JEM Journal of Experimental Medicine

REVIEW

Enlisting commensal microbes to resist antibiotic-resistant pathogens

James W. Keith[1] and Eric G. Pamer[1,2,3]

The emergence of antibiotic-resistant bacterial pathogens is an all-too-common consequence of antibiotic use. Although antibiotic resistance among virulent bacterial pathogens is a growing concern, the highest levels of antibiotic resistance occur among less pathogenic but more common bacteria that are prevalent in healthcare settings. Patient-to-patient transmission of these antibiotic-resistant bacteria is a perpetual concern in hospitals. Many of these resistant microbes, such as vancomycin-resistant *Enterococcus faecium* and carbapenem-resistant *Klebsiella pneumoniae*, emerge from the intestinal lumen and invade the bloodstream of vulnerable patients, causing disseminated infection. These infections are associated with preceding antibiotic administration, which changes the intestinal microbiota and compromises resistance to colonization by antibiotic-resistant bacteria. Recent and ongoing studies are increasingly defining commensal bacterial species and the inhibitory mechanisms they use to prevent infection. The use of next-generation probiotics derived from the intestinal microbiota represents an alternative approach to prevention of infection by enriching colonization with protective commensal species, thereby reducing the density of antibiotic-resistant bacteria and also reducing patient-to-patient transmission of infection in healthcare settings.

Introduction

Over the past three decades, medical care has increasingly involved prevention and treatment of infections caused by antibiotic-resistant microbes that are acquired in healthcare settings. Some of these microbes have acquired resistance to all currently approved antibiotics and thus have become essentially untreatable. This has raised the specter of a post-antibiotic era in which minor infections could frequently progress to death, as they did in the pre-antibiotic era. Modern-day humans have mostly lived in an era of readily treated bacterial infections, and the concept of dying from a minor cut or scratch is foreign to most of us.

The contribution of the microbiota to human health involves all organ systems, extending from the skin to the gastrointestinal tract and from hematopoietic organs to the central nervous system (Belkaid and Segre, 2014; Caballero and Pamer, 2015; Manzo and Bhatt, 2015; Sharon et al., 2016). Human and mouse studies have identified exciting and potentially clinically important correlations between microbiota composition and diseases such as obesity (Turnbaugh et al., 2009), liver disease (Henao-Mejia et al., 2013), malnutrition (Smith et al., 2013a), inflammatory bowel disease (Wlodarska et al., 2015), hypertension (Wilck et al., 2017), rheumatoid arthritis (Scher et al., 2013), cancer (Zitvogel et al., 2017), autism (Sharon et al., 2016), and Parkinson's disease (Sampson et al., 2016), and interest in exploiting the microbiota and the associated metabolome as a new approach to treating these diseases is rapidly increasing in academic, biotech, and pharmaceutical circles. The impact of microbiota composition on metabolic, inflammatory, autoimmune, and neurological diseases is readily measurable, statistically significant and, in some cases, sufficiently impressive to warrant clinical study. The effect of the microbiota on resistance to enteric infections is measured on a log scale, with susceptibility to certain infections reduced by a millionfold in the presence of a diverse microbiota. The development of next-generation probiotics derived from the commensal microbiota to reduce infections (Pamer, 2016), particularly those caused by antibiotic-resistant bacteria acquired in healthcare settings, represents the most straightforward, though arguably not the most glamorous, therapeutic target for clinical exploitation of the microbiota. However, moving from the experimental demonstration of a commensal bacterium's ability to enhance resistance against a pathogen to the development of a therapeutic probiotic will take time and extensive clinical study.

Commensal microorganisms and mechanisms of colonization resistance

Although Elie Metchnikov speculated over 100 years ago that certain bacterial species constituting the microbiota contribute

[1]Immunology Program, Louis V. Gerstner Jr. Graduate School of Biomedical Sciences, New York, NY; [2]Sloan Kettering Institute, New York, NY; [3]Infectious Diseases Service, Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY.

Correspondence to Eric G. Pamer: pamere@mskcc.org.

https://doi.org/10.1084/jem.20180399

to disease resistance and human longevity (Brown and Valiere, 2004), the role of the microbiota in resistance to infectious diseases was not fully appreciated until potent antibiotics were introduced into medical practice in the 1940s. Clinicians caring for patients treated with penicillin or streptomycin noted that the bacterial populations colonizing their patients were altered by antibiotic treatment, leading to infections with yeasts and antibiotic-resistant bacteria (Lipman et al., 1948; Keefer, 1951; Woods et al., 1951; Smith, 1952). These observations led to experimental studies with rodents in the 1950s by Miller, Bohnhoff, and Freter that demonstrated marked increases in susceptibility to infection by *Salmonella enteritidis* (Miller et al., 1957), *Shigella flexneri*, and *Vibrio cholerae* (Freter, 1956) following antibiotic treatment. Classical microbiologic studies led to the conclusion that obligate anaerobic commensal bacterial species were the most consequential contributors to resistance against *S. enteritidis* infection (Bohnhoff et al., 1964a). Autochthonous or exogenous Enterobacteriaceae were subsequently shown to markedly expand in the gastrointestinal tract of rodents and humans treated with antibiotics, and the term "colonization resistance" was coined to denote the microbiota's capacity to inhibit expansion of Enterococci and Enterobacteriaceae in the gut lumen (Clasener et al., 1987; Van der Leur et al., 1993).

