



HHS Public Access

Author manuscript

Cell Microbiol. Author manuscript; available in PMC 2016 May 13.

Published in final edited form as:

Cell Microbiol. 2014 July ; 16(7): 993–1003. doi:10.1111/cmi.12300.

The Enteric Two-Step: nutritional strategies of bacterial pathogens within the gut

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Summary

The gut microbiota is a dense and diverse microbial community governed by dynamic microbe–microbe and microbe–host interactions, the status of which influences whether enteric pathogens can cause disease. Here we review recent insights into the key roles that nutrients play in bacterial pathogen exploitation of the gut microbial ecosystem. We synthesize recent findings to support a five-stage model describing the transition between a healthy microbiota and one dominated by a pathogen and disease. Within this five-stage model, two stages are critical to the pathogen: (i) an initial expansion phase that must occur in the absence of pathogen-induced inflammation, followed by (ii) pathogen-promoting physiological changes such as inflammation and diarrhoea. We discuss how this emerging paradigm of pathogen life within the lumen of the gut is giving rise to novel therapeutic strategies.

Introduction

The human gut microbiota is an extremely dense microbial community containing 10–100 trillion bacterial cells (Savage, 1977; Turnbaugh and Gordon, 2009). Many of the resident species have likely co-evolved with human hosts for millennia. Although there is high inter-individual variability and extensive diversity at the level of species and strains, the healthy human microbiota is dominated by bacteria in the phyla Bacteroidetes and Firmicutes. Members of the microbiota play important roles in host health and disease, and altered composition, or dysbiosis, has been linked to a number of conditions including obesity, inflammatory bowel disease and cancer (Turnbaugh *et al.*, 2006; Frank *et al.*, 2007; Wang *et al.*, 2012). The presence of a healthy microbiota results in the establishment and maintenance of a robust community generally impervious to enteric pathogens, a property commonly referred to as colonization resistance. In addition to the understanding that a dense microbiota occupies the spatial and nutritional niches within the intestine, a number of specific mechanisms that contribute to colonization resistance have been elucidated, including immune modulation (Mazmanian *et al.*, 2008; Ivanov *et al.*, 2009). Here, we focus on the nutritional and competitive challenges that enteric pathogens must confront within the gut and the strategies they employ during colonization.

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The authors declare no conflicts of interest.

Disruption of a healthy microbiota provides an opportunity for several disease-causing bacteria in the gut. Antibiotic use represents one of the most common causes of microbiota disturbance and, not surprisingly, often results in the loss of colonization resistance and increased susceptibility to multiple pathogens. Many pathogens express potent virulence factors that enable them to induce an altered physiological state in the host (e.g. inflammation or diarrhoea), which places non-pathogenic competitors within the ecosystem at a disadvantage. However, one challenge that many pathogens face is achieving sufficient density so that their environment-modulating factors are effective. In the window of time before this density has been reached, pathogens must compete with commensals for nutrients and space to successfully establish a foothold. In this review, we propose a two-step model of enteric pathogen success: an initial expansion/emergence phase largely characterized by metabolic strategies employed by the pathogen to expand and colonize the intestinal ecosystem, followed by an inflammation-dependent stage in which pathogen-encoded virulence factors become crucial for pathogen maintenance and establishment of disease. This two-step model is placed in the context of five stages of microbiota–pathogen interactions (Fig. 1).

Phase 1: A healthy microbiota

A dynamic network of competitive and cooperative relationships govern interactions within the gut microbiota and between the microbiota and host. Despite compositional differences between healthy individuals, the general functional roles performed by the microbiota appear to be highly conserved (Turnbaugh *et al.*, 2009a). Bacteria that colonize a healthy microbiota have evolved under strong selective pressure to compete efficiently for available nutrients in the gut. Due to the abundance of complex carbohydrates present in host mucins and plant-derived dietary fibres, polysaccharide degradation is a prominent functional attribute found in the human microbiota (Lozupone *et al.*, 2012). Characteristic of a complex microbial ecosystem, much of this nutrient acquisition relies upon key interactions between multiple species. Examples include cross-feeding or synergistic substrate harvest, in which members of the microbiota exhibit interdependent metabolic relationships. It is likely that the inability to isolate several gut resident species in pure monoculture is, in part, due to such highly specialized and intricately linked metabolic niches that are present in the complex gut ecosystem but are difficult to replicate *in vitro*.

