REVIEW ARTICLE

https://doi.org/10.4110/in.2017.17.1.1

Immune Network

pISSN 1598-2629 · eISSN 2092-6685

Host-microbial Cross-talk in Inflammatory Bowel Disease

Hiroko Nagao-Kitamoto and Nobuhiko Kamada*

Division of Gastroenterology, Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, MI 48109, USA

A vast community of commensal microorganisms, commonly referred to as the gut microbiota, colonizes the gastrointestinal tract (GI). The involvement of the gut microbiota in the maintenance of the gut ecosystem is two-fold: it educates host immune cells and protects the host from pathogens. However, when healthy microbial composition and function are disrupted (dysbiosis), the dysbiotic gut microbiota can trigger the initiation and development of various GI diseases, including inflammatory bowel disease (IBD). IBD, primarily includes ulcerative colitis (UC) and Crohn's disease (CD), is a major global public health problem affecting over 1 million patients in the United States alone. Accumulating evidence suggests that various environmental and genetic factors contribute to the pathogenesis of IBD. In particular, the gut microbiota is a key factor associated with the triggering and presentation of disease. Gut dysbiosis in patients with IBD is defined as a reduction of beneficial commensal bacteria and an enrichment of potentially harmful commensal bacteria (pathobionts). However, as of now it is largely unknown whether gut dysbiosis is a cause or a consequence of IBD. Recent technological advances have made it possible to address this question and investigate the functional impact of dysbiotic microbiota on IBD. In this review, we will discuss the recent advances in the field, focusing on host-microbial cross-talk in IBD.

[Immune Network 2017;17(1):1-12]

Keywords: Gut microbiota, Dysbiosis, Pathobiont, Inflammatory bowel disease

INTRODUCTION

In recently years, technological advances in microbiological research, such as next generation sequencing, improved anaerobic culture methods, high-throughput analysis of luminal metabolites, and wider availability of gnotobiotic (GB) mouse models, have revolutionized our understanding of the functional impact gut microbiota has on host physiology and disease. Mounting evidence suggests that the gut microbiota interacts with the host innate and adaptive immune system. For instance, the gut microbiota plays key roles in the maturation and differentiation of Th17 cells, regulatory T (Treg) cells, innate lymphoid cells and immunoglobulin A-producing B cells (1-3). Additionally, the resident gut microbiota is crucial for the protection of the host against pathogenic

Received on November 17, 2016. Revised on January 19, 2017. Accepted on January 27, 2017.

*Corresponding Author. Nobuhiko Kamada, Division of Gastroenterology, Department of Internal Medicine, University of Michigan Medical School, 1150 W. Medical Center Dr., Ann Arbor, MI 48109, USA. Tel: 1-734-763-2142; Fax: 1-734-763-2535; E-mail: nkamada@umich.edu

Abbreviations: AIEC, adherent-invasive *E. coli*; *A. parvulum*, *Atopobium parvulum*; *B. fragilis*, *Bacteroides fragilis*; *B. vulgatus*, *Bacteroides vulgatus*; *B. adolescentis*, *Bifidobacterium adolescentis*; *B. wadsworthia*, *Bilophila wadsworthia*; CEACAM6, carcinoembryonic antigen-related cell adhesion molecule 6; *C. difficile*, *Clostridium difficile*; CDI, *Clostridium difficile* infection; *C. scindens*, *Clostridium scindens*; CD, Crohn's disease; DCs, dendritic cells; DCA, deoxycholic acid; DSS, dextran sulfate sodium; *D. invisus*, *Dialister invisus*; *E. faecalis*, *Enterococcus faecalis*; ETBF, *Enterotoxigenic Bacteroides fragilis*; *E. coli*, *Escherichia coli*; *E. rectale*, *Eubacterium rectale*; *F. prausnitzii*, *Faecalibacterium prausnitzii*; FMT, fecal microbiota transfer; FODMAPs, fermentable oligosaccharides, disaccharides, monosaccharides, and polyols; *F. nucleatum*, *Fusobacterium nucleatum*; GI, gastrointestinal; *H.hepaticus*, *Helicobacter hepaticus*; hGB, humanized gnotobiotic; H_2S, hydrogen sulfate; IBD, inflammatory bowel disease; *K. pneumoniae*, *Klebsiella pneumoniae*; *pks*, polyketide synthases; PSA, polysaccharide A; Treg, regulatory T; *R. hominis*, *Roseburia hominis*; *R. albus*, *Ruminococcus albus*; *R. bromii*, *Ruminococcus bromii*; *R. callidus*, *Ruminococcus callidus*; SCFAs, short chain fatty acids; UC, ulcerative colitis

microorganisms. The gut microbiota directly inhibits the growth and/or colonization of incoming pathogens by competing for limited nutrients and occupying the potential intestinal habitat (4). Furthermore, the resident gut microbiota protects the host against pathogen colonization through its involvement in mucus production, enhancement of epithelial cell barrier function, the release of antimicrobial peptides, and the production of immunoglobulins (5,6). Likewise, metabolites generated by the gut microbiota are essential for the maintenance of intestinal homeostasis (7). Short-chain fatty acids (SCFAs), the end products of anaerobic bacterial fermentation of dietary fiber, regulate anti-inflammatory immune responses and enhance epithelial cell barrier function (7,8). Gut dysbiosis results in the alteration of the intestinal metabolic profile, causing perturbation of gut immune homeostasis (9). Thus, the composition of the gut microbiota is strongly associated with host physiology and perturbations of the gut microbial communities lead to the development of various GI disorders, including inflammatory bowel disease (IBD) (10,11). Here, we review the recent advances in the field, focusing on host-microbial cross-talk in IBD.

IBD-ASSOCIATED DYSBIOSIS IN THE GUT

Decreased beneficial bacteria

It has been extensively reported that the abundance of beneficial commensal bacteria in IBD patients, both ulcerative colitis (UC) and Crohn's disease (CD), is reduced. Several studies using meta-genomics analysis have demonstrated that members of the phylum Firmicutes are less abundant in patients with UC or CD (12-14). Among Firmicutes, *Clostridium* clusters XIVa and IV are largely underrepresented in the gut of IBD patients (15-17). *Clostridium* cluster XIVa comprises species belonging to the *Clostridium*, *Ruminococcus*, *Lachnospira*, *Roseburia*, *Eubacterium*, *Coprococcus*, *Dorea* and *Butyrivibrio* genera. *Clostridium* cluster IV consists of *Clostridium*, *Ruminococcus*, *Eubacterium* and *Anaerofilum* genera. *Faecalibacterium prausnitzii* (*F. prausnitzii*), which belongs to *Clostridium* cluster IV, is well known to be less abundant in ileal biopsy and fecal samples isolated from CD (14,15,18-20) and UC patients compared to healthy subjects (21). Notably, the most significant reduction in the abundance of *F. prausnitzii* is observed in patients with active IBD rather than those in remission (19) and patients with recurrent IBD (18), suggesting that *F. prausnitzii* plays anti-inflammatory roles in the gut. Sokol et al. showed that human PBMC stimulation with *F. prausnitzii* induced the production anti-inflammatory IL-10, whereas it inhibited the secretion of pro-inflammatory cytokines, such as IL-12 and IFN-γ (18). Oral administration of *F. prausnitzii* in the TNBS-induced colitis model promoted IL-10 production, and reduced IL-12 and tumor necrosis factor (TNF)-α levels in the colon, thereby ameliorating experimental colitis. Furthermore, metabolites produced by *F. prausnitzii* inhibited NF-κB activation in the mucosa and induced IL-8 production (18). *F. prausnitzii* can produce butyrate by metabolizing diet-derived polysaccharides and other host-derived substrates (22,23). Butyrate is a major source of energy for colonic epithelial cells and is essential for the maintenance of colonic mucosal health. For example, microbiota-produced butyrate induces the development of regulatory T cells (24,25) and its administration restores intestinal homeostasis in rats by suppressing IL-17 production in Th17 cells, suggesting butyrate plays a critical role in the regulation of Treg/Th17 balance (26). In addition to *F. prausnitzii*, accumulating evidence indicates that the abundance of other butyrate-producing bacteria in the human gut, such as *Roseburia hominis* (*R. hominis*) and *Eubacterium rectale* (*E. rectale*), which belong to phylum Firmicutes, is reduced in IBD patients compared to control subjects (14,21). Perhaps not surprisingly, the intestinal levels of the other SCFAs, including acetate and propionate, are also known to be perturbed in IBD patients due to gut dysbiosis. *Dialister invisus* (*D. invisus*) is capable of generating both acetate and propionate (27), and the abundance of this bacterium is reduced in patients with CD (15). The most abundant bacterial families represented in the human gut microbiota are Ruminococcaceae and Lachnospiraceae (phylum Firmicutes, class Clostridia). Several studies have reported that the abundance of these families is decreased in IBD patients (23). The abundance of *Ruminococcus albus* (*R. albus*), *Ruminococcus callidus* (*R. callidus*), and *Ruminococcus bromii* (*R. bromii*) in the fecal samples of CD patients was >5-fold lower than in healthy control subjects (14). *R. albus* produces acetate by degrading dietary cellulose (28). Acetate is the most abundant SCFAs in the colon and the microbiota-produced acetate is utilized by other bacteria, such as *F. prausnitzii* and *Roseburia spp.*, to generate butyrate (29). While *R. callidus* and *R. bromii* can degrade other complex polysaccharides, including starch and xylan, and the degradation products have an impact on the gut microbial community as well as human health (30,31), dietary cellulose and polysaccharides are important