Since the advent of microscopy over three centuries ago and the description of pleomorphic "animalcules" that reside in the mouth (Lane, 2015), it has been understood that our surfaces, particularly along the gastrointestinal tract, are colonized with dense and diverse populations of microbes. The complexity of organisms inhabiting our colons was demonstrated by deep sequencing of highly variable regions of bacterial 16S ribosomal RNA genes from fecal samples, allowing for generation of phylogenetic trees. Sequencing of over 13,000 16S ribosomal RNA genes from the colons of three healthy individuals demonstrated that humans harbor highly diverse bacterial populations, with dramatic person-to-person variation in microbiota composition (Eckburg et al., 2005). The Human Microbiome Project and the MetaHit Program used next-generation sequencing platforms to characterize the microbiota of hundreds of healthy individuals, confirming substantial interindividual variation (Arumugam et al., 2011; Human Microbiome Project Consortium, 2012). A consistent message from studies spanning a wide range of human populations is that, at baseline, the adult colonic microbiota comprises predominantly bacteria belonging to the Bacteroidetes or Firmicutes phyla (Fig. 1 A). These phyla contain many different families, genera, and species of bacteria that vary in proportion between individuals but that remain remarkably constant within individuals in the absence of intestinal infection, dietary change, or antibiotic administration (David et al., 2014).

Mechanisms of colonization resistance

The bacterial species constituting the colonic microbiota provide colonization resistance via a multitude of parallel mechanisms that restrict the ability of exogenous bacterial strains to gain a foothold in the gut, thereby reducing the host's susceptibility to enteric infections (Buffie and Pamer, 2013). Direct colonization resistance restricts engraftment of exogenous microbes and limits overly robust expansion of indigenous microbes without enlisting host defenses. The major mechanisms of direct colonization resistance include bacterial competition for nutrients, direct antagonism/killing, and the production of inhibitory metabolites. Commensal bacteria derive their nutrients almost exclusively from dietary and host-derived carbohydrates, the abundance of which shapes the composition of the microbiota because bacterial strains differ in their ability to use different carbohydrates (Walker et al., 2011; Martínez et al., 2013; David et al., 2014). Competition between commensal species is best characterized for bacteria belonging to the Bacteroidetes phylum. *Bacteroides fragilis* strains encode polysaccharide utilization loci (PULs) that enable them to deprive competing *B. fragilis* strains of required nutrients and thereby maintain long-term colonization (Lee et al., 2013). *Bacteroides ovatus* and *Bacteroides thetaiotaomicron* encode and transcribe distinct PULs that endow each with the ability to metabolize distinct carbohydrates (Martens et al., 2011), with reciprocal glycan preferences enabling both species to co-inhabit a complex ecosystem by occupying distinct metabolic niches (Tuncil et al., 2017). While many bacterial species of the microbiota compete at the metabolic level, there are also examples of interspecies cooperation that facilitates carbohydrate metabolism, such as what occurs when *B. ovatus*'s ability to digest extracellular polysaccharides benefits *Bacteroides vulgatus* (Rakoff-Nahoum et al., 2016). Dietary changes, such as reduced intake of fiber, can result in enhanced utilization of mucin-associated carbohydrates by *Bacteroides* species, which thins the protective inner mucin layer and reduces host resistance to infection (Desai et al., 2016; Fig. 1 B). PULs within the Firmicutes phylum are distinct from those encoded by the Bacteroidetes, and the diversity of the Firmicutes PULs underlies their nutritional specialization and explains the fluctuations in representation of different bacterial taxa following changes in dietary fiber intake (Sheridan et al., 2016).

Commensal bacteria also produce bacteriocins, microbial products that inhibit other bacteria but to which the producing bacteria are immune. These antimicrobial products can influence the stability and composition of complex microbial populations. For example, *Lactobacillus salivarius* produces a bacteriocin that inhibits *Listeria monocytogenes* (Corr et al., 2007), and Enterococci express bacteriocins that confer competitive advantages in the intestinal tract (Kommineni et al., 2015). The human-derived commensal *Bacillus thuringiensis* produces a bacteriocin that inhibits spore-forming Gram-positive bacteria, including *Clostridium difficile*, while leaving the commensal microbiota composition intact (Rea et al., 2010, 2011). *Escherichia coli* Nissle 1917 is a probiotic that produces bacteriocins that reduce colonization by Gram-negative pathogens including *E. coli* and *Salmonella enterica* (Vassiliadis et al., 2010; Sassone-Corsi et al., 2016).

