Inflammatory Bowel Disease

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Abbreviations

Ahr	Aryl hydrocarbon receptor
ASCA	Anti-*Saccharomyces cerevisiae* antibodies
CD	Crohn disease
DSS	Dextran sulfate sodium
EEN	Exclusive enteral nutrition
FMT	Fecal microbial transplantation
FXR	Farnesoid X receptor
IBD	Inflammatory bowel disease
IL	Interleukin
ILC	Innate lymphoid cells
IPA	Indolepropionic acid
MyD88	Myeloid differentiation primary response 88
PSA	Polysaccharide A
SCFA	Short-chain fatty acids
SFB	Segmented filamentous bacteria
SpA	Spondyloarthritis
Treg	Regulatory T cells
TLR	Toll-like receptor
TNF	Tumor necrosis factor
UC	Ulcerative colitis

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Microbiota and the Immune System in Intestinal Inflammation

IBD impacts approximately 200 per 100,000 individuals [1], depending on geographic location [2]. There are two major subtypes: Crohn Disease (CD) and ulcerative colitis (UC) (Table 19.1). IBD likely results from the combination of multiple factors. On the one hand, the increase in IBD prevalence in Western countries points to a role for environmental factors, and the microbiota is likely one of them [3, 4]. On the other hand, the genetic component of susceptibility to IBD includes numerous immune-related genes, underlining the role of genetically programmed immune factors in IBD pathogenesis [5]. Our understanding of the interactions between the intestinal immune system and the microbiota

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Table 19.1 Comparison of features of CD and UC

Feature	Crohn disease	Ulcerative colitis
Location	Entire GI tract	Colon primarily
Continuity of inflammation	Skip lesions	Continuous
Depth of inflammation	Deep; can become transmural	Superficial
Pathology	Granulomas possible	Mucosal inflammation
Extraintestinal manifestations		
Arthritis	+	+
Cutaneous	+	+
Sclerosing cholangitis	+/−	+
Uveitis	+	+
Risk of colon cancer	Increased	Increased
Common symptoms	Diarrhea, weight loss, malabsorption, abdominal pain, growth failure	Bloody diarrhea, abdominal pain

Adapted from [162]

has greatly expanded over the last decade, aided by the mainstream adoption of new molecular tools allowing the molecular characterization of microbial communities. Early reports about gene mutations altering the microbiota in mouse models have to be assessed with caution, though, since the use of non-stringent controls in the early days introduced considerable errors into the system [6]. Still, the use of mice with genetic mutations has demonstrated that changes in the immune system suffice to alter the intestinal microbiota. Interestingly, the altered microbiota can then change the way the immune system responds to challenges. The pathways mediating the cross talk between the immune system and the microbiota are only beginning to be understood, and only a few specific mechanistic interactions have been demonstrated in patients or in preclinical models.

Immune Cells

Studies in recent years have highlighted the interplay between the microbiota, metabolism, and immune cells in intestinal inflammation. IBD is considered to arise from an imbalance between the inflammatory and the regulatory arms of the immune response. T cells and innate lymphoid cells (ILC) are some of the inflammatory cell types implicated in IBD, whereas $FOXP3^+$ regulatory T cells (Treg) dampen immune inflammation. Tregs could also affect intestinal immune responses by modulating IgA secretion into the intestine [7], since IgA has been shown to regulate the composition of the gut microbiota [8]. T cells and ILC do not act directly on the microbiota but appear to control it indirectly through intestinal epithelial cells and other mechanisms [9, 10]. Lymphocytes in the mucosa produce cytokines, such as IL-17 and IL-22, that act on epithelial cells enhancing their secretion of Reg3g and other antimicrobial peptides and thus altering the composition of the microbial community [11]. Intestinal myeloid immune cells, such as macrophages and dendritic cells, directly sense the microbiota but also react to changes in epithelial cells, such as increased cell death [10, 12]. They can instruct lymphocyte activity through antigen presentation and production of cytokines such as IL-23, a key player in intestinal inflammation in mice and humans, which enhances production of IL-17 and IL-22 by Th17 and ILC3 cells [13]. IL-23 mediates intestinal inflammation in animal models, and variants in *IL23R*, the gene coding the specific subunit of the IL-23 receptor, are associated with IBD susceptibility in patients [14]. Alterations in all these pathways can change the composition of the microbiota.

Intestinal Epithelial Cells

The intestinal epithelium also plays an active role in defense against pathogens and the interactions with the microbiota. It is a protective barrier as little as a single cell thick, which has a crucial role for excluding exogenous pathogens and antigens, but at the same time allowing water and nutrients to pass. Intestinal epithelial cells shape the microbial community by a variety of mechanisms including the secretion of antimicrobial peptides. It has been shown that several genes with variants associated with IBD susceptibility, including

NOD2, affect the secretion of antimicrobial peptides by epithelial cells [15]. Intestinal epithelial cells can sense the microbiota and respond to it, as seen in germ-free rats, which have reduced epithelial cell proliferation compared to conventionally raised rats [16]. Important regulators of bacteria and epithelial cell interactions are the toll-like receptors (TLRs), which recognize bacterial molecular motifs such as cell wall components and flagellin. These receptors are found on both immune and nonimmune cells, such as epithelial cells. Therefore, TLR signaling is a likely mechanism regulating bacteria-induced increases in cell proliferation. However, in the absence of intestinal injury, epithelial cell proliferation in mice deficient in either myeloid differentiation primary response 88 (MyD88, a transducer necessary for signaling by many TLRs) or TLR4-is similar to that in wild-type mice, suggesting the involvement of other bacterial signals [17].

In contrast, dextran sulfate sodium (DSS)-induced intestinal injury leads to decreased gut epithelial cell proliferation, acute inflammation, and increased mortality in MyD88-, TLR4-, or TLR2-deficient mice [18, 19]. This increased susceptibility to DSS-induced injury can be reproduced in wild-type mice by treating them with broad-spectrum antibiotics or antibodies targeting TLR2 or TLR4 [18, 19]. Administration of DSS to wild-type germ-free mice also produces greater colonic injury compared to mice that have a conventional microbiota [20, 21]. Initially, these results appear counterintuitive, as one might predict that mice that are unable to mount a TLR-dependent response against the microbiota would be less affected by DSS. However, these studies show that TLR signaling in epithelial cells is dispensable for intestinal epithelial cell proliferation under normal conditions, while in the presence of injury, both the intestinal microbiota and their interactions with TLRs are required for tissue repair.