Dietary and host glycan utilization—The influx of dietary and host mucin glycans into the colonic lumen accounts for a major proportion of nutrients available to the microbiota (Koropatkin *et al.*, 2012). The dietary fibres that reach the large intestine include resistant starch and plant cell wall polysaccharides, and are composed of complex sugar molecules containing a high diversity of complex glycosidic linkages. The human genome encodes an estimated 17 glycoside hydrolases and no polysaccharide lyases that participate in dietary carbohydrate degradation. However, an individual’s microbiota encodes thousands of glycoside hydrolases and polysaccharide lyases, which greatly expands the polysaccharide degradation capabilities and illustrates a key ‘profession’ within the gut microbiota (El Kaoutari *et al.*, 2013).

While dietary glycans can change dramatically between meals, a more stable, albeit limited source of nutrients is derived from the host. Host mucus functions as a protective barrier, separating the epithelium from the bacterial community, but also functions as the main host-derived nutrient source for the microbiota. Some commensals have evolved to preferentially consume dietary plant glycans over mucin glycans when both are present, capitalizing on the potentially transient availability of the dietary glycans (Sonnenburg *et al.*, 2010; Lynch and Sonnenburg, 2012; Rogers *et al.*, 2013).

Gut commensals can also shape the landscape of host mucin glycan production in order to provide themselves with exclusive nutritional substrates. For instance, the human gut symbiont *Bacteroides thetaiotaomicron* induces fucosylation of host mucins in gnotobiotic mice, but loses this ability when its fucose catabolism has been genetically ablated (Hooper *et al.*, 1999). In addition to specific co-evolved ‘gardening’ host–commensal relationships, host mucus secretion is generally responsive to microbial cues from pathogens and commensals (Wrzosek *et al.*, 2013). Alteration of mucin production and glycosylation patterns are likely to have cascading effects across the ecosystem, by promoting the growth of mucin utilizers which may remodel the network of syntrophic interactions.

Fermentation and microbiota metabolic output—As members of the microbiota catabolize carbohydrates they produce end-products including heat, gases (e.g. CO₂, CH₄ and H₂), and organic acids such as the short-chain fatty acids (SCFAs). The SCFAs acetate, propionate and butyrate are the most abundant microbiota fermentation end-products (Wong *et al.*, 2006). Their relative concentrations are affected by several factors including microbial composition, gut transit time and dietary substrates. While commonly viewed as metabolic end-products, SCFAs serve as important metabolic intermediates for nutrient exchange between specific gut bacteria and between the microbiota and the host (Macfarlane and Macfarlane, 2003). For instance, commensal-derived acetate is consumed by several important butyrate producers, such as *Faecalibacterium prausnitzii*, *Roseburia intestinalis* and *Eubacterium rectale* (Duncan *et al.*, 2004; Mahowald *et al.*, 2009).

Consistent with their ubiquitous production by the microbiota, SCFAs are involved in many important processes within the colon. Humans obtain an estimated 10% of their total calories from SCFAs, and butyrate, which is the preferred energy source for the colonic epithelium, has been shown to prevent colorectal cancer and colitis (Bergman, 1990; Wong *et al.*, 2006). The numerous roles that SCFAs play in microbe–microbe and microbe–host interactions make them likely candidates to impact pathogen resistance.

Phase 2: Disturbance

Antibiotic models of infection—Different antibiotic treatments shift the density and diversity of the gut microbiota in nuanced ways. While some antibiotics induce only short-term changes, others impart long-lasting alterations to the microbiota composition and function (Antonopoulos *et al.*, 2009; Buffie *et al.*, 2012). Both the personalized and dynamic properties of the microbiota complicate the predictability of how a single individual’s microbial community will react to antibiotics (Dethlefsen and Relman, 2011). An important area of investigation is aimed at understanding how changes in the microbiota composition

during and after antibiotic use influence the metabolic networks between species, the production of small molecule metabolites, and changes in host responses.