Gram-negative commensals likely also mediate colonization resistance via the Type VI secretion systems (T6SSs), a mechanism of bacterial antagonism that involves direct, contact-dependent transport of antimicrobial toxins from donor to recipient bacteria via needle-like structures (Russell et al., 2011, 2014). T6SSs are common in Gram-negative commensals, with more than half of human intestinal Bacteroidales genomes and more than a quarter of all Proteobacterial genomes possessing T6SS genes (Boyer et al., 2009; Coyne et al., 2016). The T6SS loci of hu-

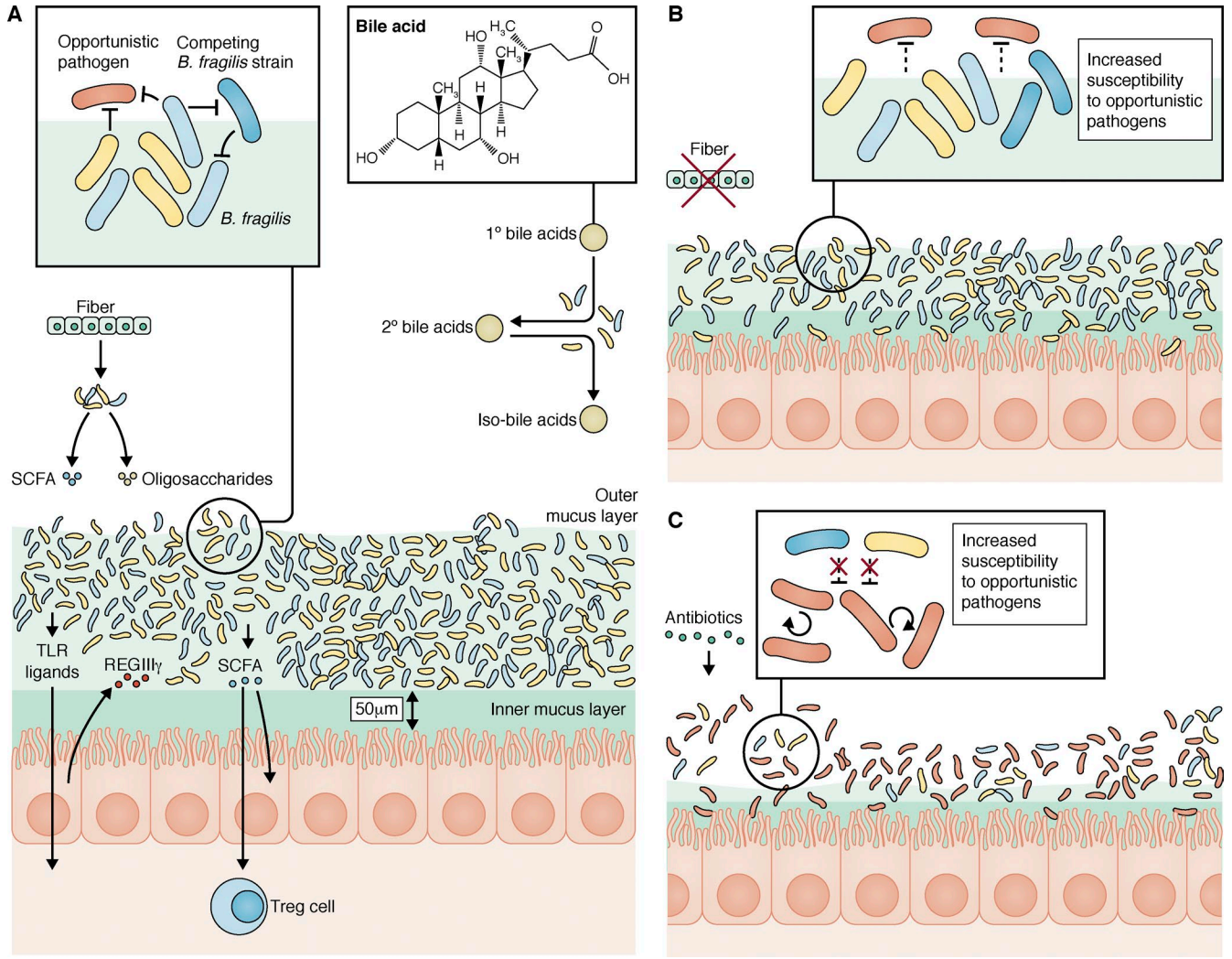

Figure 1. **The microbiota plays an important role in intestinal homeostasis and prevention of opportunistic pathogen infection.** A healthy microbiota is comprised predominantly of bacteria that are members of the Bacteroidetes (blue) and Firmicutes (yellow) phyla. These bacteria interact and cooperate to break down dietary fiber and host-derived mucus into a variety of carbohydrates that support the complex community. SCFAs are by-products of carbohydrate fermentation that promote differentiation of regulatory T cells (Treg). Bacteria-derived TLR ligands promote production of antimicrobial peptides such as RegIIIγ, helping prevent bacterial penetration into the inner mucus layer. Specific bacterial species can produce secondary and iso-bile acids, which contribute to colonization resistance against *C. difficile*. Bacteria such as *B. fragilis* maintain long-term colonization by using distinct polysaccharides so that similar strains that would otherwise use these same polysaccharides cannot engraft due to competitive exclusion. A healthy microbiota also allows for the maintenance of two distinct mucus layers: an ∼50-µm epithelium-associated inner mucus layer that is largely impenetrable by intestinal bacteria and a less dense outer layer that serves as a microbial habitat. **(A)** Dietary fiber is an important substrate of the healthy microbiota, but when dietary changes result in low fiber availability, bacteria resort to using the glycoprotein-rich mucus layer as an alternative energy source. As a result, dietary changes can lead to thinning of the mucus layer, permitting increased bacterial penetration of the mucus layer, which can lead to epithelial inflammation and increased pathogen susceptibility. **(B)** Antibiotic administration disrupts complex feedback loops that sustain the complex microbial community, causing loss of mucus due to the diminishment of microbiota-derived host factors that regulate the production and secretion of mucus. In addition, some antibiotics can cause colonization resistance to be lost, leaving the host vulnerable to opportunistic enteric pathogen (red) expansion.

man-derived Bacteroidales species segregate into three genetic architectures (GAs), denoted as GA1, GA2, and GA3. Whereas GA1 and GA2 are shared among diverse human-derived Bacteroidales species, GA3 T6SSs are limited to *B. fragilis* and do not transfer proteins to other Bacteroidales species. In vitro, T6SSs target many human gut–derived Bacteroidales strains lacking protective cognate immunity proteins (proteins produced alongside toxic effector proteins that shield the producing cell from toxicity), but they fail to inhibit *E. coli* (Chatzidaki-Livanis et al., 2016). Thus, T6SSs weaponize the competition between indigenous species, enabling some strains to persist in their niche by restricting invasion by exogenous species and limiting expansion of local competitors via direct killing (Chatzidaki-Livanis et al., 2016).