Effects of the Microbiota on the Immune System

No longer viewed as merely passengers, the gut microbiota is widely thought to play a critical role in the development and progression of IBD. Experiments in mice show that mutations in genes associated with susceptibility to IBD, such as *Nod2*, can cause an imbalance in the microbial community (dysbiosis) that exacerbates colitis [22]. However, despite extensive investigation, no single microbial agent has been proven to cause IBD. Nevertheless, some broad patterns can be discerned across many studies. These include a loss of community diversity, increased representation of some Gammaproteobacteria, and decreased relative abundance of several taxa within the Firmicutes phylum [23]; see below.

Other groups of bacteria may protect against IBD through suppression or modulation of inflammatory responses. *Bacteroides thetaiotaomicron* has been shown to attenuate intestinal epithelial cell inflammation, suppress NF-κB activation [24], increase *Gata3* and *FoxP3* gene expression, and stimulate maturation of Treg [25], effects that could be common to humans and mice.

Bacteria in close proximity to epithelial cells may play an important role in gut immune responses. In mice, segmented filamentous bacteria (SFB), which are commensals in many different animal facilities, provide a striking example of the ability of the microbiota to alter the gut immune response. About a decade ago, it was shown that the presence of this commensal drastically increases the frequency of intestinal Th17 cells [26, 27]. SFB tightly adhere to intestinal epithelial cells, and this adhesion appears to be a strong inducer of Th17 responses across species [28]. Moreover, SFB also induce IgA production in the gut. Although SFB have been detected in human ileostomy samples [29], whether they play an equivalent role in humans is still subject of investigation.

Microbiota can also trigger systemic immune responses. Patients with CD have elevated levels of antibodies against flagellin antigens [30], which when present are associated with a more complicated disease course [31]. It is currently not known if these antibodies arise before the disease or after inflammation has exposed the intestinal contents directly to the immune system. Although these findings do not necessarily implicate the antibodies as being pathogenic, the T

cells driving their production may be. Although microbiota-reactive $CD4^+$ T cells are present in the gut of healthy individuals as well as IBD patients [32], adoptive transfer of flagellin-reactive T cells into T cell receptor-deficient mice results in colitis, particularly if the T cells have a Th17 phenotype [33].

Other bacteria, such as *Faecalibacterium prausnitzii*, *Bifidobacterium*, and *Lactobacillus* spp., protect the host through a variety of mechanisms, including modulation of cytokine production [34, 35] and strengthening of the gut barrier function [36]. The evidence for the efficacy of probiotic strains like *Bifidobacterium* and *Lactobacillus* in reducing the symptoms of CD in humans remains unclear, although some beneficial effects have been shown in patients with UC [37]. Additionally, the gut microbiota may protect the host by outcompeting pathogenic bacteria that drive gastrointestinal inflammation by preventing these pathogens from occupying niches [38].

Bacterial-Derived Metabolites

Aside from physical interactions between the microbiota and the host, the products of bacterial metabolism are important regulators of intestinal immunity. The most important metabolites are short-chain fatty acids (SCFA), including butyrate, which are primarily the products of nondigestible carbohydrate fermentation. In addition, bile acid metabolism and products of tryptophan metabolism also have a role.

Activation of the inflammasome can occur via microbiota-accessible carbohydrate (MAC) modulation of the gut microbiota as well as SCFA administration, which promotes IL-18-mediated epithelial repair following DSS-induced GI inflammation [39]. Butyrate produced by the gut microbiota, most prominently by members of the *Clostridia* class, has also been shown to induce the expansion of Tregs in mice, ameliorating intestinal inflammation in an adoptive T cell transfer model of colitis [40]. Several mechanisms have been suggested to explain the anti-inflammatory effect of SCFA. First, some SCFA such as butyrate and propionate alter the epigenetic status of the cells by inhibiting histone deacetylase activity [41]; the resulting changes could induce a regulatory state in both Tregs and innate cells [40, 42, 43]. Additionally, specific receptors on immune cells can recognize SCFA. Dendritic cells and macrophages acquire regulatory activity after recognition of butyrate through Gpr109a [44].

Bacteria can also affect the host by metabolizing bile acids. Bile acids are secreted into the small intestine to aid digestion, and they are toxic to bacteria and eukaryotic cells, modulating the composition of the microbiota. Many bacteria can deconjugate bile acids through removal of taurine or glycine, leading to secondary bile acids [45]. This microbial activity not only influences the rate of bile acid reabsorption through the intestine and subsequent recycling through the enterohepatic cycle, but it can also modulate lipid metabolism [46] and intestinal immunity [47]. Bile acids interact with the intracellular farnesoid X receptor (FXR) and transmembrane receptor Takeda G-protein-coupled receptor 5, which are specific bile acid receptors present in different cell types, including innate immune cells [48]. Inactivation of FXR increases the severity of trinitrobenzenesulfonic acid or DSS-induced colitis in mice, while expression of FXR mRNA was reported to be reduced in colon biopsies from areas of macroscopically inflamed mucosa in CD disease patients [47]. Activation of FXR regulates mechanisms that affect liver and intestinal homeostasis, including reducing the expression of key inflammatory cytokines such as IL-1β, TNF-α, and IFN-γ [47, 49].

Tryptophan metabolites derived from *Lactobacilli* and other microbes are recognized by the aryl hydrocarbon (Ahr) transcription factor and promote IL-22 production by T cells and ILC in preclinical mouse models [50]. IL-22 enhances secretion of antimicrobial peptides, epithelial cell regeneration, and barrier function, and the IL-22-mediated response increases resistance to colonization by the fungus *Candida*

