

Pearls

Colonization Resistance: Battle of the Bugs or Ménage à Trois with the Host?

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The lower gastrointestinal tract is host to a dense microbial community, known as the gut microbiota, which is dominated by obligate anaerobic bacteria belonging to the phyla Bacteroidetes (class Bacteroidia) and Firmicutes (class Clostridia). This microbial community offers benefit by conferring niche protection against invading microbes, a property known as “colonization resistance” (reviewed in [1]). The concept of colonization resistance was initially derived from studies showing that an antibiotic-mediated disruption of gut-associated microbial communities greatly enhances the ability of facultative anaerobic Enterobacteriaceae, such as *Escherichia coli* or *Salmonella enterica*, to colonize the large bowel [2–4]. Conventional wisdom holds that resident Bacteroidia and Clostridia confer colonization resistance by “competitive exclusion” of Enterobacteriaceae (phylum Proteobacteria) through microbe-microbe interactions. However, recent studies draw a tantalizing new picture, which suggests that host interactions might play a central role in shaping the microbial community structure. Here we review these new hypotheses and their implications for human health.

Microbe-Microbe Interactions and Conventional Wisdom

Microbe-microbe interactions are clearly important during the competition of bacterial species that occupy similar metabolic niches. For example, rivalry between closely related Proteobacteria arises from competition for carbon sources [5] or trace elements, such as iron [6]. Changes in the availability of nutrients have also been proposed as a mechanism for increasing the relative abundance of Proteobacteria within the community. This “food hypothesis” suggests that disruption of the community of obligate anaerobic Bacteroidia and Clostridia by antibiotic treatment increases the availability of high-energy nutrients, such as carbohydrates, which supports proliferation of fast-growing Proteobacteria [7]. Consistent with this idea, antibiotic treatment of mice increases the availability in the cecal lumen of the microbiota-liberated mucosal carbohydrates sialic acid and fucose, and the ability to consume these sugars confers a two-fold fitness advantage upon *S. enterica* during growth in the large bowel [8]. While these data show that elevated levels of mucus-derived carbohydrates lead to a two-fold rise in bacterial numbers, it is also clear that additional mechanisms might be at work because streptomycin treatment of mice increases the cecal recovery of *E. coli* and *S. enterica* by more than 1,000-fold [9].

Microbe-microbe interactions have furthermore been evoked in the metabolic exclusion of members of the phylum Proteobacteria by fermentation end products of distantly related bacteria belonging to the phyla Bacteroidetes and Firmicutes. This hypothesis is based on the observation that complex polysaccharides, which cannot be broken down in the upper gastrointestinal tract of mammals, are hydrolyzed and fermented in the large bowel into short-chain fatty acids (SCFAs) by obligate anaerobic bacteria, with Clostridia being credited for producing the lion’s share (reviewed in [10]) (Figure 1A). In turn, SCFAs can reduce

intestinal inflammation, which correlates with reduced numbers of colitogenic Proteobacteria in a mouse colitis model [11]. Furthermore, the presence of SCFAs is associated with reduced growth of *E. coli* or *S. enterica* under certain *in vitro* conditions. Based on these observations, production of SCFAs by obligate anaerobic bacteria is commonly assumed to be a mechanism for metabolic exclusion of Proteobacteria from the large bowel (reviewed in [1]). Nonetheless, recent data on the role of SCFAs during microbe-host interaction provide compelling support for an alternative interpretation of these data.

Microbe-Host Interactions Maintain Immune Homeostasis

In addition to their proposed role during microbe-microbe interactions leading to metabolic exclusion, SCFAs influence several aspects of host cell signaling and metabolism. One important function of SCFAs is to provide nutrition for colonocytes, which utilize bacterially produced butyrate as their primary energy source [12]. Furthermore, SCFAs can activate host cell signaling by binding to G protein-coupled receptor 43 (GPCR43, also known as GPR43) [13], thereby altering host cell physiology. One consequence is that colonocytes oxidize carbon sources to CO₂ when butyrate is present, but in the large bowel of germ-free mice where butyrate is absent their metabolism switches toward fermentative lactate production [12]. Finally, the evidence discussed below suggests that SCFAs contribute to the maintenance of immune homeostasis in the lower gastrointestinal tract.

Colonization of the large bowel with commensal microbial communities confers protection from inflammatory disease through mechanisms that are only just beginning to be elucidated. *Bacteroides fragilis*, a prominent human commensal, synthesizes polysaccharide A (PSA), a symbiont-associated molecular pattern (SAMP) that suppresses inflammatory responses in the intestine [14] by engaging TLR2 expressed on regulatory T cells (T_{regs})