Table I. IBD-associated dysbiosis in the gut

Disease	Sample source	Phylum	Family	Species	possible mechanisms	References
Decreased beneficial bacteria						
UC and CD	Ileum, Fecal	Firmicutes	Clostridiaceae	*Faecalibacterium prausnitzii*	Butyrate↓, anti-inflammatory effect ↓ (IL-10↓, NF- IL-12↑, IFNg↑, TNF-a↑, IL-8↑, NF-κB activation)	14, 15, 18, 19, 20, 21
UC	Fecal	Firmicutes	Lachnospiraceae	*Roseburia hominis*	Butyrate↓	21
CD	Fecal	Firmicutes	Eubacteriaceae		Butyrate↓	14
CD	Fecal	Firmicutes	Veillonellaceae	*Dialister invisus*	Propinate↓, acetate↓	15
CD	Fecal	Firmicutes	Ruminococcaceae	*Ruminococcus albus*	Cellulose degradation↓, Acetate	14, 28
CD	Fecal	Firmicutes	Ruminococcaceae	*Ruminococcus callidus*	Polysaccharides degradation↓ (e.g. starch, xylan)	14, 31
CD	Fecal	Firmicutes	Ruminococcaceae	*Ruminococcus bromii*	Polysaccharides degradation↓ (e.g. starch, xylan)	14, 31
UC and CD	Ileum, Fecal	Firmicutes	Ruminococcaceae	*Butyricicoccus* spp.	Butyrate↓	20
UC and CD	Ileum, Fecal	Firmicutes	Lachnospiraceae	*Anaerostipes spp.*	Butyrate ↓	20
UC and CD	Ileum, Fecal	Firmicutes	Lachnospiraceae	*Dorea spp.*	Formate↓, acetate↓	20
UC and CD	Ileum, Fecal	Actinobacteria	Coriobacteriaceae	*Coriobacteriaceae* spp.	Lactate↓, formate↓, acetate↓	20
UC and CD	Fecal	Actinobacteria	Bifidobacteriaceae	*Bifidobacterium adolescentis* spp.	Folate↓, Treg↓	15, 17, 36, 37, 38
CD	Fecal	Bacteroidetes	Bacteroidaceae	*Bacteroides fragilis*	Immune system (Th1/Treg balance) disruption, *Helicobacter hepaticus*-induced colotis↑	40, 41, 42
CD	Fecal	Bacteroidetes	Bacteroidaceae	*Bacteroides vulgatus*	*Escherichia coli*-induced colotis↑	43
Increased pathobionts						
CD	Ileum	Proteobacteria	Enterobacteriaceae	*Escherichia coli (AIEC)*	Pro-inflammatory cytokines↑ (IL-6, IL-12, IL-23, IL-17, TNF-α)	49,50
UC and CD	Colon	Proteobacteria	Enterobacteriaceae	*pks+ Escherichia coli*	Colibactin↑, DNA damage↑	51, 52, 53
UC and CD	Colon	Proteobacteria	Enterobacteriaceae	*Klebsiella* spp.	Colitogenic bacteria in TRUC mice	56, 57, 58, 59
CD	Colon, Ileum	Proteobacteria	Pseudomonadaceae	*Pseudomonas* spp.	Epithelial cell damege↑	60
UC and CD	Fecal	Proteobacteria	Enterobacteriaceae	*Salmonella* spp.	Immune system (Th1/Th2 balance) disruption	58, 61, 62
UC and CD	Fecal	Proteobacteria	Desulfovibrionaceae	Sulfate-reducing bacteria (e.g.*Bilophila wadsworthia, Desulfovibrio* spp., *Desulfuromonas* spp)	H2S↑, toxic product↑, butyrae↓, imuune system (Th1) disruption	68, 69, 70
UC and CD	Fecal, Colon	Proteobacteria	Campylobacteraceae	*Campylobacter concisus*	IL-8↑, pro-inflammatory effect↑ (e.g. IL-6↑, IL-12, TNF-α, NF-κβ activation)	71, 72, 73, 74, 75, 76
UC and CD	Fecal	Proteobacteria	Campylobacteraceae	Campylobacter jejuni	IL-8↑, Proinflammatory effect↑ (e.g. IL-1b↑,TNF-a↑)	71, 72, 74, 76
UC and CD	Fecal	Bacteroidetes	Bacteroidaceae	Enterotoxigenic *Bacteroides fragilis*	Toxin↑, Epithlial cell function disruption, pro-inflammatory cytokines↑ (e.g. IL-8)	77, 78, 79
CD	Fecal	Actinobacteria	Coriobacteriaceae	*Atopobium parvulum*	H2S↑, mitochondrial function disruption	80, 81
UC and CD	Fecal	Firmicutes	Clostridiaceae	*Clostridium difficile*	Clostridium scindens↓, primary bile acids↑, secodary bile acids↓	82, 83, 84, 85

energy sources for intestinal microorganism (Table I).

Accumulating evidence has demonstrated that several environmental factors affect the development and progression of GI diseases, including IBD, through the induction of intestinal dysbiosis (32,33). For instance, recent meta-analysis results revealed that smoking leads to a decrease of butyrate-producing *Anaerostipes spp.*. *Anaerostipes spp.* belong to family Lachnospiraceae and convert lactate to butyrate. The abundance of these bacterial species has been shown to be reduced in IBD patients who smoke (20). Antibiotic use is recognized as the most influential factor linked to disturbances in microbial community structure and function. Some reports suggest that exposure to one or more antibiotics in the first year of life correlates with higher incidence of pediatric IBD (34,35). Disturbance of the gut microbiota could be a possible mechanism linking IBD development to antibiotic treatment. Indeed, the abundance of *Dorea spp.*, *Butyricicoccus spp.*, and *Coriobacteriaceae spp.* is reduced in patients with IBD who have been treated with antibiotics such as ciprofloxacin and metronidazole (20). Furthermore, fecal microbiota analysis of samples isolated from IBD patients has shown an attenuation of *Bifidobacterium adolescentis* (*B. adolescentis*), a folate-producing bacterium (15,17,36). Folate (also known as vitamin B9) is involved in several metabolic pathways that are required for cell division. In fact, folate supplementation is recommended for IBD patients due to its ability to regulate rectal cell proliferation (37). Moreover, folate is known to enhance the survival of $Foxp3^+$ regulatory T (Treg) cells, thus diminishing intestinal inflammation through stabilization of the intestinal Treg population (38). These results suggest that organic compound-producing bacteria regulate intestinal homeostasis. In IBD, the abundance of these beneficial bacteria is reduced, thereby resulting in impaired gut function (Table I).