albicans in a mouse model and protects the mice from intestinal inflammation. It has also been shown that tryptophan deficiency, resulting either from the diet or from intestinal malabsorption, leads to dysbiosis and enhanced susceptibility to colitis [51]. Tryptophan deficiency is associated with decreased secretion of IL-22 and IL-17 by mucosal lymphocytes and lower production of intestinal antimicrobial peptides [51]. When these antimicrobial peptides are reduced, the composition of the microbiota is changed to a community that favors intestinal inflammation. More recently, activation of Ahr by kynurenine, a tryptophan metabolite that can be produced by both the microbiota and the host, was shown to increase expression of the IL10 receptor on intestinal epithelial cells [52]. Additionally, recent data have suggested that the IBD-associated polymorphism in caspase recruitment domain family member 9 (*CARD9*) functions by altering the microbiota and tryptophan metabolism [53]. *CARD9*-deficient mice harbor an altered microbiota with decreased capacity to produce Ahr ligands from tryptophan. This dysbiotic microbiota enhances intestinal inflammation in mice, an effect that can be counteracted by tryptophan-metabolizing *Lactobacillus* strains. Importantly, analysis of feces from IBD patients in remission and healthy patients showed that patients with IBD-associated polymorphisms in *CARD9* also have lower levels of Ahr ligands in their feces [53]. More recently, indolepropionic acid (IPA) and related compounds produced by microbial metabolism of tryptophan, tyrosine, and phenylalanine were shown to influence the innate and adaptive immune system in mice. Disruption of the microbial IPA pathway led to increased intestinal permeability and higher frequencies of circulating neutrophils, monocytes, and effector/memory T cells [54]. These data underline the interdependence in the immune/microbiota dynamics. Changes in the immune system, like CARD9 dysfunction, may alter the composition of the microbiota. This altered microbiota affects then the immune response, increasing the severity of colitis.

The Contents of the Microbiota in IBD

From 2010 to the time of this writing, 44 studies using next-generation sequencing methods evaluating the microbiota or metagenome in IBD have been published (Table 19.2). The majority of the studies evaluated the bacterial populations through 16S amplicon sequencing, with a smaller number looking at the fungome or the full metagenome. There is substantial heterogeneity in the study designs, with respect to the disease under study (CD, UC, or both), subject age (pediatrics or adult), disease status (treatment-naïve, long-standing disease, remission), and sample sites (fecal or mucosal). Despite this heterogeneity in study design, several bacteria and one fungus emerged as being consistently negatively or positively associated with IBD, by appearing either over- or underrepresented in patients.

Differences in the Structure of the Microbiota

Structural differences are generally assessed through measures of alpha (within sample) or beta (between samples) diversity. Patients with CD are typically found to have diminished alpha diversity, that is, their microbiota is less diverse, as assessed by either the richness or evenness of the samples [55–71]; this is a less consistent finding in UC (e.g., [56]), although has been reported as well [72]. As discussed previously, the loss of fecal community diversity is often manifested as a decreased abundance of some members of the Firmicutes phylum, including *F. prausnitzii*, a prominent member of the healthy microbiota with significant anti-inflammatory effects [34]. Other species that appear to decrease in relative abundance in IBD include *Bacteroides fragilis*, *B. vulgatus*, *Ruminococcus albus*, Ruminococcus *callidus*, and *Ruminococcus bromii* [73].

While the focus of most studies has been on changes in taxonomic diversity and composition, more recent metagenomic studies indicate that

Table 19.2 Microbiota in IBD

Study	Subjects (n)	Prior abx	Site	Age (years)	Tx	Increased organisms/ functions	Decreased organisms/functions	Comments
Bacterial populations								
Willing et al. [78]	CD, UC	OK	Mostly fecal; some mucosal	Groups ranged from mean 47 to 55 years	N/A	*Bifidobacteriaceae* (CD), *Coriobacteriaceae* (CD), *Ruminococcaceae* (CD), *Anaeroplasmataceae* (CD)	*Ruminococcaceae incertae sedis* (CD), *F. prausnitzii* (CD)	No differences were noted between HC and UC
Lepage et al. [101]	UC	OK	Sigmoid colon	18–52	Yes	*Actinobacteria*	Subset with lower *Bacteroides* and *Prevotella*	
Walker et al. [55]	CD (6), UC (6), HC (5)	2 months	Colon	IBD: 24–73 (mean 34); HC: 45–73 (mean 57)	N/A	*Bacteroidetes* (CD, UC), *Enterobacteriaceae* (CD)	*Firmicutes* (UC)	Decreased AD (CD)
Hansen et al. [56]	CD (11), UC (11), HC (12)	3 months	Distal colon	6–16	None	*Faecalibacterium* (CD)	*Actinobacteria* (CD,UC), *Parabacteroides* (UC), *Burkholderiales* (UC), *Coriobacteriaceae* (CD)	Decreased AD in CD only
Kellermayer et al. [88]	CD (15), HC (26)	6 months	TV colon	7–17 (CD), 3–17 (HC)	None	*Sutterella*	*Roseburia*, *Eubacterium*, *Subdoligranulum*	
Michail et al. [72]	Severe UC (27), HC (26)	1 month	Fecal	13 (mean)	Yes	*Proteobacteria*, *Fusobacteria*, *Spirochaetes*	*Firmicutes*, *Lentisphaerae*, *Verrucomicrobia*	Lower AD in UC
Morgan et al. [23]	CD (121), IC (8), UC (75), HC (27)	OK	TI, colon, feces	Median 27–41	Yes	*Clostridium* (CD,UC), *Enterobacteriaceae* (CD)—especially *Escherichia/Shigella*	Butyrate-producing organisms: *Roseburia* (CD,UC), *Phascolarctobacterium* (CD,UC), *Ruminococcaceae* (CD), *Leuconostocaceae* (UC)	Among IBD patients, treatments, particularly abx, were associated with alterations in the microbiota
								Biopsy and fecal samples differed while showing similar trends
Papa et al. [117]	CD (23), UC (43), HC (24)	N/A	Feces	3–24	Yes	*Escherichia/Shigella*	Multiple rare bacteria from *Rikenellaceae*, *Porphyromonadaceae*, *Peptococcaceae*, and *Akkermansia*	Decreased AD with active disease