Metabolomic studies are providing a new broad view of changes in the spectrum of small molecules made by the gut microbiota and how this profile varies between conditions (Wikoff *et al.*, 2009; Marcobal *et al.*, 2013). Metabolomic analysis of faecal samples from mice treated with a single dose of streptomycin revealed dramatic changes in catabolism, as well as the synthesis of eicosanoid and steroid hormones and the production of bile acids (Antunes *et al.*, 2011). Additionally, SCFA concentrations decrease following antibiotic treatment, and antibiotic-associated diarrhoea has been correlated with reduced levels of SCFAs (Clausen *et al.*, 1991). The loss of SCFAs presumably results from a decrease in the density of the microbiota and a loss of microbes performing fermentation reactions. However, SCFA irrigation as a treatment for inflammatory disorders has led to inconsistent results (Wong *et al.*, 2006). It still remains unclear how altered concentrations of SCFAs and other metabolites impact pathogenesis, but with potential effects on the metabolism of the microbiota, pathogens and host (Smith *et al.*, 2013), the answers that address these relationships are likely to be complex.

Antibiotics are frequently accompanied by antibiotic-associated diarrhoea (AAD) in humans, and in hamster and mouse models antibiotics are often required for pathogens to colonize and cause disease within the gut. A single dose of clindamycin is sufficient to predispose mice to *C. difficile* infection for as long as ten days post-antibiotic treatment (Buffie *et al.*, 2012). The majority (94%) of hospital-acquired cases of *C. difficile*-associated disease (CDAD) occur after recent antibiotic treatment (Khanna *et al.*, 2012). Studies with germ-free mice which lack commensal bacteria have demonstrated the importance of the gut microbial community in keeping *C. difficile* at bay (Onderdonk *et al.*, 1980). Together, these and many other studies support the healthy commensal gut microbial community as a key in preventing pathogen-induced disease within the intestine.

What qualifies as a disturbance?—Epidemiological studies have indicated that while the majority of cases of *C. difficile*-associated disease were correlated with antibiotic use, 22% of community-acquired *C. difficile* cases had no history of antibiotic exposure within 90 days (Khanna *et al.*, 2012). These data suggest that there are other susceptibility factors that remain to be understood. Considering that antibiotic use by humans has only become a widespread treatment option over the last several decades, there are likely other factors or ecological disturbances that contribute to the establishment of opportunistic pathogens within the gut.

Shifts in diet have been shown to affect the composition of the microbiota and certainly are met with transient perturbation as the community adjusts to accommodate the new diet (Turnbaugh *et al.*, 2009b; McNulty *et al.*, 2013). Some pathogens, as well as other opportunistic commensals, may have evolved mechanisms to capitalize upon acute ecosystem alterations, such as those that occur meal-to-meal (Lozupone *et al.*, 2013). Changes in diet can affect motility and SCFAs, and are likely to affect spatial proximity of species to one another and the host (Kashyap *et al.*, 2013). How such ecosystem-wide changes influence the favourability of conditions for pathogens is currently not well

understood. One study showed that changing mice from a low-fibre to a high-fibre diet results not only in compositional and metabolite changes within the microbiota, but also increased expression of the GP3 receptor for Shiga toxin by the colonic epithelium, leading to an increase in colonization by Shiga-toxin-producing *Escherichia coli* (Zumbrun *et al.*, 2013). Consumption of live microbes (i.e. probiotics) also has the capacity to influence microbiota functional status and can directly impact pathogen viability *in vivo* (Corr *et al.*, 2007; McNulty *et al.*, 2011). Fermented foods have been part of our diet for a large part of human evolution, and much suggestive data exists for their potentially diverse roles in human health. The low quality Western diet consumed by much of the modern world, and the corresponding loss of beneficial food-borne microbes is a marked departure from the ancestral human's high-fibre diet rich in microbes, and the consequences for enteric pathogen susceptibility remain to be determined.

