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Commensal Bacteria, Traditional and Opportunistic Pathogens, Dysbiosis and Bacterial Killing in Inflammatory Bowel Diseases

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Abstract

Purpose of review—The authors present evidence published during the past two years of the roles of commensal and pathogenic bacteria in the pathogenesis of the inflammatory bowel diseases (IBD).

Recent findings—Rodent models conclusively implicate commensal enteric bacteria in chronic, immune-mediated, experimental colitis, and genetically determined defects in bacterial killing by innate immune cells are found in a subset of Crohn's disease patients. There is no evidence that a single pathogen, including *Mycobacterium avium* subspecies *paratuberculosis,* causes Crohn's disease or ulcerative colitis. However, adherent/invasive *E. coli* (AIEC) are associated with ileal Crohn's disease, with the mechanisms and genetics of AIEC virulence being elucidated. Molecular characterization of the microbiota in patients with IBD reveals decreased biodiversity of commensal bacteria, most notably the phyla Bacteroidetes and Firmicutes, including the clinically-relevant *Faecalibacterium prausnitzii*, and increased *E. coli* concentrations. VSL#3 is one probiotic preparation shown to be efficacious in certain clinical situations in small clinical trials.

Summary—Further characterization of altered microbiota in patients with IBD and linking dysbiosis with host genetic alterations in immunoregulation, innate microbial killing and barrier function are critical, so that individualized treatments to increase beneficial commensals and their metabolic products (probiotic and prebiotic administration) and diminish deleterious species such as AIEC can be tailored for defined patient subsets.

Keywords

IBD; bacteria; commensals; dysbiosis; probiotics

Introduction

Chronic idiopathic inflammatory bowel diseases (IBD), which affect 1.0 to 1.5 million Americans, [1*] are a heterogeneous group of disorders resulting from continuous enteric microbial antigenic stimulation of pathogenic T cell responses in individuals with genetic defects in mucosal barrier function, innate bacterial killing or immunoregulation. [2*] Altered microbial composition, defective clearance of bacteria and enhanced mucosal uptake in Crohn's disease (CD) and ulcerative colitis (UC) increase immune stimulation. CD and

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UC preferentially occur in areas of highest intestinal bacterial concentrations and fecal flow sustains inflammation of CD. [3] Ciprofloxacin and metronidazole treat colonic CD and experimental colitis in a number of rodent models. [4] This review describes evidence of the roles of commensal bacteria in the pathogenesis of IBD in articles published January 2007 to December 2008.

Defective bacterial killing in Crohn's disease

Pathophysiologic similarities between CD and chronic granulomatous diseases associated with defective phagocyte function, polymorphisms of genes regulating clearance of intracellular pathogens and observed defects in innate antimicrobial function have led to the hypothesis that a subset of CD is caused by defective clearance of commensal, opportunistic or pathogenic bacteria with subsequent initiation of compensatory antibacterial effector T cells that cause tissue damage. [2*, 5] Secretion of both α- and β-defensins is defective in CD, [6, 7] with decreased Paneth cell α-defensin production due to reduced expression of Tcf-4, a WNT-signaling pathway transcription factor. [8] The truncation mutation of NOD2, which is an intracellular receptor for the peptidoglycan component muramyl dipeptide, results in decreased ileal α-defensin production. [6] Furthermore, NOD2 deficient mice exhibit decreased α-defensin defcr-4, [9] while deletion of the autophagy gene ATG16L1 or the endoplasmic reticulum stress protein XBP-1 results in Paneth cell morphologic changes and decreased expression of antimicrobial peptides. [10**, 11*] CD is associated with genetic polymorphisms of at least 2 autophagy pathway components, ATG16L1 and IRGM, which, like NOD2 and NCF2, regulate intracellular bacterial killing. [12*, 13*, 14*] Decreased antimicrobial peptide secretion could lead to overgrowth, increased mucosal adherence and translocation of commensal bacteria, while defective clearance of invasive or phagocytosed bacteria promotes persistence of viable intracellular bacteria. (Figure 1.) Both mechanisms could result in excessive antigenic stimulation of pathogenic TH1/TH17 cells, chronic granulomatous inflammation and susceptibility to infection by traditional and opportunistic pathogens.