Study	Subjects (n)	Prior abx	Site	Age (years)	Tx	Increased organisms/functions	Decreased organisms/functions	Comments
Prideaux et al. [57]	CD (22), UC (30), HC (35)	OK	TI, cecum, rectum	20–63	Yes	*Bacteroidetes* (UC)	*F. prausnitzii* (CD), *Roseburia* (CD), *Firmicutes* (UC), *Coprococcus* (UC), *Dorea* (UC)	Decreased AD (CD)
Tong et al. [58]	CD (16), UC (16), HC (32)	N/A	Multiple biopsy sites	UC 36 years, CD 41 years, HC 60 years	Yes	*Actinobacteria* (IBD as a whole), *Escherichia* (CD)	*Firmicutes* (IBD as a whole), *Faecalibacterium* (CD)	Decreased AD (UC, CD)
Tyler et al. [89]	UC (34), FAP (18)	OK	Pouch and afferent limb	38 (FAP), 52 (UC without pouchitis), 41 (UC with pouchitis)	Yes	*Bacteroidetes*, *Proteobacteria*	*Blautia*, *Bacteroides*, *Parabacteroides*, *Sutterella*	Study compared fecal microbiota in UC versus FAP patients with a pouch 1 year post-IPAA
Chen et al. [59]	CD (26), UC (41), HC (21)	2 months	TI, cecum, rectum, feces	18–70	Yes	Feces: *Fusobacteria* (CD), *Proteobacteria* (CD, UC). Cecum: *Firmicutes* (UC). Rectum: *Proteobacteria* (CD)	Feces: *Faecalibacterium* (CD) TI: *Proteobacteria* (CD) Cecum: *Prevotellaceae* (CD). Rectum: *Prevotella* (UC)	Lower AD in both IBD groups in feces and mucosa Mostly reported changes in rare bacteria
Davenport et al. [163]	CD (13), UC (14), HC (27)	N/A	Multiple biopsy sites	38 ± 11 (CD), 41 ± 11 (UC), 61 ± 7 (HC)	Yes	*Bacteroidetes* (CD)		PICRUsT showed that UC and CD clustered apart, while HC clustered with both
Gevers et al. [79]	CD (468), HC (229)	N/A	Rectal, TI, feces	<17	No	*Enterobacteriaceae*, *Fusobacteriaceae*, *Neisseriaceae*	*Erysipelotrichaceae*, *Bifidobacteriaceae*, *Bacteroides*, *Faecalibacterium*, *Roseburia*, *Blautia*	Most of the findings were seen in the biopsy specimens; not stool
								Enterobacteriaceae predicted poor response to therapy, while *Fusobacterium* and *Haemophilus* predicted good response to therapy

(continued)

Table 19.2 (continued)

Study	Subjects (n)	Prior abx	Site	Age (years)	Tx	Increased organisms/ functions	Decreased organisms/functions	Comments
Kolho et al. [80]	CD (36), UC (26), HC (8), JIA (18)	OK	Fecal	9–18	Yes	*B. fragilis* (CD), *Sutterella wadsworthia* (UC), organisms related to *C. difficile*	*F. prausnitzii* (CD)	Some bacteria predicted response to TNFi, particularly *C. sphenoides* and *Haemophilus* species
								Partial normalization seen among responder to TNFi, compared to non-responders
Lavelle et al. [118]	UC (5), HC (4)	3 months	Cecum, TV colon, DC, rectum	21–58	Yes	*Clostridiaceae*, *Peptostreptococcaceae*, *Enterobacteriaceae*, *Ruminococcaceae*, *Bifidobacteriaceae*, *Actinomycetaceae*	*Bacteroidaceae*, *Akkermansia*	Only the controls underwent bowel preparation
								Within the colon, there was more variability among pts and then within sites, so the authors concluded that stool sampling likely captures a patient's microbiota
Perez-Brocal et al. [60]	CD (20), HC (20)	OK	Feces	14–72	Yes	*Actinomyces*, *P. acnes*, some *Enterobacteriaceae*, *Fusobacterium*	*Roseburia*, *F. prausnitzii*, *Ruminococcus bromii*	Lower AD
Quince et al. [61]	CD (23), HC (21)	3 months	Feces	6–15	Yes	*Peptostreptococcus*, *Escheriia/Shigella*, *Atopobium*, *E. faecalis*	*Faecalibacterium*, *Bifidobacterium adolescentis*, *Ruminococcus bromii*	Lower AD in CD. EEN further lowered AD and dropped abundance of organisms already depleted in CD, such as *F. prausnitzii*
Reshef et al. [164]	UC (131), FAP (9)	1 month	Feces	45 (UC), 57 (FAP)	Yes	Minimal differences	Minimal differences	Study compared fecal microbiota in UC versus FAP patients with a pouch
								No differences in AD

Study	Subjects (n)	Prior abx	Site	Age (years)	Tx	Increased organisms/functions	Decreased organisms/functions	Comments
Wang et al. [128]	IBD (10), HC (5)	OK	Colon	IBD 10–80. No info on the 5 HC	Yes	*Bradyrhizobiaceae*, *Enterobacteriaceae*, *Comamonadaceae*, *Moraxellaceae*		UC and CD samples were pooled for the analysis
Assa et al. [83]	CD (10), HC (15)	3 months	TI	11–16	None	*F. prausnitzii*	None	No differences in AD
Dunn et al. [62]	CD (10), HC (5)	OK	Fecal	9–16		*Bacteroidetes*, *Proteobacteria*	*Firmicutes*	Decreased AD
								Multiple organisms were predictive of therapeutic response to EEN
Forbes et al. [165]	CD (15), UC (21), HC (7)	OK	Multiple biopsy sites	Probably adults	N/A	*Firmicutes*, *Proteobacteria* (UC)	*Bacteroidetes* (UC)	Increased AD in IBD
Hasler et al. [166]	CD (19), UC (17), HC (27)	OK	TI, sigmoid	18–72	Yes	N/A	N/A	No differences in AD
Hedin et al. [63]	Inactive CD (21), HC (46)	N/A	Rectal	16–35	Yes		*F. prausnitzii*	Decreased AD in CD
Hoarau et al. [64]	CD (20), HC (49)	OK	Fecal	Children and adults	Yes	*E. coli*, *Serratia marcescens*, *Ruminococcus gnavus*	*F. prausnitzii*	Decreased AD in CD
Liguori et al. [65]	CD (23), HC (10)	2 months	Colon	Mean 38–48	Yes	*Enterococcus*, *Alicyclobacillus*, *Lactobacillus*	*Faecalibacterium*, *Blautia*, *Dorea*, *Coprococcus*, *Roseburia, Parabacteroides*	Decreased AD in CD
Mar et al. [66]	UC (30), HC (13)	OK	Fecal	22–76	Yes	*Streptococcus*, *Bifidobacterium*, *Enterococcus*	*Bacteroides*, *Prevotella*, *Lachnospiraceae*, *Ruminococcaceae*	Decreased AD in UC
Naftali et al. [81]	CD (31), HC (5)	1 month	Colon, TI	40 (mean)	Yes		*Faecalibacterium*	
Shaw et al. [67]	CD (15), UC (4), HC (10)	OK	Fecal	≤17	No	Positive dysbiosis index in both UC, CD[a]		Decreased AD (both CD, UC)

(continued)

Table 19.2 (continued)