Phase 3: Pathogen expansion

Colonization resistance and the emergence of pathogens—One of the defining features of a 'healthy' microbiota is colonization resistance to enteric pathogens. The pathogens *Shigella flexneri*, *Salmonella typhimurium* and *Vibrio cholerae* are unable to colonize mice to high densities without pre-treatment with antibiotics (Freter, 1956; Barthel *et al.*, 2003; Lawley *et al.*, 2008). This requirement of antibiotics to facilitate mouse infection models is consistent with the association between antibiotic usage and susceptibility to enteric pathogens such as *Salmonella* and *C. difficile* observed in humans (Pavia *et al.*, 1990; Doorduyn *et al.*, 2006; Khanna *et al.*, 2012). Many data suggest that commensal occupation of niches that would otherwise be exploited by the pathogen contributes to colonization resistance. In anaerobic continuous flow cultures, growth of *C. difficile* in the presence of a conventional microbiota was observed only when monosaccharides were added, indicating that efficient nutrient depletion represents a key mechanism used by the microbiota to outcompete *C. difficile* (Wilson and Perini, 1988). It is essential for pathogens to quickly benefit from niches that open up after antibiotic treatment, a transient window of opportunity given the highly efficient resource partitioning and metabolic networks in a healthy, resistant microbiota. For instance, avirulent *S. typhimurium* rapidly expands in the mouse gut, but without the ability to induce inflammation, recedes as the microbiota recovers (Stecher *et al.*, 2007). Pre-colonization with commensal strains of *E. coli* and *C. difficile* can effectively prevent colonization of their pathogenic counterparts, suggesting that careful dissection of nutritional profiles and targeted occupation of nutritional niches with commensal relatives is an effective strategy for preventing infection (Leatham *et al.*, 2009; Maltby *et al.*, 2013; Nagaro *et al.*, 2013).

The metabolic robustness and composition of a stable microbiota in the absence of external perturbations is an important predictor of pathogen invasion. *S. typhimurium* susceptibility increases in gnotobiotic mice colonized with the low complexity altered Schaedler flora, as well as conventionalized mice which harbour more Enterobacteriaceae (Stecher *et al.*, 2010). Therefore, pathogens may benefit when a microbiota has low diversity. Additionally, the presence of closely related commensals may provide a measure of overall hospitability to colonization by pathogens with similar growth requirements and selective pressures.

One important question is what happens during the transition between a healthy microbiota and one dominated by a pathogen that is causing inflammation and disruption of the gut ecosystem. Inflammation-independent (or pre-inflammatory) mechanisms allow pathogens such as *S. typhimurium* to grow to high densities before widespread inflammation is triggered. Mutants in the *Salmonella* pathogenicity islands (SPI) 1 and 2 are unable to cause inflammation, and yet on days 1 and 2 post-antibiotics are able to reach the same intestinal levels as wild-type *S. typhimurium* (Hapfelmeier *et al.*, 2004; Stecher *et al.*, 2007). Thus, there appears to be a two-step process of successful enteric pathogen colonization (Fig. 1). First, an ‘expansion’ phase, when bacteria must use specific nutritional strategies to compete for nutrients and multiply to high densities, followed by an ‘inflammatory’ phase, when these bacteria attain sufficient density to induce the inflammation and disease from which they benefit, and other strategies for survival become important.

Pathogen nutrient sources during emergence—Competition for commensal-liberated mucosal sugars is key for the expansion of many enteric pathogens. *E. coli* and *S. typhimurium* are poor mucin-degraders (i.e. possess few mucus-degrading glycoside hydrolases) and are primarily capable of metabolizing monosaccharides. Therefore, these pathogens appear to depend upon the activity of commensal anaerobes such as *Ruminococcus* and *Bacteroides* species that encode wide arsenals of glycoside hydrolases (Xu *et al.*, 2003; Pultz *et al.*, 2006). Although antibiotic treatment kills many mucin-degrading commensals, it is feasible that in addition to secreted extracellular hydrolases, membrane-associated hydrolases would continue to function after cell lysis, leading to an accumulation of monosaccharides in the absence of commensal catabolism (Shipman *et al.*, 1999). Numerous pathogens appear to capitalize upon the short-term increased nutrient availability after antibiotic treatment. A single dose of streptomycin treatment leads to a transient increase in mucus-derived sialic acids in the caecum. The distantly related pathogens *S. typhimurium* and *C. difficile* utilize this sugar during the expansion phase of infection, and mutants in sialic acid utilization are at a disadvantage relative to wild-type bacteria during infection (Ng *et al.*, 2013). Other host-derived sugars such as fucose also appear to be important during pathogen expansion (Deatherage Kaiser *et al.*, 2013; Ng *et al.*, 2013). Fucosylated glycans also appear to transiently spike in abundance after antibiotic treatment, suggesting that there is an infection-independent accumulation of fucose that *Salmonella* may capitalize on in the early stages of expansion (Deatherage Kaiser *et al.*, 2013).