Microbial pathogens

Comprehensive culture and molecular-based analyses of the microbiota of IBD patients fail to identify consistent enrichment of individual pathogenic species in IBD tissues. However, bacterial pathogens continue to reap attention because of similarities between CD, UC and enteric infections and the hope of finding a cure analogous to *Helicobacter pylori* in peptic ulcers.

Mycobacterium avium subspecies paratuberculosis—*Mycobacterium avium* subspecies *paratuberculosis* (MAP) causes spontaneous granulomatous enterocolitis (Johne's disease) with diarrhea and wasting in ruminants, making this obligate intracellular pathogen a credible etiologic agent of Crohn's disease. [15] After considerable investigation, the link between MAP and CD remains neither substantiated nor invalidated. Many investigators continue to measure the MAP-specific insertion element IS900 DNA in the tissue and/or blood of IBD patients using polymerase chain reaction (PCR) with mixed results. (Table 1.)

A study conducted at the University of Colorado reported no detectable MAP DNA in 190 tissue samples from CD, UC and control patients. [16**] Baumgart et al similarly reported lack of detectable IS900 DNA in ileal CD biopsies. [17**] Contradictory results were reported by Scanu et al, who detected MAP DNA in 87% of CD tissues and 15% of controls in a small cohort. [18] Results are also inconsistent in PCR assays for MAP DNA in blood samples. [20–22]

One recent, blinded study offered an alternative method to PCR for identifying MAP in CD patients. [25] Coded, paraffin-embedded surgical resections from CD and control subjects at two centers subjected to acid-fast staining and rRNA in situ hybridization (ISH) for MAP were visualized with oil-immersion microscopy. Both methods provided positive results in 10/17 CD subjects, (59%, CI: 36–78), contrasted with 5/35 control subjects, (OR for CD versus controls = 8.6, p = 0.002). Agreement between the two methods was good, but the time-consuming ISH probes could not discriminate between mycobacterial subspecies.

Defective bacterial killing by innate immune cells could increase risk of infection by intracellular pathogens. Two recent studies showed no association between MAP and NOD2 mutations. No significant association was seen in New Zealand CD patients for carriage of heterozygous or homozygous NOD2 mutations and MAP status, [21] or between MAP serologies and NOD2 polymorphisms in a large, population-based study in Manitoba. [26*] A recent epidemiologic report also failed to support exposure to MAP by contaminated milk or water. [27*] Furthermore, consumption of pasteurized milk and fruit was associated with a reduced risk of CD, whereas meat intake was associated with an increased risk. Finally, a well-designed, 2 year prospective trial of clarithromycin, rifabutin, and ethambutol failed to show sustained clinical response in CD patients. [28*]

Recent studies possibly linking MAP to pathogenic mechanisms of CD fuel the ongoing debate. 235 patients with CD, UC, irritable bowel syndrome (IBS) and no known disease were tested for presence of MAP IS900 DNA and cytokine secretion in intestinal biopsy samples. [23] Greater TNF levels correlated with the presence of MAP in CD patients ($p < 0.05$). However, there was no correlation of MAP with IL-2, IL-12, IL-10, and IFN-γ secretion. A separate study linked MAP to active CD via IL-4 and IL-2 cytokine profiles in peripheral blood with no differences in IFN-γ or TNF levels. [24]

Finally, MAP was recently reported to induce experimental colitis in gnotobiotic IL-$10^{-/-}$ mice. [29] Germ-free IL-$10^{-/-}$ mice receiving a single oral dose of milk containing $10^{\wedge 4}$ CFU of live MAP obtained from a CD patient developed more severe and aggressively progressive colitis, had higher serum amyloid A, IFN-γ and TNF levels, and lost more weight than did IL-$10^{-/-}$ mice that received heat-killed MAP.

Despite continued suggestions of a link between MAP and IBD, it remains doubtful that MAP is the causative agent of most CD patients, although infection of a subset of patients with intracellular killing defects caused by ATG16L1, IGRM or NCF4 needs to be investigated. CD-related NOD2 polymorphisms do not appear to be risk factors for MAP infection.