Bacteroides make up the predominant portion of the gut microbiota and play a major role in metabolic activities (39). One member of this genus, *Bacteroides fragilis* (*B. fragilis*), has been shown to have a beneficial effect on the host immune system. *B. fragilis* produces polysaccharide A (PSA), which is recognized as a symbiosis factor used to regulate pathogenic/Treg cell balance. It also exerts anti-inflammatory effects. PSA from *B. fragilis* is presented by intestinal dendritic cells (DCs) and activates $CD4^+$ T cells, thereby inducing the differentiation of $Foxp3^+$ Tregs. It also induces the secretion of an anti-inflammatory cytokine IL-10, which in turn suppresses the production of pro-inflammatory cytokines, such as IL-17, IL-23, and TNF-α (40,41). Additionally, mono-colonization of germ-free mice with PSA-producing *B. fragilis* modulated pathogenic/regulatory T cell balance and, therefore, protected the host from *Helicobacter hepaticus* (*H.hepaticus*)-induced intestinal inflammation (41,42). *Bacteroides vulgatus* (*B. vulgatus*) is the numerically predominant Bacteroides species in the human gut microbiota. A previous study has shown that *B. vulgatus* abrogates *Escherichia coli* (*E. coli*)-induced colitis in IL-2 deficient gnotobiotic mice, but the mechanism is unknown (43). Notably, the abundance of *B. fragilis* and *B. vulgatus* is lower in fecal samples of CD patients compared to those of healthy subjects (14) (Table I).

Increased pathobionts

As we have reviewed so far, the abundance of beneficial commensal bacteria is significantly decreased in IBD patients. Also, mounting evidence demonstrates that certain members of commensal bacteria, which harbor potential pathogenic features, referred to as pathobionts, are enriched in IBD patients. For example, several microbiome analyses have revealed there is an expansion of the Proteobacteria phylum in IBD patients (12,13,44). The phylum Proteobacteria is composed of six classes: Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Deltaproteobacteria, Epsilonproteobacteria and Zetaproteobacteria. Among these, class Gammaproteobacteria is largest and includes *E. coli*. Several lines of evidence indicate that certain pathogenic *E. coli* strains accumulate in the intestinal mucosa/stool of IBD patients, especially CD (14,45-47). For instance, *E. coli* strains that display adherent and invasive properties, called adherent-invasive *E. coli* (AIEC) (48), have repeatedly been isolated from IBD patients. AIEC was isolated from >35% of terminal ileum specimens isolated from CD patients, while only from 6% of healthy samples (49). AIEC can invade intestinal epithelial cells and reach the intestinal barrier, resulting in tissue damage and inflammation. AIEC colonizes the gastrointestinal tract via a type-1 pilus variant that recognizes the carcinoembryonic antigen-related cell adhesion molecule 6 (CEACAM6), which is abnormally expressed by ileal epithelial cells of CD patients (50). In the inflamed ileal mucosa, expanded expression of CEACAM6 augments the intracellular AIEC expansion, thereby leading to a massive production of pro-inflammatory cytokines, such as IL-6, IL-12, IL-23, IL-17, and TNF-α (50) (Table I). In addition to AIEC, polyketide synthase $(pks)^+$ *E. coli* is known to be pathogenic in IBD (51). It is noteworthy that pks^+ *E. coli* strains are more abundant in patients with IBD than in control subjects. The *pks* gene is a genomic

island of pathogenicity that encodes multi-enzymatic machinery for the production of a bacterial genotoxin called colibactin (52) (Table I). It has also been reported that *pks*$^{+}$ *E. coli* induces DNA damage *in vivo* (53) and promotes invasive carcinoma in the murine colorectal cancer (CRC) model (51). Given the evidence that IBD is a risk factor for CRC (54,55), the more abundant genotoxic bacteria might be responsible for the increased susceptibility to CRC associated with IBD, although the pathogenic contribution of *pks*$^{+}$ *E. coli* to intestinal inflammation per se remains unclear.

Klebsiella spp., *Pseudomonas* spp., and *Salmonella* spp. (phylum Proteobacteria, class Gammaproteobacteria) are implicated in IBD (56-58). Indeed, when *Klebsiella pneumoniae* (*K. pneumoniae*) is used to colonize T-bet Rag deficient ulcerative colitis (TRUC) mice (IBD-prone mouse model) it causes the development of colitis, indicating it is a pathobiont (59) (Table I). Bacterial 16S PCR analysis showed that *Pseudomonas* spp. were detected in 58% of ileal biopsy samples from pediatric CD patient with CD versus 33% of healthy children (58). *Pseudomonas* spp. have the ability to attach to intestinal epithelial cells and deliver toxins directly into host cells through a type 3 secretion system, thereby causing epithelial cells damage (60) (Table I). A cohort study has shown that *Salmonella* infection is associated with increased risk of IBD (58) (Table I). *Salmonella* infection is recognized as a major health problem worldwide. The infection triggers intestinal colitis by disrupting Th1/Th2 balance (61). Interestingly, *Salmonella* cannot induce colitis in Nod1 and Nod2-deficient mice (62). This suggests that the sensing of *Salmonella* is mediated by Nod1 and/or Nod2. Since loss-of-function mutations of the *NOD2* gene are associated with increased risk of CD (63), *Salmonella* infection itself may not trigger inflammation in CD patients who harbor *NOD2* mutations. Rather, luminal environmental changes elicited by *Salmonella* might increase the susceptibility of the host to diseases caused by different pathobionts. Class Deltaproteobacteria (phylum Proteobacteria) includes sulfate-reducing bacteria. The abundance of these bacteria is also known to be increased in patients with IBD (64-67) (Table I). It has been proposed that sulfate-reducing bacteria aggravate gastrointestinal diseases by producing hydrogen sulfate (H_2S) and other toxic byproducts as well as by decreasing the availability of beneficial metabolites, such as butyrate (68). Devkota et al. demonstrated that the abundance a sulfate-reducing commensal bacterium *Bilophila wadsworthia* (*B. wadsworthia*) was increased due to excessive amounts of taurocholic acid derived from dietary fat. Colonization of IBD-prone *IL-10*$^{-/-}$ mice by *B. wadsworthia* resulted in Th1-mediated colonic inflammation (69). Other sulfate-reducing bacteria, such as *Desulfovibrio* spp. and *Desulfuromonas* spp., are also more prevalent in patients with IBD compared to healthy individuals (70). The family *Campylobacteraceae* (phylum Proteobacteria, class Epsilonproteobacteria) is also involved in the pathogenesis of IBD. Recent reports indicate that the abundance of *Campylobacter concisus* (*C. concisus*) and *Campylobacter jejuni* (*C. jejuni*) in intestinal biopsies or fecal samples isolated from IBD patients is higher than those of non-IBD controls (71-73). Others have reported that *C. concisus* and *C. jejuni* infections are closely linked to IBD flare-ups (74). For instance, the invasive ratio of *C. concisus* strains isolated from patients with chronic intestinal disease was >500-fold higher than those from patients with acute intestinal disease or healthy subjects (75). Additionally, attachment of *C. concisus* and *C. jejuni* to intestinal epithelial cells prompts IL-8 secretion. The induced IL-8 mediates the recruitment of neutrophils, DCs and macrophages. The recruited cells interact with internalized *C. concisus* and *C. jejuni*, resulting in the activation of NF-κB and production of pro-inflammatory cytokines, such as IL-1, IL-6, IL-12 and TNF-α (71,72,76) (Table I). Collectively, several Proteobacteria act as putative pathobionts in IBD and multiple IBD risk factors, including genetics and diet, can induce their uncontrolled expansion.