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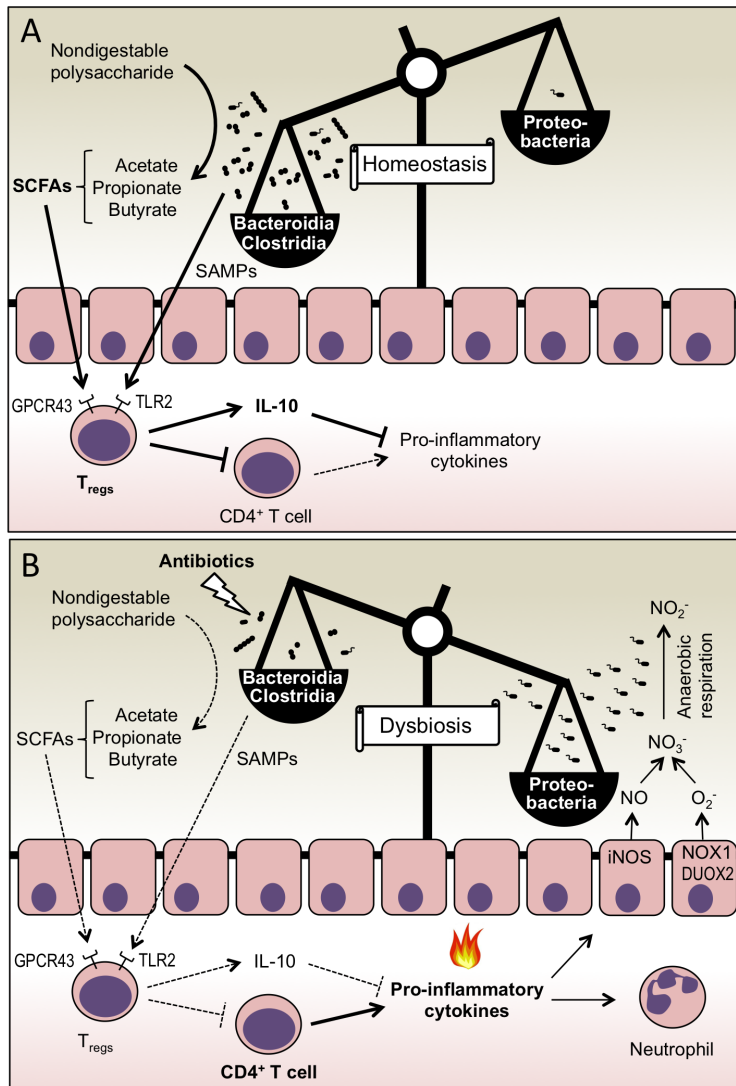


Figure 1. Colonization resistance against Proteobacteria is mediated by host-directed nutritional exclusion. (A) Microbe-host interactions maintain intestinal immune homeostasis. SCFAs and SAMPs produced by obligate anaerobic Clostridia and Bacteroidia stimulate T_{regs} , which suppress inflammation by producing IL-10 and by limiting the expansion of $CD4^+$ effector T cells. (B) Treatment with antibiotics disrupts microbial communities that produce SCFAs and SAMPs. The consequent increase in the inflammatory tone of the intestinal mucosa results in the activation of enzymes, such as iNOS, NADPH oxidase 1 (NOX1), and dual-function NADPH oxidase 2 (DUOX2), which generate reactive nitrogen species (NO) and reactive oxygen species (O_2^-). Respiratory electron acceptors (NO_3^-) generated as a by-product of the host response support growth of Proteobacteria by anaerobic respiration. The consequent bloom of Proteobacteria leads to an imbalance in the microbial community structure (dysbiosis).

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[15]. Interestingly, SCFAs produced by the gut microbiota suppress inflammation by a similar mechanism. SCFAs bind to GPCR43 expressed by colonic T_{regs} . Upon stimulation with SCFAs, colonic T_{regs} expand and mediate resolution of inflammation by increasing expression of the anti-inflammatory cytokine IL-10 (interleukin-10) and by limiting proliferation of effector $CD4^+$ T cells [16] (Figure 1A).

Disruption of the gut microbiota by vancomycin treatment reduces the levels of colonic T_{regs} , but this reduction is completely restored when mice are treated with a combination of vancomycin and SCFAs [17]. The fact that vancomycin treatment reduces intestinal SCFA concentrations [16] and enhances the ability of *S. enterica* to colonize the gut [18] supports the concept that SCFAs play a role in mediating colonization resistance against Proteobacteria. However, these novel insights also suggest that there

might be an alternative explanation for the underlying mechanism other than the microbe-microbe interaction (i.e., competitive exclusion) postulated previously. That is to say, SCFAs might contribute to colonization resistance indirectly through microbe-host interactions, which maintain colonic T_{reg} homeostasis. This new hypothesis suggests that antibiotics, by lowering SCFA concentrations, reduce the abundance and activation state of colonic T_{regs} [16], thereby increasing the inflammatory tone of the mucosa in the lower gastrointestinal tract (Figure 1B). But to explain a loss of colonization resistance after antibiotic treatment by this hypothesis, one has to consider the possibility that the ensuing inflammatory response somehow supports the growth of Proteobacteria. While this assumption has not been tested in studies reporting that SCFAs modulate host immune responses, a number of publications discussed below suggest that intestinal

inflammation does, in fact, lower colonization resistance against Proteobacteria.