Functional changes in *Escherichia coli*: Darfeuille-Marchaud reported that adherent/invasive *E. coli* (AIEC) that persist within macrophages and epithelial cells selectively colonize the ileum of CD patients. [30, 31] At least 2 separate groups have confirmed these observations. Baumgart et al demonstrated AIEC in the ileum of CD patients, documented in vivo mucosal adherence with fluorescent in situ hybridization (FISH) and identified AIEC virulence factors common to uropathic *E. coli* strains and *E. coli* strains isolated from boxer dogs with spontaneous granulomatous colitis. [17**] In a separate study, *E. coli* comprised 99% of invasive bacterial isolates in mucosal biopsies of CD patients as opposed to 42% in UC patients and 2% in normal controls. [32*] A prototypic AIEC strain, LF82, induced in vitro granulomas using blood-derived mononuclear cells. [33*] Serum antibodies directed against *E. coli* outer membrane protein C (OmpC) are present in 37–55% of patients with CD, in contrast to ≤ 5% of UC patients and subjects without IBD. High serum reactivity to *E. coli* OmpC is associated with severe CD with longer disease duration, frequent disease progression, small bowel involvement, and increased resections. [34]

Mechanisms of epithelial adherence and invasion of AIEC are being elucidated. Studies utilizing isogenic mutants of LF82 that lack OmpC and other genes suggest that increased expression of OmpC, particularly at high osmolarities reflecting the GI tract, and/or induction of the σ^E regulatory pathway promote adherence and invasion of epithelial cells by LF82 independent of the transcriptional regulator OmpR. [35*]

Flagellin is necessary for LF82's ability to exacerbate DSS-induced murine colitis. [36*] Nonflagellated LF82 mutants behaved like the nonpathogenic *E. coli* strain K-12 in this model. An LF82 mutant lacking dsbA, a gene that encodes a periplasmic oxidoreductase that determines virulence for several pathogens, expressed neither flagella nor type 1 pili, and displayed decreased survival ability. [37*] In contrast, decreased epithelial adhesion and invasion of the LF82 OmpC and OmpR mutants were not associated with decreased expression of flagella and type 1 pili. In a separate study, *E. coli* 083:H1 was dependent upon flagellin for its adherent and invasive phenotype. [38*] Increased expression of carcinoembryonic antigen-related cell adhesion molecule 6 (CEACAM6), which acts as a receptor for AIEC, by ileal epithelial cells in active Crohn's disease may mediate increased mucosal adherence of AIEC. [39*] Increased CEACAM6 expression by cultured colonic epithelial cells after IFN-γ or TNF stimulation and after exposure to LF82 indicate that AIEC may promote its own colonization.

Monocytes from CD patients carrying homozygous or heterozygous NOD2 polymorphisms displayed reduced secretion of IL-1β, IL-6, and IL-10 to LF82 in vitro compared with monocytes from CD patients without NOD2 polymorphisms. [40**] TLR4 polymorphisms did not influence monocyte response. In preliminary studies, we showed that macrophages from NOD2 deficient mice display defective clearance of a murine AIEC strain with prolonged secretion of IL-12/23 p40 and TNF. [41] These findings demonstrate the importance of genetically programmed host immune responses to disease-related bacteria in the pathogenesis of CD.

Enteric pathogens as environmental triggers: A case-control study examining medical records of 3,019 U.S. soldiers who developed IBD and 11,646 matched controls compared data using a conditional logistic regression model to determine that a single episode of infectious gastroenteritis increased the risk of IBD, (OR: 1.40, CI: 1.19–1.66). [42] These results suggest that a self-limited enteric pathogen could trigger IBD, possibly by breaking the mucosal barrier and initiating an early inflammatory response in a genetically susceptible host with immunoregulatory abnormalities. [2*] *Clostridium difficile* can reactivate quiescent IBD [43*] and induce acute experimental epithelial injury by Fas-mediated apoptosis. [44] Likewise enterotoxigenic *Bacteroides fragilis* can induce experimental colitis and IL-17 production. [45] A role for non-pylori Helicobacter in IBD pathogenesis is unlikely based on detection of species-specific serum antibodies in only 6/137 IBD patients. [46]

Dysbiosis

Recent studies in rodents confirm that bacterial composition changes with colonic inflammation and/or infection. [47] Use of molecular techniques is clarifying changes in the composition of the mucosally associated and fecal microbiota in patients with CD and UC and vastly extending previous culture based studies. (Table 2.)