Pathogen-specific metabolic strategies during expansion—Many pathogenic strains appear to exhibit distinct nutrient utilization strategies relative to their commensal counterparts. In a streptomycin mouse model of *E. coli* colonization, pathogens simultaneously metabolize multiple sugars and display different carbohydrate preferences compared to commensals *in vivo*, but not *in vitro* (Chang *et al.*, 2004; Fabich *et al.*, 2008). This differential preference and cometabolism of carbohydrates may allow the pathogen to capitalize upon niches unoccupied by its commensal relative, as well to exploit shared food sources as rapidly as possible.

Another tactic that appears to be used by intestinal pathogens is rapid growth on nutrients with the trade-off of decreased metabolic efficiency. This divergence of metabolic strategies has been observed in growth of different *E. coli* strains in chemostats and in bovine intestinal contents (Maharjan *et al.*, 2007; Bertin *et al.*, 2013). The slower but more efficient growth of commensals on mucosal sugars may translate into better adaptation to the nutrient-poor, highly competitive environment of the healthy gut and may explain the long-term nature of commensal colonization. Rapidly expanding pathogens in the antibiotic-disrupted intestine benefit from potentially inefficient metabolic strategies, as swift utilization of nutrients deprives commensal counterparts of substrates while facilitating sufficient growth to enable remodelling the environment via expression of virulence factors.

Phase 4: Pathogen-induced inflammation and disease

The ecological opportunity offered to pathogens after disturbance of the intestinal ecosystem appears to be transient. Therefore, many pathogens change the playing field, inducing high levels of inflammation and causing other physiological changes, such as diarrhoea, thus shaping the intestinal environment to promote their own growth. Since other reviews cover this phase of infection thoroughly (Stecher and Hardt, 2011; Winter *et al.*, 2013a), we will focus on a few of the key mechanisms that illustrate the importance of inflammation-derived metabolites which pathogens capitalize upon to maintain colonization.

Remodelling the gut community—After the emergence phase, some pathogens such as *C. rodentium* and *S. typhimurium* trigger a positive feedback loop that promotes a disturbed environment advantageous for their growth (Lupp *et al.*, 2007; Sekirov *et al.*, 2008; Lawley *et al.*, 2009). The defect in colonization maintenance by SPI-1 and SPI-2 effector mutants can be rescued by co-infection with a wild-type strain, suggesting that generation of the inflammatory environment is crucial for *Salmonella*'s long-term survival in the gut (Stecher *et al.*, 2007). However, the functional changes that result from pathogen-induced alterations in microbiota composition and the role altered communities play in pathogen persistence remain intriguing topics for future investigation.

Inflammation: Generation of electron acceptors and nutrients—Inflammation profoundly alters intestinal nutritional resource availability, and pathogens appear to use the products of this disruption to their advantage. During inflammation, *S. typhimurium* localizes closer to the mucosa to capitalize on increased mucus secretion (Stecher *et al.*, 2008). The immune response to many pathogens involves the production of reactive oxygen and nitrogen species, which undergo a variety of transformations including interacting with luminal metabolites to form a number of compounds, including nitrate, amine oxides and sulfur oxides. Many members of the Enterobacteriaceae are able to utilize these compounds as electron acceptors to perform anaerobic respiration. Some non-typhoidal *Salmonella enterica* strains express an effector protein, sopE, which induces caecal nitric oxide synthase 2 (Nos2) expression, providing *Salmonella* with increased nitrate and promoting growth to higher densities in the inflamed intestine (Lopez *et al.*, 2012). However, *E. coli*, another member of the Enterobacteriaceae, is also able to profit within the gut from nitrate-based anaerobic respiration (Winter *et al.*, 2013b). Thus, although nitrate is the preferred terminal

electron acceptor for *Salmonella*, it must compete with resident Enterobacteriaceae for this substrate.