Enterotoxigenic strains of *Bacteroides fragilis* (ETBF) produce *B. fragilis* toxin, which is associated with IBD (77). ETBF was detected in 13.3% of fecal samples isolated from patients with IBD versus 2.9% of control groups. Interestingly, all of the ETBF-positive specimens were isolated from UC and CD patients with active disease (78). Biochemically, the ETBF-produced toxin binds to colonic epithelial cells, inducing the cleavage of E-cadherin, decreasing mucosal barrier function and increasing pro-inflammatory cytokine (IL-8) production (77). Furthermore, ETBF has been shown to cause severe colitis in dextran sodium sulfate (DSS-commonly used to trigger IBD-like experimental colitis in mice) treated wild-type mice, while non-toxigenic *B. fragilis* did not exacerbate the DSS-induced colitis (79). These findings suggest that the ETBF colonization may contribute to the initiation and aggravation of intestinal colitis, and the enteric microenvironment associated with IBD is conducive to the overgrowth of pathogenic ETBF, resulting in the depletion of symbiotic *B. fragilis* (Table I). Impaired mitochondrial function is also a defining feature of IBD, especially in pediatric CD (80).

Mottawea, W. et al. have recently reported that the levels of one mitochondrial protein, H_2S-detoxification protein, were decreased in CD patients. In contrast, the authors observed an increase in the abundance of *Atopobium parvulum* (*A. parvulum*) and its abundance positively correlated with disease severity (Table I). *A. parvulum* is a known producer of H_2S. Excessive amounts of H_2S in the gut have been shown to cause epithelial cell damage (81). In fact, *A. parvulum* exacerbated colitis in *IL-10*$^{-/-}$ mice and the administration of a H_2S scavenger ameliorated this phenotype (80). *Clostridium difficile* (*C. difficile*) infection (CDI) is a serious complication in IBD patients, because it worsens the long-term course and outcome of disease. Accumulating data suggest that antibiotic-induced dysbiosis associated with IBD can give rise to blooms of *C. difficile*. In this context, exposure to antibiotics inhibits the conversion of primary bile acids to secondary bile acids mediated by commensal bacteria (82). Since secondary bile acids, such as deoxycholic acid (DCA), can prevent the growth and germination of *C. difficile*, impaired conversion of primary bile acids to secondary, caused by dysbiosis, weakens colonization resistance of the commensal microbiota against *C. difficile* (83,84). In fact, it has been recently shown that antibiotics decrease the abundance of *Clostridium scindens* (*C. scindens*), a bile acid 7 alpha-dehydroxylating bacterium. Decreased abundance of *C. scindens* can foster the growth of *C. difficile* due to lower production of DCA (85). Consistently, an imbalance of the microbiome-bile acid pool may lead to increased CDI susceptibility associated with IBD (Table I).

THE UTILITY OF A HUMANIZED GNOTOBIOTIC MOUSE SYSTEM

There is a growing body of evidence suggesting that gut dysbiosis contributes to the initiation and pathogenesis of IBD. However, it remains unclear whether IBD-associated gut dysbiosis is related to disease pathogenesis or is merely a secondary change caused by intestinal inflammation and/or medication. To clarify the extent to which dysbiotic microbiotas have a functional impact on disease pathogenesis, recent studies have utilized a gnotobiotic mouse system. In this system, germ-free (GF) mice are colonized with a whole or selected bacterial cocktail isolated from IBD patients. This system, referred to as humanized gnotobiotic (hGB) mouse model or human microbiota-associated mouse (HMA) model, is a powerful tool that can be used to evaluate the functional significance of colonization by putative pathobionts on the host immune system as well as the progression of disease *in vivo*. The hGB mouse model has been used to prove that mono-colonization with a bacterium or yeast, associated with IBD pathogenesis, impacts host gene expression and metabolic profiles (86). This model has revealed that colonization by *Bacteroides thetaiotaomicron* markedly facilitates the expression of genes associated with maturation of the immune system, including Treg activation. Additionally, Eun C. S. *et al*. have shown that a simplified human microbiota consortium, which comprises 7 strains of bacteria that are closely linked to IBD, induces inflammation in *IL-10*$^{-/-}$ mice through Th1 and Th17 cell responses. This report also demonstrates that colonization by AIEC and *Ruminococcus guavus* is necessary for the induction of intestinal inflammation, indicating these 2 bacteria play a key role in IBD pathogenesis (87).

In another study, putative IBD pathobionts were rationally selected as "Immunoglobulin A (IgA)-coated" bacteria (88). In both UC and CD patients, subsets of IgA-coated luminal bacteria are enriched compared to healthy control subjects. Strikingly, colonization of GF mice with this IgA-coated bacterial consortium elicits the development of more severe colitis symptoms upon treatment with DSS compared to GF mice colonized with IgA-unbound bacteria. Thus, colitogenic commensal bacteria are enriched in IBD patients and these potential pathobionts are "flagged" by IgA. Since a recent study demonstrated that depletion of IgA-coated bacteria by treatment with an anti-IgA antibody ameliorates colitis (89), therapeutic strategies that target IgA-coated bacteria might be an effective form of treatment for IBD.

In addition to these reports, we have recently used hGB mice to study defective functionality of the IBD-associated microbiota (90). In this study, we utilized a combined multi-OMICS approach to unravel the influence of dysbiosis on the host ecosystem. Structural and functional changes associated with gut dysbiosis were analyzed by 16S rRNA sequencing and functional gene (PICRUSt) analysis, unbiased luminal metabolome analysis, and host gene expression analysis. Furthermore, the impact of IBD-associated dysbiosis on disease pathogenesis was assessed by colonization of GF IL-10$^{-/-}$ mice with IBD and control microbiotas. Based on this study, it is evident that the structural and functional alterations of the gut microbiota present in IBD are, at least to some extent, recapitulated in hGB mice. For instance, α-diversity and richness of the microbiota are significantly reduced in hGB mice colonized with IBD microbiotas compared to hGB mice colonized with healthy microbiotas. These

phenotypes are also observed in donor stool samples. Likewise, there are differences between luminal metabolic profiles associated with IBD-hGB mice and healthy-hGB mice. IBD microbiotas differentially impact gene expression in the colon compared to healthy microbiotas. For example, CD patient-derived microbiotas elicit the expression of genes related to pro-inflammatory immune responses, including Th1, Th17, IL-1β pathways. At the same time, CD microbiotas also down-regulate the expression of genes related to the solute carrier group of proteins and the cytochrome families. Notably, the gene expression patterns observed as a result of colonization by CD microbiotas are similar to those occurring in newly diagnosed pediatric CD patients (90). Collectively, this evidence suggests that gut dysbiosis drives changes in intestinal immune responses. Importantly, colonization by CD microbiotas results in the development of severe colitis in GF *IL-10*$^{-/-}$ mice, an effect not seen with healthy or UC microbiotas. Thus, it is thought that gut dysbiosis in IBD patients is not only a secondary "non-functional" change. Rather, gut dysbiosis has a direct influence on the immune system, making the host more susceptible to disease. Taken together, the gnotobiotic mouse system is a powerful tool that can be used to evaluate the functional role of IBD-associated dysbiosis in the pathogenesis of IBD.

