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Commentary

Intestinal dysbiosis: Novel mechanisms by which gut microbes trigger and prevent disease

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ABSTRACT

New research has identified specific intestinal colonizing microbes that can have significant influence on health and disease. Evidence is reviewed supporting an association between *Fusobacterium nucleatum* and colon cancer and for a protective role of *Faecalibacterium prausnitzii* in inflammatory bowel disease, of *Escherichia coli* Nissle 1917 in acute intestinal inflammation, of *Bifidobacterium infantis* in neonatal necrotizing enterocolitis, and of *Akkermansia muciniphila* in obesity and diabetes. These novel bacteria are clinically relevant and present opportunities for more focused diagnosis of colon cancer and prevention of common diseases.

The intestinal microbiota is a complex community that varies dramatically between individuals and within an individual over time and is influenced by a wide variety of factors including genetics, diet, and exposure to antibiotics. Dysbiosis is an imprecise term meant to convey an alteration in the microbiota that is associated with disease or dysfunction. Species diversity can be quantified with measures including richness (the total number of species in a sample) and evenness (the relative abundance of the different species in a sample). Examples of dysbiosis include changes in diversity and blooms of specific bacterial taxa. Dysbiosis has been implicated in the pathogenesis of a wide range of diseases including inflammatory bowel disease (Sartor, 2010), necrotizing enterocolitis (Mai et al., 2011), infantile colic (de Weerth et al., 2013), allergies (Bisgaard et al., 2011), atopic dermatitis (Penders et al., 2013), *Clostridium difficile* colitis (Peniche et al., 2013), antibiotic associated diarrhea (de La Cochetiere et al., 2010), type 2 diabetes (Karlsson et al., 2013), chronic liver disease (Henao-Mejia et al., 2013), and obesity (Turnbaugh and Gordon, 2009). Five little-known bacteria exemplify the tremendous potential of identifying and shaping this community of gut microbes to prevent common diseases (Table 1).

Fusobacterium nucleatum and colon cancer

Fusobacterium species are Gram-negative bacilli (phylum Fusobacteria) associated in metagenomic studies with human colorectal carcinomas (Castellarin et al., 2012; Kostic et al., 2012; Marchesi et al., 2011). *Fusobacterium nucleatum* (*F. nucleatum*) is more common in feces of patients with colorectal carcinomas and adenomas than controls and is found in higher numbers within colorectal carcinomas and adenomas than in surrounding unaffected tissues (Kostic et al., 2013; McCoy et al., 2013). These bacteria are localized to the mucus layer and are associated with a robust local inflammatory response (McCoy et al., 2013). Given the complexity of the gut microbiota, it is unclear whether these bacteria are causally related to colon cancer or increase as a result of other changes associated with intestinal tumorigenesis. In mice prone to intestinal tumor development due to a mutation in the Apc gene, feeding of *F. nucleatum* did not cause colitis, but did cause increased numbers of tumors and expansion of myeloid-derived immune cells known to promote tumor progression (Kostic et al., 2013). It is noteworthy that these changes were not observed with feeding of *F. nucleatum* in two mouse colitis-induced tumor models, suggesting the hypothesis that *F. nucleatum*-associated tumorigenesis may be a two-hit phenomenon. In vitro studies have demonstrated that *F. nucleatum* adheres to and invades host cells through an adhesin, FadA, which binds to and modulates E-cadherin on the host cell surface activating β-catenin signaling to turn on a variety of oncogenes, Wnt genes, and inflammatory genes (Rubinstein et al., 2013). Additional proposed mechanisms

Abbreviations: F. nucleatum, *Fusobacterium nucleatum*; IBD, inflammatory bowel disease; *F. prausnitzii*, *Faecalibacterium prausnitzii*; CD, Crohn's disease; UC, ulcerative colitis; EcNissle, *Escherichia coli* Nissle 1917; NEC, necrotizing enterocolitis; *B. infantis*, *Bifidobacterium longum* subsp. *infantis*; *A. muciniphila*, *Akkermansia muciniphila*.

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Table 1
Bacteria that exemplify the tremendous potential of identifying and shaping a community of gut microbes to prevent common diseases.

Bacterium	Causative	Preventive	Mechanisms
Fusobacterium nucleatum	Colon cancer?		Pro-inflammatory, upregulation of oncogenes
Faecalibacterium prausnitzii		Inflammatory bowel disease	Anti-inflammatory, increased mucus layer
Escherichia coli Nissle 1917		Acute intestinal inflammation	Thrives in inflamed environment, immunomodulation
Bifidobacterium longum ssp. *infantis*		Necrotizing enterocolitis	Consumption of human milk oligosaccharides, anti-inflammatory, decreased gut permeability
Akkermansia muciniphila		Obesity	Increased lipid oxidation, increased mucus layer

of tumorigenesis include 1) ability to outcompete other gut microbes within the relatively hypoxic tumor microenvironment (unlike many gut microbes, Fusobacteria do not require glucose, but can metabolize amino acids and peptides; in addition Fusobacteria contain a rudimentary electron transport chain providing limited ability to respire oxygen), 2) recruitment of tumor-infiltrating myeloid cells, and 3) production of metabolites that directly promote tumor cell proliferation, immune cell infiltration and/or blood vessel growth (Kostic et al., 2013). While causality has not been established, the association between *Fusobacterium* species in the stool and colon cancer is compelling. A next reasonable step would be an adequately powered population study wherein the feces of healthy adults are tested just prior to the prep for routine screening colonoscopy to determine the predictive value of screening for fecal Fusobacteria as an indicator of how frequently colonoscopy is needed.