Study	Subjects (n)	Prior abx	Site	Age (years)	Tx	Increased organisms/ functions	Decreased organisms/functions	Comments
Shah et al. [90]	UC (10), HC (13)	N/A	DC, sigmoid	UC: 5–17, HC 11–16	No	*Haemophilus*	*Verrucomicrobia*, *Roseburia*	No differences in AD
Sokol et al. [68]	CD (149), UC (86), HC (38)	2 months	Fecal	40 (mean)	Yes	*Streptococcus anginosus* (IBD as a whole)	*Ruminococcus*, *Coprococcus*, *Blautia*, *Eubacterium Dorea* (IBD as a whole)	Decreased AD (UC, CD)
Takahashi et al. [102]	CD (10), HC (10)	OK	Fecal	Adults	Yes	*Actinomyces*, *Bifidobacterium*	*Bacteroides*, *Eubacterium*, *Faecalibacterium*, *Ruminococcus*	
Tyler et al. [167]	UC (184), FAP (≥30)	OK	TI, sigmoid, pouch	Mean 45	Off at time of surgery	No differences after controlling for antibiotic exposure	No differences after controlling for antibiotic exposure	Study compared fecal microbiota in UC versus FAP patients with a pouch 1 year post-IPAA
He et al. [82]	CD 49, HC 54	OK	Fecal	CD mean 29, HC mean 21	Not stated	*Clostridium symbiosum*, *E. coli*, *Klebsiella pneumoniae*, *Streptococcus salivarius*, and *Clostridium bolteae*	*Bifidobacterium* species, *F. prausnitzii*, *Alistipes shahii,* and *Roseburia* species	Decreased AD
Ijaz et al. [69]	CD 19, HC 31	2 months	Fecal	CD: 10–13. Ctrl: 36–50	Yes	*Enterobacteriaceae*, *Pasteurellaceae*, *Veillonella*, *Dorea*, *Anaerostipes*, *Clostridium* XVIII, *Clostridium* XIVa	*Ruminococcaceae*, *Lachnospiraceae*, *Parabacteroides*, *Akkermansia*, *Methanobrevibacter*	Lower AD in CD Lower genetic functional capacity in CD No differences in fecal SCFA
Knoll et al. [70]	CD (6), UC (6), HC (12)	2 months	Fecal	8–20	Yes	*E. coli* (CD, UC), *Ruminococcus* (CD, UC)	*E. rectale* (UC) and *F. prausnitzii* (UC)	Lower AD in IBD Similar trends were observed in both disease groups, with the findings more pronounced in UC patients
Pascal et al. [71]	IBD in remission: CD (34), UC (33), HC (111)	4 weeks	Fecal	18–58	Yes	*Streptococcus* (UC), *Collinsella* (CD,UC), *Dialister* (CD), *Sutterella* (CD)	*Sutterella* (UC), *Anaerostipes* (CD), *Methanobrevibacter* (CD), Coriobacteriaceae (CD), *Erysipelotrichaceae* (CD), *Peptostreptococcaceae* (CD), *Faecalibacterium* (CD)	Lower AD in CD

Fungal populations								
Kellermayer et al. [88]	CD (15), HC (26)	6 months	TV colon	7–17 (CD), 3–17 (HC)	None	*Malassezia* was associated with granulomatous CD		
Chehoud et al. [135]	CD (26), UC (6), HC (90)	2 weeks	Fecal	IBD: 3–21; controls were pediatric and adult	Yes	*Candida*	*Cladosporium cladosporioides*	IBD patients (CD, UC) were analyzed together Decreased AD
Mukhopadhya et al. [168]	IBD (25), HC (14)	3 months	Sigmoid/ rectum	Mostly children, although some adults	None	*Basidiomycota*	*Ascomycota*	
Hoarau et al. [64]	CD (20), HC (49)	OK	Fecal	Children and adults	Yes	*Candida*		Increased AD in CD
Liguori et al. [65]	CD (23), HC (10)	2 months	Colon	Mean 38–48	Yes	*Saccharomycetes*, *Exobasidiomycetes*, *Sordariomycetes*, *Cystofilobasidiaceae Dioszegia*, *Candida glabrata*	*Leptosphaeria* and *Trichosporon*	No differences in AD
Mar et al. [66]	UC (30), HC (13)	OK	Fecal	22–76	Yes	*C. albicans*, *Debaryomyces*	*Alternaria*, *Aspergillus flavus*, *Aspergillus*, *Cibarius*, *Candida sojae*	
Sokol et al. [68]	CD (149), UC (86), HC (38)	2 months	Fecal	40 (mean)	Yes	*Basidiomycota* (IBD)	*Ascomycota*	
El Mouzan et al. [169]	CD (15), HC (20)	6 months, except for one patient with CD	Fecal and biopsy	4–18	N/A	Biopsy: *Psathyrellaceae*, *Cortinariaceae*, *Psathyrella*, *Gymnopilus*. Fecal: *Cortinariaceae*, *Hymenochaete*, and *Gymnopilus*	Fecal: *Monilinia*	No differences in AD

(continued)

Table 19.2 (continued)

Study	Subjects (n)	Prior abx	Site	Age (years)	Tx	Increased organisms/functions	Decreased organisms/functions	Comments
Metagenome								
Erickson et al. [74]	Inactive or mild CD (8), HC (4)	1 year	Fecal	Approx. 50	N/A	Replication, recombination, and repair	COH transport and metabolism, energy production and conversion, amino acid transport and metabolism, transcription, intracellular trafficking, defense mechanisms, butyrate production	Six of eight had prior major bowel surgery Decreased functional richness in CD
Greenblum et al. [170]	IBD in remission (25), HC (99)	N/A	Fecal	Adults	N/A	Enzyme transport, phosphotransferase		
Dunn et al. [100]	CD (15), HC (5)	OK	Fecal	9–16	Yes	Butanoate, fatty acid metabolism, glyoxylate metabolism, nitrotoluene degradation	NOD-like receptor signaling, polycyclic aromatic hydrocarbon degradation, sphingolipid metabolism	Patients who went into remission following EEN were more similar at baseline to the controls, as compared to patients who did not go into remission
He et al. [82]	CD 49, HC 54	OK	Fecal	CD mean 29, HC mean 21	Not stated	Xenobiotic degradation	SCFA production, carbohydrate metabolism	