DISCUSSION

Several lines of evidence support the notion that gut dysbiosis contributes to the pathogenesis of IBD. As described above, beneficial bacteria are less abundant while pathogenic bacteria are increased in IBD patients (Table I). This imbalance of the gut microbiota is not merely a secondary consequence of disease. Rather, it is thought that a dysbiotic microbiota has inflammatory potential and/or interfere with protective/regulatory function of the immune system. Hence, novel therapeutic strategies that selectively target dysbiosis have attracted considerable interest and offer hope for more effective IBD treatment options. For instance, fecal microbiota transplantation (FMT), which aims to restore normal gut microbial composition and function appears to be an effective treatment for IBD (91,92). It has been shown that FMT can be effective for the treatment of CDI in IBD patients, resulting in the normalization of altered microbial composition and metabolic function, and elimination of *C. difficile* (93,94). Indeed, microbial α-diversity and richness in IBD patients treated with FMT increase and become similar to those of healthy donors (95). Additionally, FMT restores secondary bile acid metabolism in the intestine and ameliorates gut damage. Thus, FMT seems to be a promising therapeutic approach that restores/enhances microbiota-mediated resistance to pathogens. Likewise, dietary intervention is another approach used to restore normal microbial composition and function, as it has been reported that diet is a powerful driver of microbial structure in the gut. Elemental diet has been used in the treatment of IBD, in particular to manage the symptoms of CD. Cohort studies have shown that elemental diet has an impact on the composition of the gut microbiota, and induces mucosal healing and clinical remission of CD (96,97). Mounting evidence suggests that a diet low in fermentable oligosaccharides, disaccharides, monosaccharides, and polyols (FODMAPs) can effectively reduce GI symptoms in patients with irritable bowel syndrome by altering microbial composition and luminal metabolism (98). In this context, pilot studies have demonstrated that a low-FODMAPs diet can significantly ameliorate gut dysfunction in some IBD patients (98,99).

Collectively, recent technological advances have provided evidence that gut dysbiosis is one of the triggers and/or mediators of progression of intestinal inflammation in IBD. The increased appreciation of the role the microbiota plays in host physiology and disease has resulted in a vigorous effort to understand the precise mechanism underlying the involvement of the microbiota in the pathogenesis of IBD. Undoubtedly, new discoveries in the field will lead to the development of novel therapeutic approached that aim to restore normal function of the microbiota.

ACKNOWLEDGEMENTS

This work was supported by JSPS Postdoctoral Fellowship for Research Abroad (to H. N.-K.), the Crohn's and Colitis Foundation of America (to H. N.-K. and N.K.), Young Investigator Grant from the Global Probiotics Council, Kenneth Rainin Foundation, and NIH R21 DK110146 (to N. K).

CONFLICTS OF INTEREST

The authors have no financial conflict of interest.

REFERENCES

1. Kamada, N., and G. Nunez. 2014. Regulation of the immune system by the resident intestinal bacteria. *Gastroenterology* 146: 1477-1488.
2. Honda, K., and D. R. Littman. 2016. The microbiota in adaptive immune homeostasis and disease. *Nature* 535: 75-84.
3. Thaiss, C. A., N. Zmora, M. Levy, and E. Elinav. 2016. The microbiome and innate immunity. *Nature* 535: 65-74.
4. Kamada, N., G. Y. Chen, N. Inohara, and G. Nunez. 2013. Control of pathogens and pathobionts by the gut microbiota. *Nat. Immunol.* 14: 685-690.
5. Kamada, N., S. U. Seo, G. Y. Chen, and G. Nunez. 2013. Role of the gut microbiota in immunity and inflammatory disease. *Nat. Rev. Immunol.* 13: 321-335.
6. Nagao-Kitamoto, H., S. Kitamoto, P. Kuffa, and N. Kamada. 2016. Pathogenic role of the gut microbiota in gastrointestinal diseases. *Intest. Res.* 14: 127-138.
7. Rooks, M. G., and W. S. Garrett. 2016. Gut microbiota, metabolites and host immunity. *Nat. Rev. Immunol.* 16: 341-352.
8. Sonnenburg, J. L., and F. Backhed. 2016. Diet-microbiota interactions as moderators of human metabolism. *Nature* 535: 56-64.
9. Lee, W. J., and K. Hase. 2014. Gut microbiota-generated metabolites in animal health and disease. *Nat. Chem. Biol.* 10: 416-424.
10. Manichanh, C., N. Borruel, F. Casellas, and F. Guarner. 2012. The gut microbiota in IBD. *Nat. Rev. Gastroenterol. Hepatol.* 9: 599-608.
11. Nell, S., S. Suerbaum, and C. Josenhans. 2010. The impact of the microbiota on the pathogenesis of IBD: lessons from mouse infection models. *Nat. Rev. Microbiol.* 8: 564-577.
12. Frank, D. N., A. L. St Amand, R. A. Feldman, E. C. Boedeker, N. Harpaz, and N. R. Pace. 2007. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. *Proc. Natl. Acad. Sci. U. S. A.* 104: 13780-13785.
13. Gophna, U., K. Sommerfeld, S. Gophna, W. F. Doolittle, and S. J. Veldhuyzen van Zanten. 2006. Differences between tissue-associated intestinal microfloras of patients with Crohn's disease and ulcerative colitis. *J. Clin. Microbiol.* 44: 4136-4141.
14. Kang, S., S. E. Denman, M. Morrison, Z. Yu, J. Dore, M. Leclerc, and C. S. McSweeney. 2010. Dysbiosis of fecal microbiota in Crohn's disease patients as revealed by a custom phylogenetic microarray. *Inflamm. Bowel Dis.* 16: 2034-2042.
15. Joossens, M., G. Huys, M. Cnockaert, P. De, V, K. Verbeke, P. Rutgeerts, P. Vandamme, and S. Vermeire. 2011. Dysbiosis of the faecal microbiota in patients with Crohn's disease and their unaffected relatives. *Gut* 60: 631-637.
16. Sokol, H., P. Seksik, L. Rigottier-Gois, C. Lay, P. Lepage, I. Podglajen, P. Marteau, and J. Dore. 2006. Specificities of the fecal microbiota in inflammatory bowel disease. *Inflamm. Bowel Dis.* 12: 106-111.
17. Vigsnaes, L. K., A. P. van den, K. Sulek, H. L. Frandsen, C. Steenholdt, J. Brynskov, J. Vermeiren, W. T. van de, and T. R. Licht. 2013. Microbiotas from UC patients display altered metabolism and reduced ability of LAB to colonize mucus. *Sci. Rep.* 3: 1110.
18. Sokol, H., B. Pigneur, L. Watterlot, O. Lakhdari, L. G. Bermudez-Humaran, J. J. Gratadoux, S. Blugeon, C. Bridonneau, J. P. Furet, G. Corthier, C. Grangette, N. Vasquez, P. Pochart, G. Trugnan, G. Thomas, H. M. Blottiere, J. Dore, P. Marteau, P. Seksik, and P. Langella. 2008. Faecalibacterium prausnitzii is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. *Proc. Natl. Acad. Sci. U. S. A.* 105: 16731-16736.
19. Sokol, H., P. Seksik, J. P. Furet, O. Firmesse, I. Nion-Larmurier, L. Beaugerie, J. Cosnes, G. Corthier, P. Marteau, and J. Dore. 2009. Low counts of Faecalibacterium prausnitzii in colitis microbiota. *Inflamm. Bowel Dis.* 15: 1183-1189.
20. Morgan, X. C., T. L. Tickle, H. Sokol, D. Gevers, K. L. Devaney, D. V. Ward, J. A. Reyes, S. A. Shah, N. LeLeiko, S. B. Snapper, A. Bousvaros, J. Korzenik, B. E. Sands, R. J. Xavier, and C. Huttenhower. 2012. Dysfunction of the intestinal microbiome in inflammatory bowel disease and treatment. *Genome Biol.* 13: R79.
21. Machiels, K., M. Joossens, J. Sabino, P. De, V, I. Arijs, V. Eeckhaut, V. Ballet, K. Claes, I. F. Van, K. Verbeke, M. Ferrante, J. Verhaegen, P. Rutgeerts, and S. Vermeire. 2014. A decrease of the butyrate-producing species Roseburia hominis and Faecalibacterium prausnitzii defines dysbiosis in patients with ulcerative colitis. *Gut* 63: 1275-1283.
22. Barcenilla, A., S. E. Pryde, J. C. Martin, S. H. Duncan, C. S. Stewart, C. Henderson, and H. J. Flint. 2000. Phylogenetic relationships of butyrate-producing bacteria from the human gut. *Appl. Environ. Microbiol.* 66: 1654-1661.
23. Lopez-Siles, M., T. M. Khan, S. H. Duncan, H. J. Harmsen, L. J. Garcia-Gil, and H. J. Flint. 2012. Cultured representatives of two major phylogroups of human colonic Faecalibacterium prausnitzii can utilize pectin, uronic acids, and host-derived substrates for growth. *Appl. Environ. Microbiol.* 78: 420-428.
24. Furusawa, Y., Y. Obata, S. Fukuda, T. A. Endo, G. Nakato, D. Takahashi, Y. Nakanishi, C. Uetake, K. Kato, T. Kato, M. Takahashi, N. N. Fukuda, S. Murakami, E. Miyauchi, S. Hino, K. Atarashi, S. Onawa, Y. Fujimura, T. Lockett, J. M. Clarke, D. L. Topping, M. Tomita, S. Hori, O. Ohara, T. Morita, H. Koseki, J. Kikuchi, K. Honda, K. Hase, and H. Ohno. 2013. Commensal microbe-derived butyrate induces the