Faecalibacterium prausnitzii and inflammatory bowel disease (IBD)

Faecalibacterium prausnitzii (*F. prausnitzii*) is one of the most common commensal organisms in the healthy adult intestinal tract. *F. prausnitzii* is a Gram-positive coccus of the family Clostridiaceae (phylum Firmicutes). Adults with Crohn's disease (CD) have lower numbers of *F. prausnitzii* in their feces (Manichanh et al., 2006) and associated with their ileal mucosa (Frank et al., 2007; Martinez-Medina et al., 2006) than healthy controls, and lower levels of *F. prausnitzii* are associated with increased markers of severity of disease (Fujimoto et al., 2013). Similarly, adults with ulcerative colitis (UC) and their first degree relatives have decreased *F. prausnitzii* in their stools compared to controls, and an increased number of fecal *F. prausnitzii* in early remission is associated with sustained remission whereas decreased fecal *F. prausnitzii* is associated with an increased risk of relapse (Varela et al., 2013). This pattern is not seen in pediatric patients with newly diagnosed CD and UC compared to controls (Hansen et al., 2012), suggesting that decreased *F. prausnitzii* may be a result of long-standing inflammation. It is likely that IBD is a heterogeneous group of disease processes that differ in etiology based on anatomic location, age of onset, and severity. Indeed, significant differences in the microbiota of children and adults with IBD have been demonstrated (Cucchiara et al., 2009).

F. prausnitzii appears to have significant anti-inflammatory activity with decreased production of pro-inflammatory IL-8, IL-12 and IFN-γ and increased production of anti-inflammatory IL-10 in cell cultures (Sokol et al., 2008). In a colitis model, mice fed either *F. prausnitzii* or supernatant from *F. prausnitzii* have less macroscopic and histologic colitis and less weight loss than controls (Sokol et al., 2008). Studies in germ free mice suggest that *F. prausnitzii* in conjunction with another common commensal, *Bacteroides thetaiotaomicron*, plays a role in differentiation of goblet cells and production of the mucus layer (Wrzosek et al., 2013). An intact mucus layer is an important component of the intestinal barrier that limits exposure of the epithelial monolayer to pro-inflammatory bacteria in the gut lumen.

Given the current evidence, a reasonable next step would be phase 1 trials of purified oral *F. prausnitzii* and/or supernatant from *F. prausnitzii* in adults (but not children) with newly diagnosed IBD. Dose escalation trials with outcomes including analysis of the fecal microbiota, fecal markers of inflammation, and colonoscopy with biopsies would be invaluable.

Escherichia coli Nissle 1917 and acute inflammation

Escherichia coli (*E. coli*) Nissle 1917 (EcNissle) was first isolated by Alfred Nissle during the first world war during an outbreak of *Shigella*. Dr. Nissle postulated that bacteria in the stool of soldiers that did not develop dysentery were protective. He isolated this strain of *E. coli* from the stool of one of the healthy soldiers and then fed it to soldiers with dysentery who improved (Nissle, 1959). Recent investigations have identified four novel mechanisms that suggest that this strain may have particular usefulness in the setting of acute gut inflammation.

In the healthy adult, γ-Proteobacteria (including *E. coli*) represent a very small proportion of the intestinal microbiota; however in the inflamed gut, a dysbiosis with prominence of γ-Proteobacteria is observed. Some γ-Proteobacteria, including *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) and EcNissle are able to thrive in a pro-inflammatory environment by utilizing nitrogen, produced by the host as part of the inflammatory response, through an alternate respiratory pathway (Winter et al., 2010, 2013). These organisms are thus able to outcompete commensal intestinal bacteria in the presence of inflammation (Thiennimitr et al., 2012).

Second, EcNissle is better able to sequester iron than many gut microbes. Free iron is tightly controlled by host proteins such as heme, ferritin, transferrin, and lactoferrin. Bacteria that are starved for iron synthesize high-affinity iron chelators called siderophores to compete with host sequestration of iron. In the presence of inflammation, the host secretes lipocalin 2, an antimicrobial peptide that sequesters many bacterial siderophores. *S.* Typhimurium produces a siderophore that is too large to be captured by lipocalin 2 giving this pathogen a second effective strategy to thrive in a pro-inflammatory environment. EcNissle has multiple iron uptake systems that are lipocalin 2 resistant and is able to outcompete *S.* Typhimurium in an inflamed and iron-starved environment (Deriu et al., 2013).

Third, EcNissle has immunomodulatory effects that are unique to this strain including activation of γδ T cells (Guzy et al., 2008), inhibition of peripheral blood T cell proliferation (Sturm et al., 2005), suppression of pro-inflammatory cytokine production in the gut mucosa in an acute colitis mouse model and in the plasma and lung in a sepsis mouse model (Arribas et al., 2009), and increased production of tight junction proteins (Hering et al., 2013), and IgA and IgM (Cukrowska et al., 2002).

Fourth, the flagellin protein of EcNissle stimulates production of the potent antimicrobial peptide human beta defensin 2 (Mondel et al., 2009; Schlee et al., 2007), and serves as an adhesin to facilitate binding to gluconate in human intestinal mucus (Troge et al., 2012).

A clinical trial of EcNissle in infants and toddlers showed efficacy in reducing the severity and duration of acute diarrhea (Henker et al., 2007); however, a recent meta-analysis of trials of EcNissle in IBD showed only a small but significant benefit in maintaining remission in adults with UC (Jonkers et al., 2012). It may be that EcNissle is more effective in the setting of acute rather than chronic intestinal inflammation; this hypothesis should be testable in animal models. Further investigations in acute inflammatory conditions such as *C. difficile*