-induced TNF-α production in RAW264.7 cells by inhibiting NF-κB nuclear translocation [58].

VSL#3 had immunomodulatory properties in an IL-10 −/− mouse colitis model, reducing histopathological inflammation in the cecum, but not in the colon, through down-regulation of Th1 response transcription factors [47, 139]. Reduced inflammation in primary cecal epithelial cells belonging to IL-10 −/− mice was observed in vitro as well, suggesting a protective effect of VSL#3 at the cellular level. However, this effect was disease- and tissue-specific: TNF-α-induced ileitis was unresponsive to the probiotic culture in $TNF^{\Delta ARE}$ mice [140]. VSL#3 also reduced histopathological inflammation in an IL-10 −/− mouse colitis model in another study, through a similar down-regulation of Th1 cytokines [139]. This probiotic preparation also reduced TNF-α expression in response to the injection of *Escherichia coli* DNA in mice, IFN-γ expression in response to *Bacteroides vulgatus* in splenocytes, and IL-8 secretion in response to bacterial LPS or TNF-α in HT-29 cells, coupled with delayed NF-κB activation and IκB stabilization [139].

Probiotic cultures decrease the levels of pro-inflammatory cytokines and increase those of inducers of regulatory T cells and tolerance mechanisms [141]. Several probiotic organisms have been found to reduce the levels of pro-inflammatory cytokines and proteins [41, 43–45, 47–52, 56–60, 62, 64–67, 69, 70, 72–76]. VSL#3 reduced the expression of the neutrophil chemoattractant chemokine IL-8, the Th1 cytokine IFN-γ and the pro-inflammatory cytokine IL-1β in mucosal tissue in UC patients [141].

A recombinant strain of *Lactobacillus casei* bioengineered to secrete the anti-inflammatory neuropeptide α-melanocyte-stimulating hormone deactivated colonic inflammatory responses in a DSS mouse colitis model [52]. The anti-inflammatory effects of the recombinant strain were superior to those of the wild-type strain [52]. Blueberry husks or rye bran were found to enhance the anti-inflammatory effects of a probiotic mixture containing *Lactobacillus crispatus* DSM 16743, *L. gasseri* DSM 16737 and *Bifidobacterium infantis* DSM 15158 in a DSS rat colitis model [142].

VSL#3 and *Lactobacillus rhamnosus* GG reduced the expression and activity of inflammatory proteins in an iodoacetamide rat colitis model, coupled with reduced recruitment and activity of T cells and granulocytes in the mucosal layer, yet were ineffective against DNBS colitis [44].

SCFAs are an important source of energy for enterocytes as well as a stimulator of increased sodium and water absorption and increased mucus secretion [127]. Some nutritional supplements (Di-D-fructose dianhydrides and enzyme-treated rice fiber) [28, 31], as well as certain probiotic strains (*Lactobacillus fermentum* and *Clostridium tyrobutyricum*) [57, 75], can enhance the levels of SCFAs (mainly acetate, propionate and butyrate). Butyrate, a product of bacterial fermentation of carbohydrates, is believed to modulate the immune response triggered by pro-inflammatory cytokines [143], while its anti-inflammatory properties ameliorate disease symptoms [57].

The production of the pro-inflammatory cytokine IL-8 in the course of IBD is enhanced by immune responses to pathogen-associated molecular patterns [143]. Butyrate lowered IL-8 secretion in Caco-2 cells pre-treated with the TLR-2 ligand Pam3CSK4 (Pam3CysSerLys4), a synthetic tripalmitoylated lipopeptide that mimics the acylated NH_2-terminus of bacterial lipoprotein, compared with that in Caco-2 cells treated with Pam3CSK4 alone [143]. An additional study with segments of human intestine cultured ex vivo failed to confirm the ability of Pam3CSK4 to induce IL-8 secretion [143]. While somewhat efficacious in vitro, the feasibility of butyrate therapy in humans is unknown as, compared to healthy controls, resistance to the anti-inflammatory properties of this molecule has been observed in IBD patients, [144].

Clostridium tyrobutyricum has been found to up-regulate IL-18 production in severe combined immunodeficiency mice, with no association between IL-18 levels and severity of colitis. A possible explanation is the effect of butyrate on IL-18 gene expression [75]. *C. butyricum* MIYAIRI 588 was observed to greatly inhibit the toxicity of *C. difficile* in vitro, a pathogenic bacterium associated with diarrhea and colitis development. The mechanism is thought to involve the inhibition of toxin production brought about by close contact between the two species. *C. butyricum* MIYAIRI 588 was also effective in vivo against *Clostridium difficile* toxicity [145].

Lactobacillus bulgaricus OLL1181 increased secreted alkaline phosphatase activity in HeXS34 cells and prostaglandin E2 production in Caco-2 cells through an aryl hydrocarbon receptor-dependent pathway associated with COX-2 production. No such effects were observed with *Lactobacillus gasseri* MEP222701 [51].

Heat-killed *Lactobacillus brevis* SBC8803 and *Bacillus subtilis* had cytoprotective and anti-inflammatory effects in a DSS mouse colitis model through the induction of heat shock proteins (Hsp) [50]. Polyphosphate, a structure made up of phosphate units repeats produced by lactobacilli and other members of the intestinal microbiota, has been identified as the bacterial component responsible for Hsp27 induction [50, 146]. Polyphosphate aids in the maintenance of the intestinal barrier through a mitogen-activated protein kinase-dependent pathway [146]. The use of heat-killed *L. brevis* SBC8803 is promising as it can even be used under conditions unsuitable to bacterial colonization as it does not employ a live microorganism [50].