AD alpha diversity, *CD* Crohn disease, *DC* descending colon, *DMARD* disease-modifying antirheumatic drugs, *EEN* exclusive enteral nutrition, *FAP* familial adenomatous polyposis, *HC* healthy control, *IBD* inflammatory bowel disease, *IC* indeterminate colitis, *IPAA* ileal pouch-anal anastomosis, *N/A* not available, *PICRUsT* Phylogenetic Investigation of Communities by Reconstruction of Unobserved States, *SCFA* short-chain fatty acids, *TI* terminal ileum, *TV* transverse, *UC* ulcerative colitis

[a]Dysbiosis index in the Shaw study was based upon the Gevers 2014 study: the increased in CD taxa comprise *Enterobacteriaceae*, *Pasteurellaceae*, *Fusobacteriaceae*, *Neisseriaceae*, *Veillonellaceae*, and *Gemellaceae*. Decreased-in-CD taxa are *Bacteroidales*, *Clostridiales* (excluding *Veillonellaceae*), *Erysipelotrichaceae*, and *Bifidobacteriaceae*

the overall quantity of bacteria is also reduced in IBD. In patients with IBD, the fecal metagenome has been shown to possess up to 25% fewer microbial genes, suggesting a lower functional diversity [74]. Metagenomic changes include a loss of genes encoding amino acid and carbohydrate metabolism in IBD compared to healthy controls, while genes involved in transport, secretion, and virulence factors were increased [23]. This raises the possibility that the key factor in IBD is a loss of metabolic pathways, rather than differences in actual taxonomic abundances [23]. Indeed, diminished diversity of fecal metabolomics has also been observed in IBD [75]. A feature of a healthy, diverse microbiome is a high degree of functional redundancy [76]. It is conceivable that a loss of functional redundancy could render the microbiome less able to adapt to adverse perturbations and/or allow potentially pathogenic bacteria to take over previously occupied niches. The concept of protection through niche occupation has been demonstrated in mouse studies in which disruption of the microbiota using oral antibiotics enabled the expansion of pathogenic *Salmonella enterica* serovar *Typhimurium* and *Clostridium difficile*, which are able to utilize host-derived sugars that were previously monopolized by commensal bacteria [38]. In line with this experimental result, infection with opportunistic pathogens such as *C. difficile* is a significant cause of morbidity in IBD patients [77], indicating that they may present an unoccupied niche in their intestinal environment. The *Enterobacteriaceae*, members of the Proteobacteria phylum, have a remarkably diverse pan-genome, and, therefore, they may be well placed to take advantage of any newly vacated niches [76].

Faecalibacterium prausnitzii (Depleted in CD)

Of the 38 studies in CD that included assessments of the bacterial populations, 15 of them reported depletion of *F. prausnitzii* [57–61, 63–65, 70, 71, 78–82], with only two showing the opposite result [56, 83]. This has been observed in both fecal and biopsy specimens, in recent-onset and long-standing disease. Abundance of *F. prausnitzii* also appears to be higher in CD patients in remission versus those with active disease [84], and low abundance of *F. prausnitzii* is predictive of future flares among CD patients undergoing surgical resection [85]. This depletion of *F. prausnitzii* is thus among the most consistent findings of any bacterial species in any disease state. *F. prausnitzii* may have direct regulatory properties; when added to cultures of human peripheral blood mononuclear cells, it upregulated the generation of Tregs and interleukin (IL)-10 [34, 86, 87].

Another mechanism by which *F. prausnitzii* may protect against gut inflammation is through generation of SCFA, including but not limited to butyrate. Indeed, another five studies that did not report depletion of *F. prausnitzii* in IBD patients did identify depletion of other butyrate-producing organisms, such as *Roseburia* and *Blautia* [23, 68, 88–90]. Notably, some of these organisms were also depleted in UC [23, 89, 90]. As reviewed [91], the generation of SCFAs occurs through the metabolism of so-called non-digestible carbohydrates. Branched-chain carbohydrates, which constitute nondigestible fiber, can in fact be metabolized by certain bacteria, constituting their energy source. The breakdown product is the SCFA, which act as proton sinks for the regeneration of NAD+ from NADH during glycolysis [92]. Because bacteria lack mitochondria, they are largely unable to metabolize SCFA any further, thus leaving them to the human host. However, it is important to note that while certain SCFAs may be the metabolic endpoint for some bacteria, SCFAs can act as a substrate for others. For example, acetate and lactate produced by lactic acid bacteria, such as *Bifidobacterium* and *Lactobacillus* spp., can be used as a carbon and energy source by bacteria such as *Eubacterium rectale*, *Roseburia faecis*, and *Faecalibacterium prausnitzii*, which in turn produce butyrate as their metabolic by-product [93–95]. Beneficial properties of SCFAs include inhibition of enteropathogens, increased intestinal epithelial cell health, increased mucin production, and induction of regulatory T cells [96, 97]. It is thus not surprising that fecal metabolomics studies have also shown diminished pro-

duction of SCFAs in patients compared to controls [98, 99]. Additionally, two studies looking at the IBD metagenome showed decreased genetic potential for butyrate or other SCFA production [74, 82], although another study reported the reverse [100].

Bacteroides (Depleted in CD, UC)

Several studies have demonstrated that the *Bacteroides* genus is depleted in both CD and UC [66, 79, 89, 101, 102]. This conclusion was also reached by a review article that, despite being published in 2016, was limited to studies using older technologies such as culture and restriction-length fragment polymorphism and thus has no overlapping studies with the present chapter [103]. A limitation of some of the widely used sequencing technologies is the inability to identify organisms at the species level. However, it is plausible that the depleted organism is *B. fragilis*. This organism prevents intestinal inflammation in mouse models of colitis, mostly through its component polysaccharide A (PSA) [104]. PSA has been reported to induce Foxp3+ Tregs that suppress Th17-mediated intestinal inflammation [105, 106]. In humans, PSA also enhances in vitro Treg induction [107]. A beneficial effect of *Bacteroides* may not be limited to IBD; diminished fecal abundance of *Bacteroides* has also been observed in rheumatoid arthritis [108, 109] and spondyloarthritis (SpA) [110].