Respiration of a different electron acceptor has been demonstrated to promote *S. typhimurium* densities in the inflamed intestine, potentially a strategy employed to sidestep the competition from commensals for nitrate. In the presence of inflammation, tetrathionate is formed by the oxidation of thiosulfate, a product of host detoxification of the microbiota metabolite hydrogen sulfide. The inability of *E. coli* to utilize tetrathionate provides an exclusive substrate for *Salmonella*'s use, and the presence of tetrathionate allows *Salmonella* to gain energy through anaerobic respiration of compounds that it cannot ferment, such as ethanolamine (Price-Carter *et al.*, 2001; Thiennimitr *et al.*, 2011). Thus, it appears that after expansion in the gut, *Salmonella* induces inflammation and harnesses its unique capability to utilize nutrients that most commensals cannot use.

Phase 5: Recovery

After a pathogenic insult, the microbiota and the host face the challenge of rebounding from a disturbed and inflammatory state to a normal healthy state. Host innate and adaptive immune responses often play a large role in pathogen clearance, and microbiota recovery may be aided by therapeutic intervention (e.g. antibiotics or faecal transplantation) (Bry and Brenner, 2004; Lawley *et al.*, 2009). During this recovery phase, the microbiota begins to regain its density and diversity, consuming available resources, increasing its metabolic outputs, and outcompeting the invading pathogen (Fig. 1). The new healthy state that excludes the pathogen may or may not resemble the original ecological configuration and microbial species composition (Costello *et al.*, 2012; Lee *et al.*, 2013). The factors that determine the resilience of different disturbed microbial communities to resist transition back to a normal state will be an interesting frontier for future research to explore and may reveal new treatment strategies (Dethlefsen and Relman, 2011).

Probiotics—Probiotics, or intentionally consumed microbial species that exert a positive health impact, have been touted for protection or alleviation from pathogenic insults. Currently, the molecular mechanisms underlying the impact that probiotics exert on the host, pathogens, and the microbiota are largely lacking, with some notable exceptions (Corr *et al.*, 2007; Yan *et al.*, 2013). Probiotic studies vary widely in design, which has led to difficult to interpret and sometimes conflicting results (McFarland, 2006; Hempel *et al.*, 2012). Although several studies have found probiotic consumption to be useful in treating diarrhoea and antibiotic-associated diarrhoea (Siitonen *et al.*, 1990; Pochapin, 2000), the mode of activity of probiotic species, which often do not reach substantial densities *in vivo*, remains a mystery. Whether individual-specific aspects of host microbiome or genome may influence the effectiveness of a probiotic remain to be understood, and whether probiotics are effective in combination with other therapies or only for certain types of disease is also of great interest. Soon, we may be able to select novel probiotics based on their capabilities to fill certain niches that are exposed after antibiotic treatment to preventatively exclude potential invading pathogens.

Diet as a treatment—A variety of studies have shown that dietary non-digestible oligosaccharides, known as prebiotics, present a useful approach for promoting a healthy indigenous microbiota. Prebiotic interventions have shown a variety of effects in human studies, including altering abundance of metabolites such as phenols, ammonia and SCFAs (Cummings *et al.*, 1996; Muir *et al.*, 2004). Altering host diet has also been shown to promote recovery from pathogen-induced disease. Consumption of cooked green bananas has been shown to effectively treat severe *Shigella*-induced dysentery in children in two independent studies (Alvarez-Acosta *et al.*, 2009; Rabbani *et al.*, 2009); the increased SCFA production suggests that providing resistant starch and pectin to the microbiota may provide a path from a pathogen-dominated state back to a healthy microbiota. Starch-based solutions have been effective in treating cholera and diarrhoea, presumably by promoting recuperation of the commensal microbiota, although the mechanism is unknown and may differ between studies (Ramakrishna *et al.*, 2000; Alam *et al.*, 2005).

Faecal microbiota transplantation—Faecal microbiota transplantation (FMT), or the exogenous administration of a healthy human donor's faeces into a sick patient, is the most successful treatment for recurrent *C. difficile* infections in humans and is far more effective than the standard vancomycin treatment (van Nood *et al.*, 2013). The success of FMT supports the notion that the pathogen creates a self-potentiating dysbiotic inflammatory state. Studies on FMT in humans and mice demonstrate that pathogen eradication is accompanied by restoration of a 'healthy' microbiota resembling the composition of the donor microbiota (Khoruts *et al.*, 2010). Although the complexity of the donor microbiota required to re-boot a diseased microbiota is not well-defined, administration of a community of six bacterial species has recently been shown to be sufficient to displace *C. difficile* in a mouse model of persistent *C. difficile* disease. Notably, several other six species cocktails were ineffective, and colonizations with individual species from the 'magic' combination of six species were not able to outcompete *C. difficile* (Lawley *et al.*, 2012).