Examining DNA libraries of mucosal-associated microbiota, which may be more relevant than the fecal microbiota to the pathogenesis of IBD, reveals that patients with CD and UC have decreased complexity of commensal bacteria. [16**] Most notably, members of the phyla Bacteroidetes and Firmicutes are decreased in CD and UC. These organisms promote GI health in multiple ways. [59] Reduced *Faecalibacterium prausnitzii*, a major member of

the family Firmicutes, in CD patients [16**, 50**] was confirmed and associated with a higher risk of post-resection recurrence of ileal CD. [49**] In vitro peripheral blood mononuclear cell stimulation by *F. prausnitzii* decreased IL-12 and IFN-γ production and stimulated secretion of IL-10. Oral administration of either live *F. prausnitzii* or its supernatant reduced the severity of TNBS colitis and corrected the associated dysbiosis. In parallel, the abundance of *E. coli* is increased in IBD, particularly the B2+D phylogenetic group. [17**, 48*] *E coli* isolated from CD patients express uropathic-like virulence factors that are postulated to facilitate mucosal invasion. [17**] The number of mucosal *E. coli* in situ correlates with the severity of ileal disease and invasive *E. coli* are restricted to inflamed mucosa. Compositional changes of the microbiota (dysbiosis) in IBD subsets may contribute to disease severity, since abnormal microbiotas correlated with the occurrence of abscesses in CD patients, and IBD patients with dysbiosis underwent surgery at a younger age than those with normal microbiotas. [16**] Finally, fecal and mucosally associated microbial communities of patients with CD and UC are consistently less diverse with increased temporal instability. [16**, 53*, 54*, 55*, 56*]

Nonpathogenic bacteria can cause colitis in hosts with immunoregulatory and mucosal barrier deficits. When germ-free IL-$10^{-/-}$ and wild type (WT) mice were inoculated with nonpathogenic *E. faecalis* and/or *E. coli*, dual-associated IL-$10^{-/-}$ (but not WT) mice developed aggressive TH1/TH17-mediated colitis within 3 weeks that progressed to severe pancolitis by 7 weeks. [60*] A separate study revealed that uptake of nonpathogenic *E. coli* strains K-12 and HB101 by specialized follicle-associated epithelial cells overlying Peyer's patches, (the site of the earliest observable microscopic lesions of recurrent CD), is increased in CD patients, but not in UC subjects. [61*] Increased *E. coli* localized within dendritic cells of CD mucosa correlated with augmented tissue release of TNF.

Metabolic products of the microbiota have important effects on mucosal epithelial cell and immune function. [2*] Butyrate and other short-chain fatty acids are the primary metabolic substrates of colonocytes, with rectal epithelial cells dependent on this fuel source, while hydrogen sulfide (HS), nitric oxide (NO) and serine proteases produced by a subset of commensal microbiota can injure epithelial cells and matrix components. [62*] HS and NO block butyrate metabolism and could lead to a state of epithelial starvation relevant to the pathogenesis of UC.

Probiotics

Animal models and human studies suggest that therapeutically manipulating the balance between beneficial and detrimental intestinal bacterial species can influence health and disease. [63] Use of probiotics, (viable, nonpathogenic microorganisms that exert health benefits beyond basic nutrition), to shift this balance to favor protective species and treat IBD, has been extensively reviewed [63, 64*, 65, 66, 67*], yet the role for probiotics in treating CD and UC remains undetermined because most trials are underpowered and therefore not definitive.

In a TNBS colitis model, oral *Lactobacillus acidophilus*, *Bifidobacterium lactis*, and *Lactobacillus casei* reduced intestinal inflammation. [68] *L. casei* had more modest protection than the other probiotic species, yet in a separate in vitro study, live *L. casei* decreased TNF, IFN-γ, IL-2, IL-6, IL-8, and CXCL1 secretion by explanted CD mucosa and counteracted the proinflammatory effects of commensal *E. coli* ATCC 35345. [69] This and other studies emphasize host-specific responses to probiotics. Other probiotic species control aberrant immune responses in intestinal tissue. *Bifidobacterium bifidum* (BGN4) prevented colitis in a murine $CD4^{+}$ $CD45RB^{high}$ T cell transfer model. [70*] *Lactobacillus gasseri* expressing manganese superoxide dismutase ameliorated colitis in IL-$10^{-/-}$ mice, demonstrating the therapeutic potential of recombinant bacteria engineered to secrete