Microbial metabolic products, such as short chain fatty acids (SCFAs), also contribute to colonization resistance. The Firmicutes phylum encompasses a wide range of bacterial species that includes facultative anaerobes such as *Lactobacillus* and spore-forming obligate anaerobes such as the Clostridia. Given their prevalence in the colonic microbiota, it is not surprising that these bacterial classes are major contributors to the over-

all metabolism of the lower gastrointestinal tract. It is now appreciated that bacteria belonging to the Lachnospiraceae and Ruminococcaceae families are the major producers of butyrate in the lower gastrointestinal tract (Barcenilla et al., 2000; Louis and Flint, 2009), thereby impacting colonic health, immune system development, and colonization resistance. Butyrate production by commensal bacteria influences host mucosal immune development by promoting differentiation of regulatory T cells (Arpaia et al., 2013; Furusawa et al., 2013; Smith et al., 2013b) and likely contributes to colonization resistance against enteric pathogens. A small subset of colonic Firmicutes, represented by *Clostridium scindens*, encodes operons that modify primary bile acids in the lower intestinal tract, generating secondary bile salts (Ridlon et al., 2006), which can enhance resistance against *C. difficile* infection (Buffie et al., 2015). *Ruminococcus gnavus* converts the secondary bile acid deoxycholic acid to a less cytotoxic iso-bile acid that allows for preferential growth of some *Bacteroides* species, thereby potentially contributing to colonization resistance (Devlin and Fischbach, 2015; Fig. 1 A).

Antibiotic-induced changes to the microbiota

Antibiotic treatment, while often remarkably effective at curing bacterial infections, can cause collateral damage to the patient's microbiota and markedly reduce resistance to colonization and infection by pathogens. Classic studies of the 1940s and 1950s demonstrated the occurrence of antibiotic-induced changes in the microbiota, and next-generation sequencing has since provided a more comprehensive picture of the impact of antibiotics on the microbiota (Dethlefsen et al., 2008; Dethlefsen and Relman, 2011), the extent of which often extends beyond their antibacterial spectra. For example, vancomycin, an antibiotic that interferes with bacterial cell wall synthesis, exclusively kills Gram-positive bacteria in vitro but also markedly reduces the prevalence of Gram-negative Bacteroidetes in vivo (Ubeda et al., 2010; Isaac et al., 2017). Other antibiotics, such as clindamycin and metronidazole, have broad, detrimental effects on microbiota composition in the mouse gut (Buffie et al., 2012; Lewis et al., 2015). Given the interdependencies of bacterial species in the microbiota, it is possible that direct elimination of antibiotic-sensitive bacterial species leads to indirect loss of dependent, albeit antibiotic-resistant, species (Fig. 1 C). Our knowledge of the impact of antibiotics on the commensal microbiota, however, is far from complete, and we are likely to learn much from longitudinal clinical studies of microbiota changes following initiation and completion of specific antibiotic treatments.

