Therapeutic correction of bacterial dysbiosis discovered by molecular techniques

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High-throughput, automated DNA-sequencing techniques have provided deeper understanding of the incredibly complex, largely uncultivatable microbiota populating the mammalian gastrointestinal tract in concentrations that outnumber mammalian cells by 10-fold (1–3). These molecular techniques identify component bacterial species by sequencing PCR-amplified products of primers that target highly conserved regions of the 16S rDNA subunit gene. Variation in these sequences is then used to phylogenetically represent genera and species (4). Alternatively, directly sequencing DNA of stool, luminal samples, or mucosal specimens can provide a metagenomic analysis of the structure and function of microbial communities and their interactions with the habitats that they occupy (Committee on Metagenomics, 2007) (4, 5). Metagenomic studies have led to the concept of a "superorganism" whose genome and metabolism are mutalistic aggregates of microbial and host components (6). Molecular analysis of fecal and mucosal samples have increased culture-based estimates of 200–300 individual colonic bacterial species to as high as 1,800 genera and 15,000–36,000 species (7). This remarkable diversity of intestinal bacteria is confined largely (99%) to 4 phyla: Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria, with the dominant Firmicutes (64% of mucosally associated colonic species) primarily composed of *Clostridium* XIVa and IV groups (7). Key advances in recognizing the impact of intestinal microbiota on human diseases were provided by recent observations that the relative proportions of these phyla are altered in obesity (3), inflammatory bowel diseases (7), and experimental type 1 diabetes (8). Expansion of Firmicutes and contraction of Bacteroidetes occurs in obesity (3, 5), whereas diabetic mice have a reduced Firmicutes/Bacteroidetes ratio (8) and a subset of Crohn disease patients and ulcerative colitis exhibit impressive contraction of Firmicutes and Bacteroidetes with concomitant relative expansion of Proteobacteria (7). Functional relevance of these observations is provided by the onset of fat accumulation and weight gain in germ-free lean mice colonized with fecal microbiota of obese mice (5) and attenuated diabetes in NOD murine recipients colonized with bacteria from nondiabetic mice (8). A number of investigators have reported decreased diversity of fecal microbiota in human Crohn disease (9), with specific decreases in the *Clostridium* XIVa and IV groups, including the *Clostridium leptum* group (7, 10). Further analysis demonstrated a selective decrease in *Faecalibacterium prausnitzii*, a predominant member of the *C. leptum* group, in mucosal biopsies of Crohn disease patients (11). In this issue of PNAS, these observations have been extended by Sokol *et al.* (12), who report that a low population of mucosally associated *F. prausnitzii* at the time of ileal resection predicts recurrence of Crohn disease, that a secreted product of this bacterial species exhibited immunomodulatory activity in vitro, and that oral administration of this organism or its supernatant reduced severity of acute experimental colitis in mice.

Table 1. Balance of beneficial vs. detrimental commensal enteric bacterial species helps determine intestinal homeostasis vs. inflammation

Potentially injurious species in susceptible hosts	Protective species
Bacteroides vulgatus, B. thetaiotaomicron	*Lactobacillus species*
Escherichia coli (adherent/invasive)	*Bifidobacterium species*
Enterococcus faecalis (nonpathogenic)	*Escherichia coli*
Klebsiella pneumoniae	*Bacteroides thetaiotaomicron*
Fusobacterium varium	*Faecalibacterium prausnitzii*
Helicobacter hepaticus and other intestinal species	
Bifidobacterium animalis	