biologically active molecules. [71*] Orally administered *Lactobacillus suntoryeus* HY7801 ameliorated TNBS-induced colitis, decreased colonic IL-1β, IL-6, and TNF expression and inhibited TLR4-linked NFκB activation. [72*] Bifidobacterium-fermented milk enhanced IL-10 production in peripheral blood mononuclear cells from UC patients and inhibited IL-8 secretion by intestinal epithelial cells. [73] *Lactobacillus rhamnosus GR-1* and *Lactobacillus reuteri RC-14* fed in yogurt to IBD patients had peripheral blood anti-inflammatory effects. In an in vitro study, *L. reuteri* potently suppressed human TNF production by lipopolysaccharide-activated monocytes and monocyte-derived macrophages from children with CD in a strain-dependent manner by inhibiting activation of MAP kinase-regulated c-Jun and the transcription factor AP-1. [74*]

Several groups have investigated the probiotic combination VSL#3 as a treatment for IBD based on its ability to maintain remission in relapsing pouchitis. [75, 76] This combination of 4 live Lactobacillus strains, (*L. casei, bulgaricus, plantarium and acidophilus*), 3 live Bifidobacterium strains, (*B. longum, breve and infantis*), and 1 live Streptococcus strain, (*thermophilus*), dose-dependently ameliorated DSS-induced colitis in weanling rats. [77] In a small (N=18) open-label study, VSL #3 induced remission in 56% of pediatric patients with mild to moderate UC. 6% showed a response, and 39% had no response or did worse with treatment. [78*] Daily oral VSL #3 significantly reduced the pouchitis disease activity index and expanded the number of mucosal regulatory T cells following colectomy with ileal pouch anal anastomosis. [79*] Two components of VSL #3, *B. longum* and *B. breve*, in combination with the prebiotic psyllium, were well-tolerated at high doses and induced remission in 6/10 patients with Crohn's disease who had failed a regimen of aminosalicylates and prednisolone. [80*]

Similar to Sokol et al's studies with *F. prausnitzii*, [49**] an Israeli group isolated and characterized a new putative protective bacterial species, *Enterococcus durans*, from the fecal microbiota of a healthy human vegetarian. [81*] In a DSS model of colitis, oral administration of *E. durans* significantly ameliorated colitis compared to controls and mice fed *Lactobacillus delbrueckii*. To properly note negative studies, oral administration of *Lactobacillus johnsonii* failed to prevent early postoperative endoscopic recurrence of CD 12 weeks after ileo-caecal resection. [82]

To date, probiotics appear to be more effective in preventing relapse of UC and pouchitis, with poor results in CD. Examining *F. prausnitzii* in preventing postoperative recurrence of CD will be quite interesting and could lay the foundation for targeted correction of dysbiotic microbiota in IBD. Use of prebiotics, which are poorly absorbed oligosaccharides that stimulate growth and metabolic activity of beneficial microbiota, could be a low cost, nontoxic strategy to correct dysbiosis in IBD patients.

Conclusions and future directions

There remains no evidence that MAP is a causative agent in the pathogenesis of IBD. A more likely scenario involves specific *E. coli* strains and other species with virulence factors that allow them to adhere to and invade epithelial cells and persist within macrophages. These strains could be viewed as opportunistic pathogens in genetically susceptible hosts with innate killing, mucosal barrier or immunoregulatory defects, with no disease induced in normal hosts. Identifying and then eliminating these strains, blocking expression of their virulence factors or altering their metabolism and identifying host susceptibility factors could be essential keys to treating IBD. Likewise, identifying changes in microbiota composition, gene expression and metabolic profiles in different IBD patient subsets using rapidly evolving molecular tools will provide important new insights into the pathogenesis and novel treatment of IBD using the example of *F. prausnitzii*. [49**] Meanwhile, using

probiotics and prebiotics to enhance concentrations of beneficial species remains promising, although clinical trials lack statistical power due to small numbers. Treatments need to be individualized based on compositional alterations in patient subsets. Understanding bacterial and host mutalistic interactions will determine success in this area.