Pathogens of the healthcare environment

Although intestinal infections with bacterial pathogens such as *S. enteritidis*, *S. flexneri*, and *V. cholerae* remain major threats to human health, particularly in settings with limited resources, infections caused by less pathogenic but more antibiotic-resistant bacterial species have become an increasing problem in the developed world. Indeed, a recent Centers for Disease Control and Prevention publication lists the most threatening antibiotic-resistant pathogens (https://www.cdc.gov/drugresistance/biggest_threats.html), many of which are acquired in healthcare settings and can become problematic when the host's microbiota is dysregulated, most often by antibiotic administration itself. In the following sections, we will discuss the role of the intestinal microbiota in defense against these hospital-acquired pathogens and describe experimental studies and clinical trials that are revealing new approaches to reducing the risk of infection with and transmission of antibiotic-resistant bacteria. Finally, we propose that reconstitution of the microbiota following broad-spectrum antibiotic treatment should become a routine part of medical practice.

Enterococcus faecalis *and* Enterococcus faecium

Enterococci are common commensal bacteria that colonize the intestine of nearly all terrestrial animals (Lebreton et al., 2017). *E. faecalis* and *E. faecium*, the main enterococcal species inhabiting the human gut, are nonpathogenic in the gastrointestinal tract but cause severe infections if they enter the bloodstream; such infections are challenging to treat because of antibiotic resistance (Arias and Murray, 2012). Vancomycin-resistant *E. faecium* (VRE), for example, is one of the most common causes of bloodstream infection in patients undergoing treatment for leukemia or following bone marrow transplantation (Kamboj et al., 2010), and recent studies have demonstrated that the intestinal microbiota becomes dominated by VRE before invasion of the bloodstream (Ubeda et al., 2010; Taur et al., 2012). Antibiotics that kill obligate anaerobic bacteria of the colon predispose patients to dense intestinal colonization with VRE (Donskey et al., 2000; Taur et al., 2012), suggesting that commensal anaerobes are critical for suppression of VRE and likely Enterococci in general. Commensal bacterial inhibition of VRE is mediated, in part, by stimulation of innate immune defenses (e.g., release of Toll-like receptor ligands) that promote intestinal epithelial cell expression of regenerating islet-derived protein IIIγ (RegIIIγ), an antimicrobial C-type lectin that inhibits VRE growth in the small intestine (Brandl et al., 2008; Fig. 2 A). In a randomized trial of children with VRE infection, oral administration of *Lactobacillus rhamnosus* GG reduced intestinal colonization with VRE (Szachta et al., 2011), potentially by competing with VRE at the level of binding to intestinal mucus, given that the pili of these two bacterial species share sequence similarities (Tytgat et al., 2016). Fecal transplantation can clear VRE from the mouse intestine and correlates with the presence of *Barnesiella* (Ubeda et al., 2013) in the colon. Direct inhibition of VRE is mediated by obligate anaerobes, including *Blautia producta* and *Clostridium bolteae* (Caballero et al., 2017), by mechanisms that remain to be defined. The endogenous commensal *E. faecalis* can also directly inhibit competing Enterococcus strains by expressing bacteriocins (Kommineni et al., 2015).

C. difficile

The most common hospital-acquired pathogen is *C. difficile*, and infection is generally associated with previous antibiotic administration (Abt et al., 2016). *C. difficile* can cause severe colitis and often occurs in patients with a compromised microbiota. The global rise of two epidemic *C. difficile* strains was recently correlated with their distinct ability to metabolize the disaccharide trehalose, which was introduced as a food additive just before the emergence of the antibiotic-resistant strains (Collins et al., 2018).