A protective effect of *Bacteroides* may be limited to adults. While virtually all studies in adults with IBD that reported differential abundance of *Bacteroides* found it to be protective ([103] and Table 19.2), the pediatrics data are mixed. Of the two studies in pediatric CD that reported differential abundance, one found it to be depleted [79], and the other elevated [80]. Consistent with this observation is that a study that was limited to specific bacteria, including *Bacteroides*, reported decreased abundance in older as compared to younger subjects with CD [111]. Interestingly, studies in juvenile idiopathic arthritis have also shown elevated abundance of fecal *Bacteroides* [112–114], and an increase in *B. ovatus* may precede the onset of type I diabetes in high-risk children [115]. The implications of these findings are not clear. However, an explanation may have been provided by Vatanen et al., who compared the ability of *B. dorei* and *Escherichia coli* to induce endotoxin tolerance, which refers to diminished immunologic response to endotoxin following initial exposure. The authors showed that *B. dorei* had diminished ability to induce endotoxin tolerance, and showed as well that injection of this organism, as compared to injection of *E. coli*, failed to delay the onset of diabetes in a mouse model of the disease [116]. Thus, *Bacteroides* in children may be a two-edged sword, both providing benefit through the PSA tail of *B. fragilis* but also providing increased risk of autoimmunity through altered immunologic maturation.

Akkermansia muciniphila (Depleted in CD, UC)

The third and final organism consistently depleted in IBD is *A. muciniphila*, which was found to be depleted in four studies [69, 72, 117, 118]. This organism was isolated in 2004 and given its name based upon its ability to thrive on intestinal mucins [119]. Most of the literature on this organism focuses on a potentially beneficial role in obesity and metabolic syndrome (e.g., [120]); there is very little literature on its role in inflammatory disease. Asquith et al. demonstrated that in the HLA-B27+ rat model of SpA and IBD, *A. muciniphila* emerges at onset of clinical disease [121], and Stoll et al. reported increased abundance of *A. muciniphila* in a subset of pediatric SpA patients [112]. As patients with SpA and IBD have altered intestinal permeability [122, 123], it is possible that by increasing intestinal permeability, *A. muciniphila* results in increased bacterial invasiveness, which in turn promotes intestinal inflammation. These authors speculate that the decreased abundance of *A. muciniphila* in patients with IBD may be an epiphenomenon reflecting loss of substrate, as previously suggested [90]. That is, as the inflammatory process progresses, the mucin content is lost as has been reported [124], resulting in depletion of *A. muciniphila*.

Other mucus-associated bacteria that may have a role in IBD are sulfate-reducing bacteria such as *Desulfovibrio piger* [125]. Sulfate-reducing

bacteria compete with acetogens and methanogens for hydrogen to produce energy by reducing sulfated mucus glycans, leaving H_2S as a by-product [126]. H_2S has genotoxic properties and can disrupt the mucus structure, as sulfides are potent reducers of disulfide bonds [127].

Enterobacteriaceae, Especially *E. coli*/*Shigella* (Increased in CD, UC)

Thirteen studies have reported increased abundance of the *Enterobacteriaceae* family or specifically of *E. coli*/*Shigella* (which often cannot be distinguished by 16S sequencing), in patients with CD or UC [23, 55, 58, 60, 61, 64, 69, 70, 79, 82, 117, 118, 128]; none have revealed depletion of this organism. The increased *Enterobacteriaceae* abundance may stem from their capacity to use sialic acid and fucose liberated from mucus [38]. Among this family, adherent-invasive *E. coli* (AIEC) has gained particular interest [118]. Pathogenic bacteria such as AIEC may have virulence factors allowing them to interact with M cells, specialized epithelial cells on the surface of Peyer's patches. AIEC could use this interaction to translocate across the epithelial cell barrier into the mucosa [129]. In support of the hypothesis that AIEC contributes to disease by translocating through the intestinal wall barrier, Knoll et al. reported that abundance of *E. coli* correlated with genes implicated in bacterial adhesion to the intestinal mucosa [70]. Additionally, AIEC contains virulence factors such as α-hemolysins that can contribute to impairment of the intestinal wall barrier function, in essence by punching holes in the wall [130]; colonization of colitis-prone IL-10 deficient mice with *E. coli* containing α-hemolysin induced active disease, significantly less so if the bacteria lacked this virulence factor [130]. As reviewed [118], other mechanisms by which AIEC has been linked to IBD include impairment of autophagy as well as of the ubiquitin proteasome activity, the latter resulting in increased activation of NF-κB. Importantly, it has also been proposed that the inflammatory process itself promotes the growth of *Enterobacteriaceae* and thus that the increased abundance of this family may be the consequence not the cause of the underlying disease process [131].

Bifidobacteriaceae (Increased in CD, UC)

Four studies reported increased abundance of the *Bifidobacteriaceae* family in IBD [66, 78, 102, 118], with two reporting it to be depleted [79, 82]. This finding of increased abundance of the *Bifidobacteriaceae* family in IBD, particularly in UC, appears to be a counterintuitive finding, as several species of *Bifidobacterium* are widely incorporated into probiotics, including VSL # 3, which is widely used as therapy for UC (see treatment, below). Indeed, the possibility that these findings reflected prior use of probiotics cannot be entirely excluded. However, in some model systems, *Bifidobacterium* can demonstrate pro-inflammatory effects in vitro, with variation at the species or even the strain level. Specifically, He et al. noted variations among *Bifidobacteria* species to induce IL-12 and tumor necrosis factor (TNF) production from a cell line [132], while Medina et al. demonstrated differences among strains within the *Bifidobacterium longum* species in their ability to induce production of TNF by human peripheral blood mononuclear cells [133]. Conversely, a protective role for *Bifidobacterium longum* in murine colitis has been demonstrated [134]. In light of this contradictory information, there are insufficient data upon which to draw firm conclusions regarding the role of the *Bifidobacteriaceae* family in IBD.

Candida (Increased in CD, UC)

As shown in Table 19.2, most of the studies focused on bacteria. However, just as bacteria can be amplified through sequencing of the 16S ribosomal DNA, so can fungi through their counterpart, the 18S ribosomal DNA. Of the eight studies that evaluated the fungome in patients with IBD, only one consistent result has been reported: increased abundance of *Candida* in patients with CD and to a lesser extent UC; this has been reported in four studies [64–66, 135]. In addition to demonstrating increased fecal abundance of *Candida*, Hoarau et al., also reported an association between abundance of *C. tropicalis* and presence of anti-*Saccharomyces cerevisiae* antibodies (ASCA), which they stated could be triggered by *Candida* as well as by *Saccharomyces cerevisiae*. Despite this finding, the role of fungal organisms

in the pathogenesis of IBD is yet unknown. It is possible that they reflect fungal overgrowth secondary to antibiotics, although findings that ASCA appear prior to development of symptoms suggest that the fungal dysbiosis may be upstream of clinical disease [136]. In addition, mice deficient in dectin-1, a pattern recognition receptor specific for fungi, developed a more severe form of chemical colitis, and polymorphisms in the dectin-1 gene were likewise associated with increased severity of UC in humans [137], suggesting an important role for fungi in the pathogenesis of IBD.