The potential of two *Lactobacillus delbrueckii* subsp. *bulgaricus* strains to ameliorate colitis was found to be dependent on the level of exopolysaccharide secreted, showing the therapeutic potential of exopolysaccharide in an acetic acid rat colitis model. Higher levels of exopolysaccharide are associated with increased antioxidant activities in a colitis model characterized by oxidative damage [55]. *L. fermentum* CECT 5716 is also believed to have antioxidant abilities [56].

Bifidobacterium lactis reduced inflammatory and T cells mediators and was able to promote regulatory T cell-specific markers [70]. *B. longum* HY8004 and *Lactobacillus plantarum* AK8-4 reduced the glycosaminoglycan degradation induced by *Bacteroides stercoris* in vitro and in vivo [65].

VSL#3 reduced TNF-α-mediated induction of IFN-γ-inducible protein-10 (IP-10, also known as CXCL10), suggesting that VSL#3 acts via an IP-10-specific pathway [140]. In another study, *L. casei* affected the intracellular protein level of IP-10 through post-translational degradation. Co-stimulation with *L. casei*, the bioactive component of VSL#3, blocked the chemotactic response exhibited by TNF-α in the small intestine Mode-K cell line [140].

Lactobacillus plantarum was observed to interfere with CAM up-regulation [60], while *L. reuteri* decreased leukocyte– and platelet–endothelial cell interactions [61], thus attenuating symptoms of colitis. *L. acidophilus* and *L. rhamnosus* CS led to increased P-glycoprotein expression in differentiated Caco-2 cells, a drug efflux transporter whose dysregulation is associated with IBD. P-glycoprotein expression was down-regulated in a DSS mouse colitis model, while *L. acidophilus* or *L. rhamnosus* CS probiotic treatment reversed this effect [147].

VSL#3 delayed the transition to dysplasia and cancer in a TNBS rat colitis model, showing a potential beneficial effect in IBD patients with long-standing colitis [82]. Chronic inflammation is a risk factor for the development of colorectal cancer in IBD patients. The use of probiotics, prebiotics and synbiotics therefore shows promising prophylactic results in preventing colorectal cancer, with an apparent lack of toxicity [148].

Harding et al. argue that an adequate diet is more important for the management of colitis than probiotic supplementation [149]. In a DSS piglet colitis model, piglets were fed a diet containing less than the recommended levels of macronutrients or one containing less than the recommended levels of macronutrients supplemented with probiotics; piglets on both diets were found to have lower weight gains, lower growth and lower skeletal muscle protein fractional synthesis rates than those fed a diet containing adequate levels of macronutrients [149].

Future Considerations

Palileo and Kaunitz have recently reviewed the pathophysiological mechanisms relating to GI homeostasis [150]. There is a fine balance between factors that contribute to the failure of gastroduodenal mucosal defenses and mediators of mucosal healing. An increased understanding of these systems may help prevent mucosal injury, eliminating the need for treatment. There is a strong association between diet, intestinal microbiota and IBD. Intestinal microbiota manipulations using prebiotic nutritional supplements and probiotics have the potential to alter the composition of the intestinal microbiota, with direct and indirect effects on intestinal immunity [151]. An increasing awareness that intestinal bacterial communities have an important impact on the host immune response can lead to beneficial new diets that included prebiotics and probiotics in IBD patients. It is also imperative that these individuals do not ignore the importance of an adequate diet rich in essential nutrients. Such knowledge helps to identify new therapeutic targets, thus creating the possibility of developing new non-invasive, non-medication ways to reduce inflammation. Furthermore, identification

of specific nutrient receptors and their respective ligands can provide novel therapeutic targets for the treatment of intestinal mucosal injury. Nguyen et al. argue that the interaction between nutrient receptors and their preferred ligands evokes neurohormonal responses [152]. Taste receptors also play an important role in intestinal chemosensing, such that sweet, bitter and umami evoke responses in the GI system [152].

This review shows that there is currently a significant interest in identifying alternatives to traditional medications. It could be particularly interesting to perform a systematic study aimed at identifying additional patterns of disease behavior and response to treatment with standard medication in the presence of an adequate diet or nutritional supplements. The task of choosing the right medication for an individual IBD patient will likely become more complex in the future. Although much work is still needed before phenotype, genotype and serological parameters can predict treatment response, nutritional and probiotic supplementation may help maximize efficacy, minimize delays to effective treatment and improve safety and tolerability.

This review is limited by the lack of animal models combining chemically induced colitis with infectious enteritidis. Infectious sources may also occur together with IBD or be the cause of UC (*Campilobacter jejuni*, *Salmonella*, *Shigella*, invasive *Esherichia coli* or *Clostridium difficile*) or CD *(Yersinia enterocolitica*, *Micobacterium tuberculosis* and *M. avium*) [153].

In conclusion, our study shows that colitis models can help select an adequate diet, independent of disease localization, thus minimizing the need for surgery in human IBD patients.

Conflict of interest None.

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