Prospectus—Research in microbiota–pathogen nutritional interactions has progressed significantly over the past 5 years. With numerous -omics technologies and increasingly cheap sequencing technology, researchers are able to probe new aspects of complex ecosystems at unprecedented depth (Medini *et al.*, 2008; Lichtman *et al.*, 2013; Marcobal *et al.*, 2013). Germ-free mice colonized with defined or complete human gut communities will continue to provide valuable tools in the mechanistic understanding of microbiota–pathogen dynamics. In the coming age of personalized medicine, it is likely that microbiome information will help to identify resident opportunistic pathogenic strains or other predictors of disease risk and inform individualized treatments, such as custom diets or defined cocktails of microbiota-derived bacterial strains (i.e. 'second generation' probiotics).

As the process of FMT becomes more refined, it still remains to be seen whether certain individuals are endowed with more pathogen-resistant or sensitive microbiotas and would serve as better donor (or recipient) candidates for FMT, and whether FMT is appropriate for use in conditions other than *C. difficile* recurrence. Alternatively, faecal transplants may even be simplified to a defined community of bacteria. Reaching this stage will require much more data to understand the specific species, molecular determinants and important

ecological principles which are key to the success of FMT, as well as a better assessment of the still uncertain potential risks.

A major problem faced by patients undergoing antibiotic treatment is the unintended indiscriminate ablation of the commensal microbes. Antibiotics with increased precision in targeting specific pathogens may help to solve the problem of microbiota disruption (Tannock *et al.*, 2010). Application of principles from synthetic biology to create genetically engineered gut resident microbes that secrete specific effectors, complement metabolic pathways, block pathogen adherence or neutralize harmful toxins will likely be among the arsenal of new tools. For instance, bacteriocins have shown promise in directly killing pathogens *in vivo* with exceptional species specificity (Corr *et al.*, 2007). Additionally, ecological engineering approaches to strengthen the existing microbial community may sprout from increased insight into the rules that govern microbial ecosystem stability and resilience. Gut microbiota manipulations could be designed based on co-occurrence principles or syntrophic relationships between different commensal microbes, relationships whose characterization is well underway.

As this review highlights, new therapeutic strategies aimed to undercut the nutritional and functional niches used by pathogens are a new frontier. There may be an opportunity to leverage new drugs to target recently elucidated nutritional pathways exploited by antibiotic-associated pathogens, such as administration of a sialidase inhibitor to block sialic acid liberation by commensal microbes after antibiotic treatment (Ng *et al.*, 2013). By considering the dynamics of nutrient use and metabolite production by the microbiota, we may discover novel treatments or improve existing treatments for various opportunistic and antibiotic-associated pathogens.

Acknowledgments

This research was supported by R01-DK085025 (to J.L.S.), NSF graduate fellowships (to K.M.N. and J.A.F.) and a Stanford Graduate Fellowship (to K.M.N.). Justin L. Sonnenburg holds an Investigators in the Pathogenesis of Infectious Disease Award from the Burroughs Wellcome Fund.