differentiation of colonic regulatory T cells. *Nature* 504: 446-450.
25. Smith, P. M., M. R. Howitt, N. Panikov, M. Michaud, C. A. Gallini, Y. Bohlooly, J. N. Glickman, and W. S. Garrett. 2013. The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. *Science* 341: 569-573.
26. Zhang, M., Q. Zhou, R. G. Dorfman, X. Huang, T. Fan, H. Zhang, J. Zhang, and C. Yu. 2016. Butyrate inhibits interleukin-17 and generates Tregs to ameliorate colorectal colitis in rats. *BMC Gastroenterol.* 16: 84.
27. Downes, J., M. Munson, and W. G. Wade. 2003. Dialister invisus sp. nov., isolated from the human oral cavity. *Int. J. Syst. Evol. Microbiol.* 53: 1937-1940.
28. Miller, T. L., and M. J. Wolin. 1995. Bioconversion of cellulose to acetate with pure cultures of Ruminococcus albus and a hydrogen-using acetogen. *Appl. Environ. Microbiol.* 61: 3832-3835.
29. Duncan, S. H., A. Barcenilla, C. S. Stewart, S. E. Pryde, and H. J. Flint. 2002. Acetate utilization and butyryl coenzyme A (CoA):acetate-CoA transferase in butyrate-producing bacteria from the human large intestine. *Appl. Environ. Microbiol.* 68: 5186-5190.
30. Flint, H. J., E. A. Bayer, M. T. Rincon, R. Lamed, and B. A. White. 2008. Polysaccharide utilization by gut bacteria: potential for new insights from genomic analysis. *Nat. Rev. Microbiol.* 6: 121-131.
31. Leitch, E. C., A. W. Walker, S. H. Duncan, G. Holtrop, and H. J. Flint. 2007. Selective colonization of insoluble substrates by human faecal bacteria. *Environ. Microbiol.* 9: 667-679.
32. Mukherjee, P. K., B. Sendid, G. Hoarau, J. F. Colombel, D. Poulain, and M. A. Ghannoum. 2015. Mycobiota in gastrointestinal diseases. *Nat. Rev. Gastroenterol. Hepatol.* 12: 77-87.
33. Rautava, S., R. Luoto, S. Salminen, and E. Isolauri. 2012. Microbial contact during pregnancy, intestinal colonization and human disease. *Nat. Rev. Gastroenterol. Hepatol.* 9: 565-576.
34. Shaw, S. Y., J. F. Blanchard, and C. N. Bernstein. 2010. Association between the use of antibiotics in the first year of life and pediatric inflammatory bowel disease. *Am. J. Gastroenterol.* 105: 2687-2692.
35. Kronman, M. P., T. E. Zaoutis, K. Haynes, R. Feng, and S. E. Coffin. 2012. Antibiotic exposure and IBD development among children: a population-based cohort study. *Pediatrics* 130: e794-e803.
36. Pompei, A., L. Cordisco, A. Amaretti, S. Zanoni, D. Matteuzzi, and M. Rossi. 2007. Folate production by bifidobacteria as a potential probiotic property. *Appl. Environ. Microbiol.* 73: 179-185.
37. Biasco, G., U. Zannoni, G. M. Paganelli, R. Santucci, P. Gionchetti, G. Rivolta, R. Miniero, L. Pironi, C. Calabrese, F. G. Di, and M. Miglioli. 1997. Folic acid supplementation and cell kinetics of rectal mucosa in patients with ulcerative colitis. *Cancer Epidemiol. Biomarkers Prev.* 6: 469-471.
38. Kinoshita, M., H. Kayama, T. Kusu, T. Yamaguchi, J. Kunisawa, H. Kiyono, S. Sakaguchi, and K. Takeda. 2012. Dietary folic acid promotes survival of Foxp3$^+$ regulatory T cells in the colon. *J. Immunol.* 189: 2869-2878.
39. Wexler, H. M. 2007. Bacteroides: the good, the bad, and the nitty-gritty. *Clin. Microbiol. Rev.* 20: 593-621.
40. Round, J. L., and S. K. Mazmanian. 2009. The gut microbiota shapes intestinal immune responses during health and disease. *Nat. Rev. Immunol.* 9: 313-323.
41. Mazmanian, S. K., J. L. Round, and D. L. Kasper. 2008. A microbial symbiosis factor prevents intestinal inflammatory disease. *Nature* 453: 620-625.
42. Mazmanian, S. K., C. H. Liu, A. O. Tzianabos, and D. L. Kasper. 2005. An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system. *Cell* 122: 107-118.
43. Waidmann, M., O. Bechtold, J. S. Frick, H. A. Lehr, S. Schubert, U. Dobrindt, J. Loeffler, E. Bohn, and I. B. Autenrieth. 2003. Bacteroides vulgatus protects against Escherichia coli-induced colitis in gnotobiotic interleukin-2-deficient mice. *Gastroenterology* 125: 162-177.
44. Rehman, A., P. Lepage, A. Nolte, S. Hellmig, S. Schreiber, and S. J. Ott. 2010. Transcriptional activity of the dominant gut mucosal microbiota in chronic inflammatory bowel disease patients. *J. Med. Microbiol.* 59: 1114-1122.
45. Baumgart, M., B. Dogan, M. Rishniw, G. Weitzman, B. Bosworth, R. Yantiss, R. H. Orsi, M. Wiedmann, P. McDonough, S. G. Kim, D. Berg, Y. Schukken, E. Scherl, and K. W. Simpson. 2007. Culture independent analysis of ileal mucosa reveals a selective increase in invasive Escherichia coli of novel phylogeny relative to depletion of Clostridiales in Crohn's disease involving the ileum. *ISME J.* 1: 403-418.
46. Lapaquette, P., A. L. Glasser, A. Huett, R. J. Xavier, and A. rfeuille-Michaud. 2010. Crohn's disease-associated adherent-invasive E. coli are selectively favoured by impaired autophagy to replicate intracellularly. *Cell Microbiol.* 12: 99-113.
47. Mukhopadhya, I., R. Hansen, E. M. El-Omar, and G. L. Hold. 2012. IBD-what role do Proteobacteria play? *Nat. Rev. Gastroenterol. Hepatol.* 9: 219-230.
48. Boudeau, J., A. L. Glasser, E. Masseret, B. Joly, and A. rfeuille-Michaud. 1999. Invasive ability of an Escherichia coli strain isolated from the ileal mucosa of a patient with Crohn's disease. *Infect. Immun.* 67: 4499-4509.
49. Darfeuille-Michaud, A., J. Boudeau, P. Bulois, C. Neut, A. L. Glasser, N. Barnich, M. A. Bringer, A. Swidsinski, L. Beaugerie, and J. F. Colombel. 2004. High prevalence of adherent-invasive Escherichia coli associated with ileal