Host-Microbe Interactions Feed Proteobacteria

The concept that host responses can reduce colonization resistance against Proteobacteria was first introduced by reports demonstrating that colitis elicited in response to an enteric pathogen, a chemical trigger, or genetic predisposition markedly increases the prevalence of Enterobacteriaceae within murine gut-associated microbial communities [19–21]. Consistent with this idea, a microbial imbalance (dysbiosis) characterized by an increased prevalence of Proteobacteria is observed during intestinal inflammatory disorders in humans, such as Crohn's disease [22], enteropathy in human immunodeficiency virus (HIV)-infected subjects [23], or necrotizing enterocolitis in preterm infants [24]. This enrichment for Proteobacteria during inflammation has been arguably the most consistent and robust ecological pattern observed in the mammalian lower gastrointestinal tract.

A host-microbe interaction responsible for a loss of colonization resistance against Proteobacteria was recently identified as the generation of respiratory electron acceptors, which are produced as a by-product of the host inflammatory response [25]. For example, iNOS (inducible nitric oxide synthase) and NADPH oxidases induced during inflammation produce nitric oxide (NO) and superoxide radicals (O_2^-), respectively, which can react to form nitrate (NO_3^-). In the anaerobic environment of the healthy large intestine, obligate anaerobic Clostridia and Bacteroidia have a competitive growth advantage over the facultative anaerobic Proteobacteria (reviewed in [26]). But by using host-derived nitrate as a terminal electron acceptor for anaerobic respiration, both commensal *E. coli* and the pathogen *S. enterica* serovar Typhimurium can bloom in the lumen of the inflamed lower gastrointestinal tract (Figure 1B) [27,28]. The ability to respire nitrate or other electron acceptors generated as by-products of the host inflammatory response is widely conserved among the facultative anaerobic Proteobacteria. In contrast, the obligate anaerobic Clostridia and Bacteroidia lack the terminal oxidoreductases needed to use these exogenous electron acceptors (reviewed in [10]). As a result, Clostridia and Bacteroidia compete for a limited amount of fermentable carbohydrates and amino acids, while Proteobacteria can sidestep this competition during inflammation by using anaerobic respiration to grow on fermentation end products or on other non-fermentable carbon sources [29]. Through this mechanism, the host inflammatory response selectively feeds facultative anaerobic Proteobacteria, thereby increasing their prevalence within communities inhabiting the large bowel.

How Antibiotics Lower Colonization Resistance

One remaining question is whether this mechanism contributes to the antibiotic-induced reduction in colonization resistance against

Proteobacteria first reported in 1954 [2]. The recent discovery that a disruption of the gut microbiota by vancomycin lowers intestinal SCFA concentrations and reduces colonic T_{regs} [16,17] suggests that antibiotics might increase the inflammatory tone of the mucosa in the large bowel. Consistent with this prediction, mild infiltrates of inflammatory monocytes, neutrophils, and natural killer cells are observed in the cecal mucosa of mice treated orally with metronidazole or streptomycin [9,30]. More importantly, streptomycin treatment markedly increases expression of *NOS2*, the gene encoding iNOS, in the cecal mucosa. The consequent streptomycin-induced generation of host-derived nitrate boosts luminal growth of *E. coli* by anaerobic respiration [9]. Collectively, these publications strongly support the idea that antibiotics lower colonization resistance against Proteobacteria by increasing the inflammatory tone of the intestinal mucosa (Figure 1B).

Assembling the Bigger Picture

The recent publications reviewed above have each contributed key pieces to a puzzle that can now, for the first time, be assembled into a coherent model. The emerging image suggests that colonization resistance against Proteobacteria is mediated by microbe-host interactions that maintain intestinal immune homeostasis (Figure 1A). Antibiotics can diminish this microbe-host interaction by disrupting the microbial community of obligate anaerobic SCFA producers [16]. Through this mechanism, antibiotics can increase the inflammatory tone of the intestinal mucosa, thereby initiating host-microbe interactions that selectively feed Proteobacteria (Figure 1B) [9,27]. Further work is needed to test this hypothesis, for instance by determining whether a disruption of SCFA-mediated immune homeostasis is indeed responsible for the antibiotic-induced loss of colonization resistance against Proteobacteria. Should such studies provide support for the model depicted in Figure 1B, this would establish the concept that colonization resistance involves a ménage à trois between obligate anaerobic bacteria, the host immune system, and Proteobacteria. We propose the term “host-directed nutritional exclusion” to differentiate this novel concept from the preceding image of microbe-microbe interactions, which is commonly described as “competitive exclusion” or “metabolic exclusion.”

Furthermore, the proposed model (Figure 1B) might have significant implications for understanding the pathogenesis of irritable bowel syndrome (IBS), a condition characterized by low-level intestinal inflammation and diarrhea, which often follows surgery or repeated courses of antibiotics. IBS patients have a lower concentration of total SCFAs [31,32], and their fecal microbial population is characterized by an increased abundance of Proteobacteria [33–37]. The streptomycin-treated mouse exhibits many features that are characteristic of human IBS and might thus represent a useful animal model for exploring the therapeutic value of approaches aimed at increasing SCFA concentrations for treatment.

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