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Papers of particular interest, published within the annual period of review, are highlighted as:

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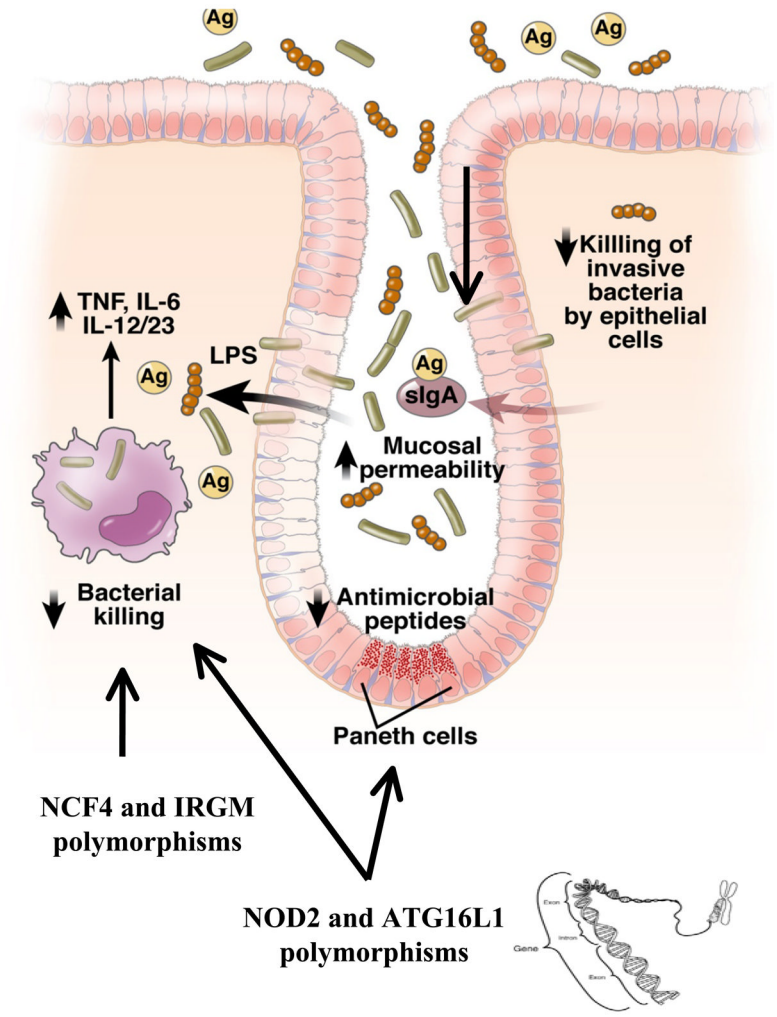

Figure 1. Defective containment of commensal bacteria in IBD

Crohn's disease genes that affect bacterial killing result in defective containment of commensal bacteria. NOD2 polymorphisms result in defective α-defensin production and clearance of intracellular bacteria. Overgrowth of mucosal bacteria occurs with defective secretion of antimicrobial peptides (NOD2 and ATG16L1 polymorphisms) or secretory IgA. Inefficient killing of phagocytosed bacteria (NOD2, ATG16L1, NCF4 and IGRM polymorphisms) results in persistent intracellular bacteria and ineffective clearance of bacterial antigens. Increased mucosal permeability leads to overwhelming exposure of bacterial TLR ligands and antigens that activate pathogenic innate and T-cell immune responses. Modified from ref 2 and used with permission of Elsevier Publishing Company.

Table 1

MAP-specific IS900 DNA PCR data from peer-reviewed articles published in 2007–2008

Recent data does not support an association between IBD and the presence of MAP DNA in gastrointestinal tissues or blood.