mucosa in Crohn's disease. *Gastroenterology* 127: 412-421.
50. Barnich, N., F. A. Carvalho, A. L. Glasser, C. Darcha, P. Jantscheff, M. Allez, H. Peeters, G. Bommelaer, P. Desreumaux, J. F. Colombel, and A. rfeuille-Michaud. 2007. CEACAM6 acts as a receptor for adherent-invasive E. coli, supporting ileal mucosa colonization in Crohn disease. *J. Clin. Invest.* 117: 1566-1574.
51. Arthur, J. C., E. Perez-Chanona, M. Muhlbauer, S. Tomkovich, J. M. Uronis, T. J. Fan, B. J. Campbell, T. Abujamel, B. Dogan, A. B. Rogers, J. M. Rhodes, A. Stintzi, K. W. Simpson, J. J. Hansen, T. O. Keku, A. A. Fodor, and C. Jobin. 2012. Intestinal inflammation targets cancer-inducing activity of the microbiota. *Science* 338: 120-123.
52. Nougayrede, J. P., S. Homburg, F. Taieb, M. Boury, E. Brzuszkiewicz, G. Gottschalk, C. Buchrieser, J. Hacker, U. Dobrindt, and E. Oswald. 2006. Escherichia coli induces DNA double-strand breaks in eukaryotic cells. *Science* 313: 848-851.
53. Cuevas-Ramos, G., C. R. Petit, I. Marcq, M. Boury, E. Oswald, and J. P. Nougayrede. 2010. Escherichia coli induces DNA damage *in vivo* and triggers genomic instability in mammalian cells. *Proc. Natl. Acad. Sci. U. S. A.* 107: 11537-11542.
54. Itzkowitz, S. H., and D. H. Present. 2005. Consensus conference: Colorectal cancer screening and surveillance in inflammatory bowel disease. *Inflamm. Bowel Dis.* 11: 314-321.
55. Kornbluth, A., and D. B. Sachar. 2010. Ulcerative colitis practice guidelines in adults: American College Of Gastroenterology, Practice Parameters Committee. *Am. J. Gastroenterol.* 105: 501-523.
56. Horing, E., D. Gopfert, G. Schroter, and G. U. von. 1991. Frequency and spectrum of microorganisms isolated from biopsy specimens in chronic colitis. *Endoscopy* 23: 325-327.
57. Plessier, A., J. Cosnes, J. P. Gendre, and L. Beaugerie. 2002. Intercurrent Klebsiella oxytoca colitis in a patient with Crohn's disease. *Gastroenterol. Clin. Biol.* 26: 799-800.
58. Wagner, J., K. Short, A. G. Catto-Smith, D. J. Cameron, R. F. Bishop, and C. D. Kirkwood. 2008. Identification and characterisation of Pseudomonas 16S ribosomal DNA from ileal biopsies of children with Crohn's disease. *PLoS One* 3: e3578.
59. Garrett, W. S., C. A. Gallini, T. Yatsunenko, M. Michaud, A. DuBois, M. L. Delaney, S. Punit, M. Karlsson, L. Bry, J. N. Glickman, J. I. Gordon, A. B. Onderdonk, and L. H. Glimcher. 2010. Enterobacteriaceae act in concert with the gut microbiota to induce spontaneous and maternally transmitted colitis. *Cell Host Microbe* 8: 292-300.
60. Deng, Q., and J. T. Barbieri. 2008. Molecular mechanisms of the cytotoxicity of ADP-ribosylating toxins. *Annu. Rev. Microbiol.* 62: 271-288.
61. Ruby, T., L. McLaughlin, S. Gopinath, and D. Monack. 2012. Salmonella's long-term relationship with its host. *FEMS Microbiol. Rev.* 36: 600-615.
62. Geddes, K., S. Rubino, C. Streutker, J. H. Cho, J. G. Magalhaes, B. L. Le, T. Selvanantham, S. E. Girardin, and D. J. Philpott. 2010. Nod1 and Nod2 regulation of inflammation in the Salmonella colitis model. *Infect. Immun.* 78: 5107-5115.
63. Hugot, J. P., M. Chamaillard, H. Zouali, S. Lesage, J. P. Cezard, J. Belaiche, S. Almer, C. Tysk, C. A. O'Morain, M. Gassull, V. Binder, Y. Finkel, A. Cortot, R. Modigliani, P. Laurent-Puig, C. Gower-Rousseau, J. Macry, J. F. Colombel, M. Sahbatou, and G. Thomas. 2001. Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature* 411: 599-603.
64. Verma, R., A. K. Verma, V. Ahuja, and J. Paul. 2010. Real-time analysis of mucosal flora in patients with inflammatory bowel disease in India. *J. Clin. Microbiol.* 48: 4279-4282.
65. Duffy, M., L. O'Mahony, J. C. Coffey, J. K. Collins, F. Shanahan, H. P. Redmond, and W. O. Kirwan. 2002. Sulfate-reducing bacteria colonize pouches formed for ulcerative colitis but not for familial adenomatous polyposis. *Dis. Colon Rectum* 45: 384-388.
66. Pitcher, M. C., and J. H. Cummings. 1996. Hydrogen sulphide: a bacterial toxin in ulcerative colitis? *Gut* 39: 1-4.
67. Loubinoux, J., J. P. Bronowicki, I. A. Pereira, J. L. Mougenel, and A. E. Faou. 2002. Sulfate-reducing bacteria in human feces and their association with inflammatory bowel diseases. *FEMS Microbiol. Ecol.* 40: 107-112.
68. Muyzer, G., and A. J. Stams. 2008. The ecology and biotechnology of sulphate-reducing bacteria. *Nat. Rev. Microbiol.* 6: 441-454.
69. Devkota, S., Y. Wang, M. W. Musch, V. Leone, H. Fehlner-Peach, A. Nadimpalli, D. A. Antonopoulos, B. Jabri, and E. B. Chang. 2012. Dietary-fat-induced taurocholic acid promotes pathobiont expansion and colitis in Il10-/- mice. *Nature* 487: 104-108.
70. Rowan, F., N. G. Docherty, M. Murphy, B. Murphy, C. J. Calvin, and P. R. O'Connell. 2010. Desulfovibrio bacterial species are increased in ulcerative colitis. *Dis. Colon Rectum* 53: 1530-1536.
71. Man, S. M., N. O. Kaakoush, S. T. Leach, L. Nahidi, H. K. Lu, J. Norman, A. S. Day, L. Zhang, and H. M. Mitchell. 2010. Host attachment, invasion, and stimulation of proinflammatory cytokines by Campylobacter concisus and other non-Campylobacter jejuni Campylobacter species. *J. Infect. Dis.* 202: 1855-1865.
72. Man, S. M., L. Zhang, A. S. Day, S. T. Leach, D. A. Lemberg, and H. Mitchell. 2010. Campylobacter concisus and other Campylobacter species in children with newly diagnosed Crohn's disease. *Inflamm. Bowel Dis.* 16: 1008-1016.