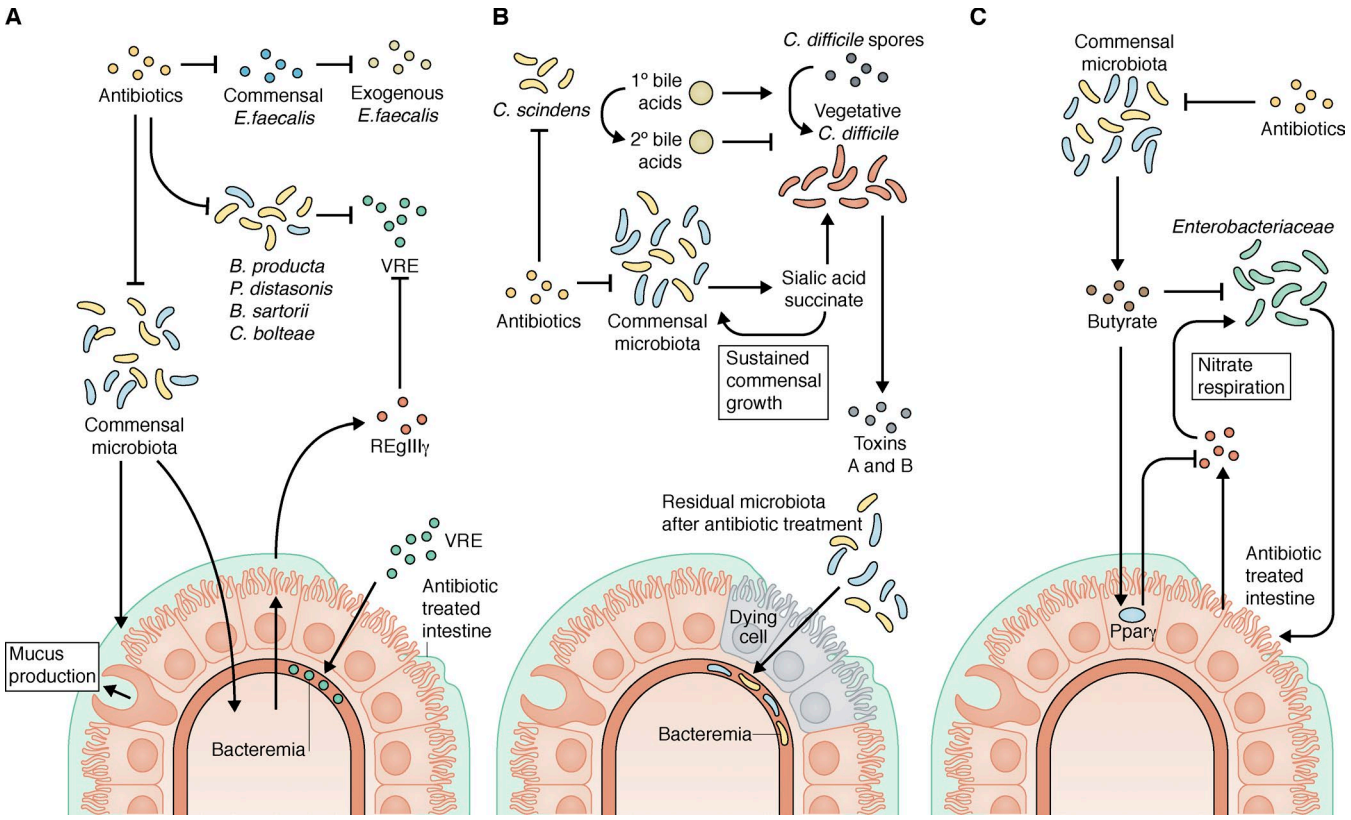

Figure 2. **The microbiota drives defense against nosocomial bacterial pathogens. (A)** Enterococcal infections can be deleterious to the antibiotic-treated host, as Enterococci can translocate into the bloodstream. Loss of colonization resistance is an important component in the manifestation of these infections, and colonization resistance is mediated through several mechanisms, including direct inhibition by commensal strains of *E. faecalis* and obligate anaerobes such as *B. producta*, *Parabacteroides distasonis*, *Bacteroides sartorii*, and *C. bolteae*. Bacteria-derived TLR ligands drive indirect inhibition by stimulating production of RegIIIγ. **(B)** *C. difficile* infection can cause severe colitis by inducing epithelial cell death, and this loss of epithelial integrity allows for residual bacteria remaining after antibiotic treatment to spill into the underlying tissue and bloodstream. Spores of *C. difficile* are ingested by the host and germinate into vegetative cells upon stimulation by primary bile acids. When the microbiota is unperturbed by antibiotics, bacteria such as *C. scindens* are present and can convert primary bile acids into secondary bile acids, which inhibit vegetative cell growth. Succinate is a metabolic by-product of commensal bacteria, and sialic acid is a host-derived carbohydrate that is cleaved from epithelial cells by commensals and released into the intestinal lumen. At steady state, succinate and sialic acid support sustained growth of various commensal species, but when antibiotics are administered, the commensal species that would benefit from these factors are eliminated, leaving them to be used by vegetative *C. difficile* to facilitate its own growth instead. **(C)** Enterobacteriaceae are a family of bacteria that are adept at exploiting the antibiotic treated intestine by inducing inflammation, a setting in which Enterobacteriaceae can exploit to facilitate their own expansion. Commensal bacteria produce butyrate as a by-product of carbohydrate fermentation, which in turn prevents inflammation and also directly kills Enterobacteriaceae in the presence of acidified pH. Loss of butyrate production reduces PPARγ signaling in epithelial cells, inducing inducible nitric oxide synthase (iNOS) expression that can be used as a substrate for nitrogen respiration in Enterobacteriaceae. This increased availability of iNOS is exploited by Enterobacteriaceae, creating a positive feedback loop that enables expansion of these opportunistic pathogens since the increased presence of Enterobacteriaceae can in turn lead to increased expression of iNOS.