In summary, numerous studies have identified abnormalities in the contents of the human intestinal microbiota in patients with IBD. That the same microbiota are consistently identified as being present in abnormal quantities, either high or low, and are often observed at disease onset, gives credence to the possibility that some of these abnormalities may contribute to the pathogenesis of the disease. Even within the disease, the extent of the microbiota-based abnormalities often correlates with disease severity [84] and can be used to predict response to therapy [85], underscoring a potential pathogenic role. The potential for microbiota-based therapy will be discussed below.

Therapeutic Manipulation of the Microbiota

In practice, there are four ways that the microbiota can be therapeutically altered: diet, antibiotics, probiotics, and fecal microbial transplant. Each of those modalities has been reviewed in depth elsewhere [138–141] and will be summarized briefly below and in Table 19.3.

Table 19.3 Microbial interventions in IBD

	Crohn disease		Ulcerative colitis	
Intervention	Pediatric	Adult	Pediatric	Adult
Antibiotics	+	+	+/−	+/−
Probiotics, e.g., VSL # 3	−	−	+	+
EEN	+	+/−	−	−
FMT	+	+	+	+

Adapted from [153]

Diet

One dietary intervention that has a clearly established place in the treatment of IBD is exclusive enteral nutrition (EEN), which consists of a complete replacement of typical solid foods with liquid nutritional supplements for a period of 4–12 weeks, either orally or via nasogastric tube [142]. EEN appears to be more effective in CD as compared to UC and possibly more effective in children than adults [143]. In children with CD, EEN is as effective as are corticosteroids at inducing remission [144], is thus standard of care for induction therapy in Europe [145], and is increasingly being offered or recommended to patients in the United States in lieu of corticosteroids. The mechanism by which EEN is effective is unclear. While it has striking effects on the microbiota, the net effect is seemingly to make the microbiota even more dysbiotic than its baseline state, with lower alpha diversity and even lower abundance of *F. prausnitzii* [146].

Other dietary approaches have been considered, although most were not necessarily designed with a specific intent of altering the microbiota, so will not be discussed herein. One exception is a diet high in nonabsorbable carbohydrates, such as fructo-oligosaccharides. The rationale behind such a diet is that it may result in increased abundance of butyrate-producing organisms, such as *F. prausnitzii*, which are capable of digesting fiber. In practice, however, studies have not supported this approach [147].

Antibiotics

Antibiotics are a double-edged sword in IBD. Epidemiologic data indicate that early-childhood exposure to antibiotics is associated with an increased risk of acquiring the disease [148], and antibiotics are a risk factor for development of *Clostridium difficile* infection, an important cause of morbidity in patients with IBD [149]. However, there is also an important role for antibiotics as induction and maintenance therapy, particularly in CD, where several studies have demonstrated an important role as induction

therapy as well as postoperative management [150]. They are also used to treat pouchitis, which consists of an inflammatory process of the ileal pouch that occurs with colectomy followed by ileal pouch-anal anastomosis [150]. In UC, antibiotics are less effective, although they may have benefit as add-on therapy to standard treatments [151]. In addition to their therapeutic role, antibiotics are often required to treat infectious complications, including but not limited to abscess development in CD and *C. difficile* infections.

Probiotics

Probiotics are defined as live organisms that are administered in order to have a therapeutic effect on a disease state. In addition to altering the contents of the microbiota, they are postulated to have beneficial effects on gut barrier wall function, immunity, and production of antimicrobial metabolites, among others [152, 153]. A widely used probiotic in patients with UC is VSL # 3, which is a mixture of eight bacterial strains including four species within the *Lactobacillus* genus, three within the *Bifidobacterium* genus, and *Streptococcus thermophilus*. As reviewed [152], randomized and open-label studies in both children and adults with UC have generally found that addition of VSL # 3 to standard treatment reduces disease activity. These findings are not generalizable to all probiotics, as the same review reported that *E. coli Nissle* 1917 was generally ineffective [152]. In addition, while probiotics may be beneficial in the management of pouchitis, they are not otherwise considered to be beneficial in the treatment of CD [153]. While generally considered safe, serious infectious events associated with probiotic strains have been reported [154].

Fecal Microbial Transplantation (FMT)

Although it has been reported that the idea behind FMT dates to nearly two millennia ago [155], this is a relatively recent development in IBD. The initial purpose behind FMT was as a therapeutic alternative to subjects with recurrent *C. difficile* infections [156], although improvements were subsequently noted in the underlying bowel disease of subjects who had both IBD and *C. difficile* [157]. Thus, subsequent studies were geared toward using FMT as a therapy for IBD itself. After some positive case reports [158, 159], randomized trials were conducted, with mixed results [160]. However, studies that used multiple donors and also that involved pretreatment with antibiotics, presumably to clear out the existing microbiota to allow the normal microbiota to take root, appeared to have shown particular benefit [141, 160]. In the United States, the Food and Drug Administration has deemed FMT to be experimental for any purpose other than treatment of recurrent *C. difficile* infection, so this procedure is only available in the context of a clinical trial. Multiple parameters, including whether the transplants should consist of donor samples or defined consortium of microbiota, and whether they should be administered via upper (e.g., by gavage) or lower (endoscopy) delivery, have yet to be definitively established. In addition, as with probiotic therapy, this treatment carries with it a rare but non-zero risk of serious infections caused by the introduced bacteria [161]. Thus, the precise role of FMT in the management of CD and UC has yet to be fully defined.

Conclusions

In this chapter, we have presented compelling evidence that the microbiota is altered in patients with IBD, particularly CD. It is likely that at least some of these changes, such as increased abundance of pathogenic bacteria including adherent-invasive *E. coli* and depletion of butyrate-producing organisms such as *F. prausnitzii*, contribute to the disease. The microbiota has a profound impact on intestinal immune responses, which drive intestinal inflammation. In turn, the immune system can impact the microbiota and cause dysbiosis. This resulting dysbiosis could lead to exacerbation of inflammation in IBD. Therapeutic manipulation of the microbiota through EEN, antibiotics, and probiotics is a routine part of clinical care for both CD and UC. We hope that

the future holds in store more targeted means of altering the microbiota that can safely and effectively restore a more normal state.

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