Subject location	# CD samples	% MAP +	# UC samples	% MAP +	# healthy samples	% MAP +	Sample sources	Notes	Study/reference
New York	68	0	61	0	61	0	S.I.- 71 Colon- 114 MLN- 5	*M. avium*- specific primer PCR also negative	Frank [16**]
New York	21	0			7	0	Ileum	Aim: Investigate local changes in microbiota in ileal CD	Baumgart [17**]
Italy	23	87			20	15	Ileum Ascending colon Descending colon	Aim: Investigate association between MAP and IBS	Scanu [18]
New YorkHouston	20	5 inside granulomas 0 outside granulomas					Ileum- 4 Colon- 16	Retrospective study: Samples collected from 1997–2006	Toracchio [19]
India	5	Tissue- 80 Blood- 100 Stool- 80			71	Blood- 38 Stool- 27.2	Tissue (CD only) Blood Stool	ELISA, culture, and acid-fast staining also utilized	Singh [20]
New Zealand	361	33.8			200	21.5	Blood	No association between MAP and NOD2 mutations	Bentley [21]
Spain	132	16	103	16	100	47	Blood	All IBD patients in the study taking medications shown to have anti-MAP activity	Juste [22]
Australia	63	23.8	53	11.3	74	14.9	Ileum Colon Inflamed areas	High TNF in MAP+ CD patient tissues	Clancy [23]
Australia	46	26.1	30	10	18	16.7	Ileum Colon Inflamed areas	High IL-2 and IL-4 in MAP+ CD patient blood	Ren [24]

Table 2

Changes in the composition of the mucosa-associated microbiota (MAM) and/or fecal microbiota in patients with CD and UC compared with normal controls, as published in 2007–2008 in peer-reviewed articles

Biodiversity is decreased in the microbiota of IBD patients, while Firmicutes including *F. prausnitzii* are decreased in patients with CD. *E. coli* species are increased in the microbiota of CD patients.

↑IN CD	↓IN CD	↑IN UC	↓IN UC	# patients	Techniques	Source	Study/reference
E. coli	Clostridiales *(Faecalibacteria, Subdoligranula)* *Lachnospiraceae*			28	qPCR[1], FISH	MAM Ileum	Baumgart [17**]
Enterobacteriaceae *E. coli* B2 and D Serine protease autotransporters		Enterobacteriaceae *E. coli* B2 and D Serine protease autotransporters		47	RISA[2]	MAM Cecum Rectum	Kotlowski [48*]
Proteobacteria Actinobacteria	Biodiversity Bacteroidetes Firmicutes (*Lachnospiraceae* subgroups) *F. prausnitzii*	Proteobacteria Actinobacteria	Biodiversity Bacteroidetes Firmicutes *(Lachnospiraceae* subgroups) *F. prausnitzii*	190	qPCR	MAM Ileum Colon MLN	Frank [16**]
	Firmicutes (*F. prausnitzii, C. coccoides*)			7	FISH	MAM Ileum	Sokol [49**]
Enterobacteriaceae *Eubacterium halii* *E. cylindroides*	*F. prausnitzii*	*F. prausnitzii* *Bifidobacteria* *Atopobium*	Enterobacteriaceae	236	FISH	Feces	Swidsinski [50**]
		Gammaproteobacteria *Bacteroides* *Eubacterium* *Fusobacterium* *Lactobacillus* *Ruminococcus*		87	T-RFLP[3]	Feces	Andoh [51*]
	B. vulgatus *B. fragilis* *C. coccoides* *C. leptum* subgroup*Atopobium* *Bifidobacterium*		*B. vulgatus* *B. fragilis* *B.ovatus* *C. coccoides* *C. leptum* subgroup *Atopobium* *Bifidobacterium*	96	FISH	Feces	Takaishi [52]
Temporal instability *B. vulgatus* *B. ovatus*	Biodiversity *B. uniformis*			36	T-RFLP	Feces	Dicksved [53*]
			Biodiversity Firmicutes *Ruminococcus obeum* *Ruminococcus gnavus*	27	T-RFLP	MAM Rectum	Nishikawa [54*]

↑IN CD	↓IN CD	↑IN UC	↓IN UC	# patients	Techniques	Source	Study/reference
		Temporal instability	Biodiversity *Bacteroides* *Eubacterium* *Lactobacillus*	18	PCR-DGGE[4]	MAM Colon	Ott [55*]
		Temporal instability	Biodiversity	33	PCR-DGGE	Feces	Martinez [56*]
		Localized dysbiosis		24	PCR-DGGE	MAM Colon Ulcerated (1/patient) Non-ulcerated (1/pt.)	Zhang [57]
	Dysbiosis (absent)			22	FISH	MAM Ileum Inflamed Not inflamed	Vasquez [58]

[1] qPCR= quantitative polymerase chain reaction;

[2] RISA= ribosomal intergenic spacer analysis;

[3] T-RFLP= terminal restriction fragment length polymorphism;

[4] DGGE= denaturing gradient gel electrophoresis.