Several recent studies have identified mechanisms by which the intact intestinal microbiota confers resistance to *C. difficile* colitis. Spores of *C. difficile*, which can survive for long periods of time on dry surfaces, express a receptor that responds to primary bile salts such as taurocholate in the mammalian gastrointestinal tract, inducing germination (Francis et al., 2013). Certain commensal bacteria, such as *C. scindens*, combat *C. difficile* colonization in part by converting primary bile salts to secondary bile salts, leading to the production of deoxycholic acid and lithocholic acid, which inhibit vegetative growth of *C. difficile* (Wilson, 1983; Buffie et al., 2015; Fig. 2 B). Another inhibitory mechanism involves microbiota-mediated depletion of monosaccharides, such as sialic acid, that promote *C. difficile* growth. Antibiotic treatment can eliminate commensals that metabolize sialic acid, thereby increasing sialic acid concentrations in the colon to the benefit of *C. difficile* (Ng et al., 2013). Commensal organisms also cleave sialic acids from host glycoproteins, and thus *C. difficile* growth depends on antibiotic-mediated elimination of bacteria that catabolize sialic acid while preserving bacteria that liberate sialic acid from mucosal glycoconjugates. Antibiotic treatment also leads to transient increases in the luminal concentration of succinate, which can also boost growth of *C. difficile* in the lower gastrointestinal tract (Ferreyra et al., 2014). SCFAs have also been implicated in resistance to *C. difficile* infection (Rolfe, 1984), with dietary fiber and consequent production of the SCFAs acetate, propionate, and butyrate enhancing *C. difficile* clearance from the mouse gut (Hryckowian et al., 2018).

The high rate of recurrence following antibiotic treatment of *C. difficile* infection likely results from persistent damage to the microbiota, regardless of which antibiotic regimen is used

(Cornely et al., 2014). A randomized clinical trial demonstrated that fecal microbiota transplantation (FMT) is highly effective at curing recurrent *C. difficile* infection (van Nood et al., 2013). A key factor in preventing recurrence and achieving remission is restoring a "healthy" microbiota, specifically *Bacteroides*, Lachnospiraceae, and Ruminococcaceae species (Schubert et al., 2014, 2015). Provision of strains of Lachnospiraceae, *Lactobacillus*, *Bifidobacterium*, and *Lactococcus* have also shown varying degrees of success in preventing *C. difficile* recurrence in vitro and in mouse models, but further work needs to be done to optimize which consortia of strains are optimal for prevention (Reeves et al., 2012; Schoster et al., 2013; Le Lay et al., 2016). Recent analyses of donor and recipient microbiota, in the setting of FMT in patients with recurrent *C. difficile* infection, led to a model whereby the abundance and phylogeny of the donor and pre-transplant recipient microbiota could be used to predict successful microbial engraftment and might ultimately facilitate the assembly of a specific bacterial consortia that optimizes engraftment (Smillie et al., 2018).

Enterobacteriaceae

Infections caused by Gram-negative rods belonging to the Enterobacteriaceae family of the Proteobacterium phylum are particularly problematic in healthcare settings. This family includes pathogenic organisms such as *S. enteritidis*, *S. flexneri*, and *Yersinia enterocolitica*, but also many other members that are less virulent and are common residents of the mammalian intestinal tract, including *E. coli*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, and *Enterobacter cloacae*. As facultative anaerobes, these bacteria inhabit the length of the gastrointestinal tract, from the oral cavity to the anaerobic colon. The density of Enterobacteriaceae colonization is generally low, rarely contributing more than a fraction of 1% to the colonic microbiota. However, following antibiotic treatment, these organisms can undergo marked expansion and can achieve over 90% occupancy of the lower gastrointestinal tract in some settings (Taur et al., 2012). This scenario has become increasingly common in clinical settings as organisms like *E. coli* and *K. pneumoniae* have acquired resistance against a wide range of antibiotics, in some circumstances all those that are clinically available.