73. Mahendran, V., S. M. Riordan, M. C. Grimm, T. A. Tran, J. Major, N. O. Kaakoush, H. Mitchell, and L. Zhang. 2011. Prevalence of Campylobacter species in adult Crohn's disease and the preferential colonization sites of Campylobacter species in the human intestine. *PLoS One* 6: e25417.
74. Gradel, K. O., H. L. Nielsen, H. C. Schonheyder, T. Ejlertsen, B. Kristensen, and H. Nielsen. 2009. Increased short- and long-term risk of inflammatory bowel disease after salmonella or campylobacter gastroenteritis. *Gastroenterology* 137: 495-501.
75. Kaakoush, N. O., N. P. Deshpande, M. R. Wilkins, C. G. Tan, J. A. Burgos-Portugal, M. J. Raftery, A. S. Day, D. A. Lemberg, and H. Mitchell. 2011. The pathogenic potential of Campylobacter concisus strains associated with chronic intestinal diseases. *PLoS One* 6: e29045.
76. Siegesmund, A. M., M. E. Konkel, J. D. Klena, and P. F. Mixter. 2004. Campylobacter jejuni infection of differentiated THP-1 macrophages results in interleukin 1 beta release and caspase-1-independent apoptosis. *Microbiology* 150: 561-569.
77. Sears, C. L. 2009. Enterotoxigenic Bacteroides fragilis: a rogue among symbiotes. *Clin. Microbiol. Rev.* 22: 349-369.
78. Prindiville, T. P., R. A. Sheikh, S. H. Cohen, Y. J. Tang, M. C. Cantrell, and J. Silva, Jr. 2000. Bacteroides fragilis enterotoxin gene sequences in patients with inflammatory bowel disease. *Emerg. Infect. Dis.* 6: 171-174.
79. Rabizadeh, S., K. J. Rhee, S. Wu, D. Huso, C. M. Gan, J. E. Golub, X. Wu, M. Zhang, and C. L. Sears. 2007. Enterotoxigenic bacteroides fragilis: a potential instigator of colitis. *Inflamm. Bowel Dis.* 13: 1475-1483.
80. Mottawea, W., C. K. Chiang, M. Muhlbauer, A. E. Starr, J. Butcher, T. Abujamel, S. A. Deeke, A. Brandel, H. Zhou, S. Shokralla, M. Hajibabaei, R. Singleton, E. I. Benchimol, C. Jobin, D. R. Mack, D. Figeys, and A. Stintzi. 2016. Altered intestinal microbiota-host mitochondria crosstalk in new onset Crohn's disease. *Nat. Commun.* 7: 13419.
81. Linden, D. R., M. D. Levitt, G. Farrugia, and J. H. Szurszewski. 2010. Endogenous production of H2S in the gastrointestinal tract: still in search of a physiologic function. *Antioxid. Redox Signal.* 12: 1135-1146.
82. Cho, I., S. Yamanishi, L. Cox, B. A. Methe, J. Zavadil, K. Li, Z. Gao, D. Mahana, K. Raju, I. Teitler, H. Li, A. V. Alekseyenko, and M. J. Blaser. 2012. Antibiotics in early life alter the murine colonic microbiome and adiposity. *Nature* 488: 621-626.
83. Theriot, C. M., M. J. Koenigsknecht, P. E. Carlson, Jr., G. E. Hatton, A. M. Nelson, B. Li, G. B. Huffnagle, Z. Li, and V. B. Young. 2014. Antibiotic-induced shifts in the mouse gut microbiome and metabolome increase susceptibility to Clostridium difficile infection. *Nat. Commun.* 5: 3114.
84. Sorg, J. A., and A. L. Sonenshein. 2008. Bile salts and glycine as cogerminants for Clostridium difficile spores. *J. Bacteriol.* 190: 2505-2512.
85. Buffie, C. G., V. Bucci, R. R. Stein, P. T. McKenney, L. Ling, A. Gobourne, D. No, H. Liu, M. Kinnebrew, A. Viale, E. Littmann, M. R. van den Brink, R. R. Jenq, Y. Taur, C. Sander, J. R. Cross, N. C. Toussaint, J. B. Xavier, and E. G. Pamer. 2015. Precision microbiome reconstitution restores bile acid mediated resistance to Clostridium difficile. *Nature* 517: 205-208.
86. Hoffmann, T. W., H. P. Pham, C. Bridonneau, C. Aubry, B. Lamas, C. Martin-Gallausiaux, M. Moroldo, D. Rainteau, N. Lapaque, A. Six, M. L. Richard, E. Fargier, M. E. Le Guern, P. Langella, and H. Sokol. 2016. Microorganisms linked to inflammatory bowel disease-associated dysbiosis differentially impact host physiology in gnotobiotic mice. *ISME J.* 10: 460-477.
87. Eun, C. S., Y. Mishima, S. Wohlgemuth, B. Liu, M. Bower, I. M. Carroll, and R. B. Sartor. 2014. Induction of bacterial antigen-specific colitis by a simplified human microbiota consortium in gnotobiotic interleukin-$10^{-/-}$ mice. *Infect. Immun.* 82: 2239-2246.
88. Palm, N. W., M. R. de Zoete, T. W. Cullen, N. A. Barry, J. Stefanowski, L. Hao, P. H. Degnan, J. Hu, I. Peter, W. Zhang, E. Ruggiero, J. H. Cho, A. L. Goodman, and R. A. Flavell. 2014. Immunoglobulin A coating identifies colitogenic bacteria in inflammatory bowel disease. *Cell* 158: 1000-1010.
89. Okai, S., F. Usui, S. Yokota, I. Hori, M. Hasegawa, T. Nakamura, M. Kurosawa, S. Okada, K. Yamamoto, E. Nishiyama, H. Mori, T. Yamada, K. Kurokawa, S. Matsumoto, M. Nanno, T. Naito, Y. Watanabe, T. Kato, E. Miyauchi, H. Ohno, and R. Shinkura. 2016. High-affinity monoclonal IgA regulates gut microbiota and prevents colitis in mice. *Nat. Microbiol.* 1: 16103.
90. Nagao-Kitamoto, H., A. B. Shreiner, M. G. Gillilland, III, S. Kitamoto, C. Ishii, A. Hirayama, P. Kuffa, M. El-Zaatari, H. Grasberger, A. M. Seekatz, P. D. Higgins, V. B. Young, S. Fukuda, J. Y. Kao, and N. Kamada. 2016. Functional Characterization of Inflammatory Bowel Disease-Associated Gut Dysbiosis in Gnotobiotic Mice. *Cell Mol. Gastroenterol. Hepatol.* 2: 468-481.
91. Borody, T. J., and A. Khoruts. 2011. Fecal microbiota transplantation and emerging applications. *Nat. Rev. Gastroenterol. Hepatol.* 9: 88-96.
92. Khoruts, A., and M. J. Sadowsky. 2016. Understanding the mechanisms of faecal microbiota transplantation. *Nat. Rev. Gastroenterol. Hepatol.* 13: 508-516.
93. van, N. E., A. Vrieze, M. Nieuwdorp, S. Fuentes, E. G. Zoetendal, W. M. de Vos, C. E. Visser, E. J. Kuijper, J. F. Bartelsman, J. G. Tijssen, P. Speelman, M. G. Dijkgraaf, and J. J. Keller. 2013. Duodenal infusion of donor feces for recurrent Clostridium difficile. *N. Engl. J. Med.* 368: 407-415.
94. Hourigan, S. K., L. A. Chen, Z. Grigoryan, G. Laroche,

M. Weidner, C. L. Sears, and M. Oliva-Hemker. 2015. Microbiome changes associated with sustained eradication of Clostridium difficile after single faecal microbiota transplantation in children with and without inflammatory bowel disease. *Aliment. Pharmacol. Ther.* 42: 741-752.
95. Rossen, N. G., S. Fuentes, M. J. van der Spek, J. G. Tijssen, J. H. Hartman, A. Duflou, M. Lowenberg, G. R. van den Brink, E. M. Mathus-Vliegen, W. M. de Vos, E. G. Zoetendal, G. R. D'Haens, and C. Y. Ponsioen. 2015. Findings from a randomized controlled trial of fecal transplantation for patients with ulcerative colitis. *Gastroenterology* 149: 110-118.
96. Voitk, A. J., V. Echave, J. H. Feller, R. A. Brown, and F. N. Gurd. 1973. Experience with elemental diet in the treatment of inflammatory bowel disease. Is this primary therapy? *Arch. Surg.* 107: 329-333.
97. Akobeng, A. K., and A. G. Thomas. 2007. Enteral nutrition for maintenance of remission in Crohn's disease. *Cochrane. Database. Syst. Rev.* 18: CD005984.
98. Halmos, E. P., C. T. Christophersen, A. R. Bird, S. J. Shepherd, P. R. Gibson, and J. G. Muir. 2015. Diets that differ in their FODMAP content alter the colonic luminal microenvironment. *Gut* 64: 93-100.
99. Croagh, C., S. J. Shepherd, M. Berryman, J. G. Muir, and P. R. Gibson. 2007. Pilot study on the effect of reducing dietary FODMAP intake on bowel function in patients without a colon. *Inflamm. Bowel. Dis.* 13: 1522-1528.