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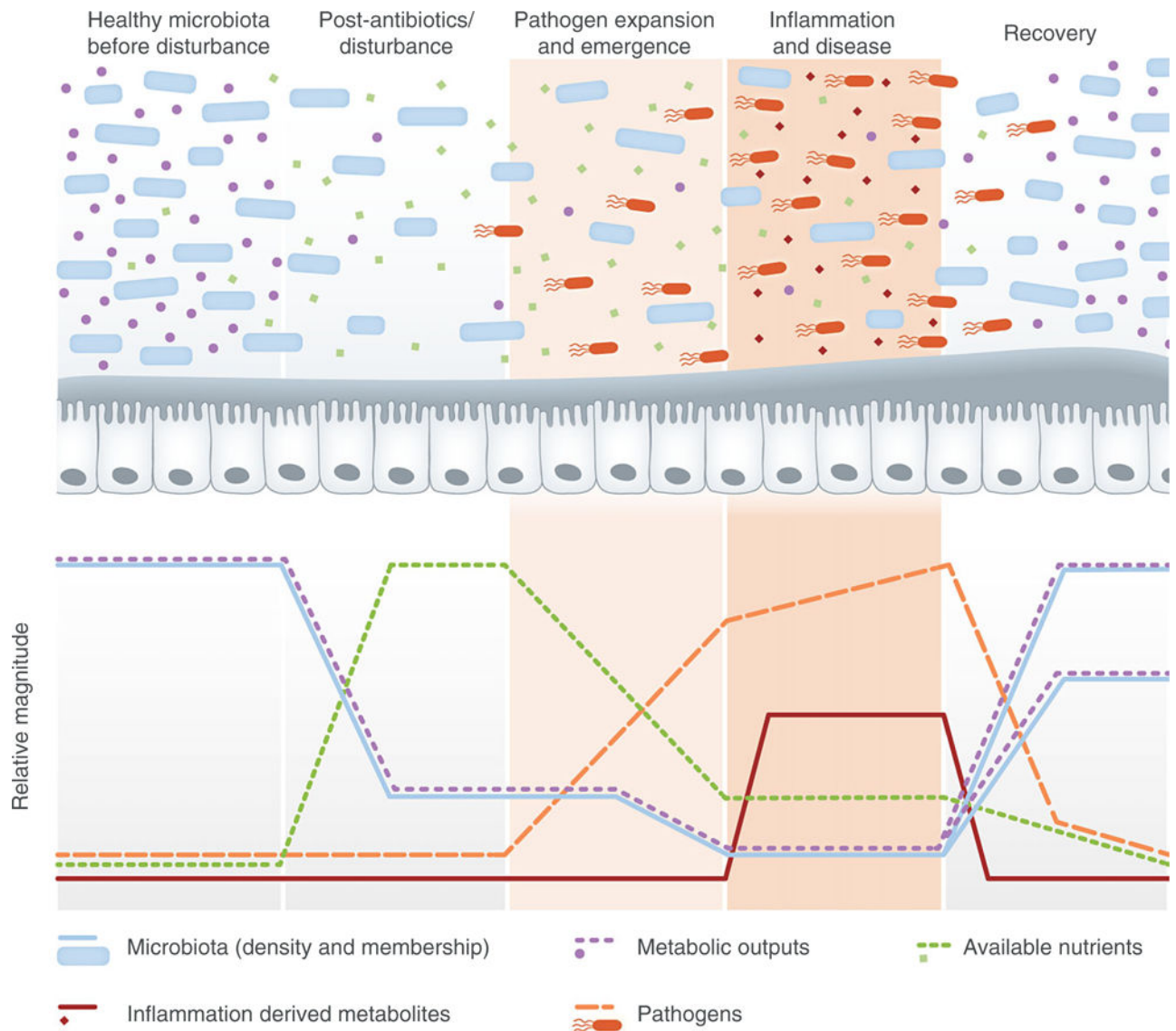


Fig. 1. The five stages of microbiota–pathogen interactions. In stage one, a normal, healthy intestinal ecosystem is characterized by a metabolically active microbiota, which consumes available nutrients, produces high levels of metabolic outputs, and keeps pathogens at bay. Upon disturbance (e.g. with antibiotics) in stage two, the density and diversity of the gut commensal microbial community are dramatically decreased, leading to a spike in available nutrients and a decrease in metabolic outputs. In stage three, pathogens capitalize upon the transient increase in available nutrients to expand within the environment. The fourth stage is characterized by pathogen-induced physiological changes, often including inflammation, which continues to shift the balance by further altering the commensal microbial community and the nutrient landscape of the gut. Finally, in the fifth stage, the microbiota begins to recover and expand, consuming available nutrients and increasing metabolic outputs while outcompeting the disease-causing pathogen. Although commensal density will likely increase, the composition and diversity of the microbiota may or may not return to a state

identical to the initial healthy state, indicated by the dotted lines at the end of the recovery phase. Light and dark tan shading highlight the enteric two-step, the two distinct stages of pathogen nutritional strategy.

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