Several novel features of this study have important pathophysiologic and clinical implications for Crohn disease, a relapsing chronic inflammatory disorder that appears to be caused by overly aggressive T cell responses directed against a subset of commensal bacteria that inhabit the distal ileum and colon of genetically susceptible hosts (9, 13). Extensive investigations in gnotobiotic rodents demonstrate an essential role for enteric microbiota in chronic TH1/TH17-mediated experimental colitis with host and bacterial species specificity (9, 14). Selective colonization of germ-free (sterile) rodents with several enteric bacterial species induces chronic colitis and antigen-specific TH1/TH17 responses (Table 1). In contrast, other bacterial species, primarily exogenously administered probiotic agents either alone or in combination, treat experimental colitis and prevent relapse of ulcerative colitis or inflammation in ileal reservoirs (pouchitis) created after colectomy for ulcerative colitis (9). This raises the possibility that a disturbed balance of beneficial and detrimental bacteria (dysbiosis) could promote intestinal inflammation, including Crohn disease, and that therapeutically manipulating microbial composition and function by antibiotics, probiotics, and/or prebiotics could restore mucosal homeostasis (9, 15). Sokol *et al.* (12) uniquely demonstrate that a commensal enteric bacterial species identified to be decreased in Crohn disease patients by molecular techniques (11) has functional in vitro and in vivo protective effects, with dominant protective activities mediated by a yet-to-be-defined secreted product(s). These in vitro effects include preferential induction of IL-10, a protective cytokine, with minimal expression of proinflammatory IL-12 and IFN-γ by peripheral blood mononuclear cells exposed to the intact bacteria. The supernatant, but not intact viable bacteria, suppressed constitutive and IL-1β-induced IL-8 secretion and NFκB activation in cultured Caco-2 colonic epithelial cells in vitro. Oral administration of either culture supernatant or viable *F. prausnitzii* attenuated acute (2 day) trinitrobenzene sulfonic acid

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(TNBS)-induced colitis and decreased colonic TNF and IL-12 production while stimulating IL-10. Activity within the intestinal lumen was not required, because i.p. delivered bacteria or supernatant were effective over 20 days of administration. This systemic effect of bacterial products is similar to parenteral treatment of experimental colitis with the toll-like receptor (TLR)-9 agonist, CpG, through induction of IFNα/β production by plasmacytoid dendritic cells (16).

The biologically active protective molecule secreted by *F. prausnitzii* was not identified, but was shown not to be butyrate, which is actively produced by members of the *Clostridium* XIVa and IV groups (7), and to lack direct microbiocidal function by in vitro assays. Several groups have likewise demonstrated immunoregulatory protective effects of products secreted by common probiotic *Lactobacillus* species (17, 18). Yan *et al.* (18) purified 40- and 75-kDa proteins secreted by *Lactobacillus rhamnosus* GG that have epithelial homeostatic responses mediated through AKT. It will be important to characterize the immunosuppressive molecule(s) secreted by *F. prausnitzii* and establish its mechanism(s) of action, including possible mediation by IL-10. Part of this characterization will need to verify that these protective effects are not due in part to residual effects of culture media used to grow *F. prausnitzii*, because the sterile culture media alone had modest in vivo protective effects, with no significant difference between media and bacterial culture supernatant in most readouts of clinical disease activity (12). Unfortunately, a media control was not included in the 20-day survival study (figure 7 of the study).

The observation that patients who developed endoscopic evidence of recurrent mucosal ulceration in the neoterminal ileum had lower mucosal concentrations of *F. prausnitzii* at the time of surgery compared with these subjects who did not develop recurrent ulceration could have important clinical implications, if replicated in a larger independent population. Persistently low mucosal Firmicutes (*Clostridium coccoides* and *F. prausnitzii*) concentrations persisted for 6 months in relapsing patients, raising the provocative possibility that exogenous administration of *F. prausnitzii* could restore normal levels of Firmicutes and prevent the onset of recurrent disease, as was seen with therapeutic oral administration of *F. prausnitzii* in the murine colitis model. Discovering low mucosal concentrations of *F. prausnitzii* at resection could target a high-risk population that may respond to prophylactic therapy with either established immunosuppressive agents, or perhaps more mechanistically, by *F. prausnitzii* administration. This could be particularly important in light of the poor therapeutic response of traditional probiotic bacterial formulations in Crohn disease (9, 15). Thus, this study opens possible new therapeutic paradigms in Crohn disease and other diseases driven by commensal microbiota. It raises the realistic hope of clinical use of molecular diagnostic techniques to identify altered microbial composition in an individual, then reversing the observed dysbiosis by selectively restoring missing protective bacterial species and/or eliminating detrimental organisms. It remains to be determined whether orally administered *F. prausnitzii* or its purified secreted biologically active immunosuppressive product will have greater therapeutic benefit to patients with low mucosal *F. prausnitzii* concentrations, compared with those patients with normal levels of this common mucosal species. It is also extremely important to determine whether low mucosal concentrations of *F. prausnitzii* at resection will predict postoperative recurrence in a broad population of patients.

A disturbed balance of beneficial and detrimental bacteria (dysbiosis) could promote intestinal inflammation.

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