The mechanisms by which antibiotic-naive microbiota confers colonization resistance against Enterobacteriaceae are manifold but can be divided into three main groups: direct microbe-to-microbe inhibition; competition for nutrients such as carbohydrates, iron, zinc, and manganese; and indirect inhibition via activation of the host immune system or modification of host factors. Because the Enterobacteriaceae family includes important gastrointestinal pathogens, much work on microbiota-mediated colonization resistance has focused on pathogens (*Salmonella* and *Yersinia* in particular), but findings from these studies likely apply to Enterobacteriaceae in general.

Of particular recent interest has been the finding that Enterobacteriaceae undergo expansion during inflammation of the gut (Lupp et al., 2007). Deeper studies of this phenomenon revealed that *S. typhimurium*, for example, exploits inflammation and associated reactive oxygen species by using tetrathionate as a respiratory electron acceptor (Winter et al., 2010). More recent studies have demonstrated that antibiotic-induced loss of butyrate reduces PPARγ signaling and thereby induces gut inflammation and inducible nitric oxide synthase (iNOS) expression, providing *E. coli* with a growth advantage because it can use nitrates as a respiratory electron acceptor (Byndloss et al., 2017; Fig. 2 C). The host inflammatory response includes production of calprotectin, a molecule that sequesters zinc and manganese, thereby depriving pathogenic microbes of essential nutrients. But *S. enterica* combats this by encoding metal transporters that outcompete host-mediated chelation of manganese (Liu et al., 2012; Diaz-Ochoa et al., 2016). In the setting of intestinal inflammation, competition between members of the Enterobacteriaceae family can be mediated by small bacterial proteins called microcins that enable certain strains of *E. coli*, for example, to expand in the intestinal lumen (Sassone-Corsi et al., 2016). This form of colonization resistance has recently been exploited by engineering an *E. coli* strain that encodes a tetrathionine-inducible microsin, resulting in resistance to *Salmonella* infection (Palmer et al., 2018).

One of the most important mechanisms of growth restriction of Enterobacteriaceae is mediated by SCFAs such as acetate and butyrate, particularly at low pH. Early studies showed that expansion of *Salmonella* in the mouse colon was inhibited by acetate at low pH, but not high pH, and that antibiotic treatment increased the luminal pH (Bohnhoff et al., 1964b). The widespread use of *E. coli* for the production of recombinant proteins led to the discovery that acetate and butyrate are protonated at low pH, allowing them to diffuse across the bacterial membrane and subsequently acidify the bacterial cytoplasm, inhibiting bacterial growth (Booth, 1985). This general process of fermentative acidification has been used for centuries to preserve food by inhibiting the growth of pathogens during storage (Levine and Fellers, 1940). Acetate production by *Bifidobacteria* protects mice against enteropathogenic *E. coli* infection, with inhibition attributed to acetate-mediated enhancement of mucosal epithelial resistance to secreted bacterial enterotoxins (Fukuda et al., 2011).

The identity of commensal bacterial species that inhibit Enterobacteriaceae in the lower gastrointestinal tract has been investigated most extensively with *S. enterica*. The importance of obligate anaerobes in inhibition of *S. enterica* was recognized by Bohnhoff over 50 years ago (Bohnhoff et al., 1964a), and more recent studies have correlated the presence of specific commensal species with enhanced resistance to *S. enterica* infection (Brugiroux et al., 2016; Sassone-Corsi et al., 2016). FMT has been demonstrated to clear dense intestinal colonization with *K. pneumoniae* in mice (Caballero et al., 2015), and some examples of FMT-mediated clearance of antibiotic-resistant bacteria suggest that this may extend to humans (Crum-Cianflone et al., 2015; Biliński et al., 2016). Further studies, however, are necessary to identify the mechanisms by which specific commensal bacteria inhibit the expansion of Enterobacteriaceae in the intestinal lumen.

Current status of microbiota-mediated inhibition of intestinal pathogens

Over the past decade, the growing focus on the microbiota has greatly increased our understanding of colonization resistance, in part by revealing that the infectiousness of intestinal patho-

gens can be reduced by multiple parallel mechanisms. In some cases, recent studies using new experimental platforms and technologies have confirmed old ideas and findings. But novel mechanisms are also being discovered. Undoubtedly we are far from completely understanding microbiota-mediated defenses, in part because there are mechanisms that await discovery but also because the relative contributions of known mechanisms have, so far, been inadequately quantified. Not surprisingly, the most recently discovered mechanisms tend to gain center-stage attention for a while, only to be replaced by the next discovery, which, though more recent, may be quantitatively less impactful. An ongoing challenge, therefore, is to temporally, quantitatively, and biogeographically (e.g., inhibition in ileum versus colon) stitch together the various inhibitory mechanisms.

Although the enormous impact of antibiotic treatment on human health and longevity is difficult to overstate, recognition that antibiotics can have adverse effects on health and paradoxically result in increased susceptibility to infection is increasing (Pamer, 2016). While serious bacterial infections require antibiotic administration, remediating post-treatment damage to a patient's microbiota represents a logical, if challenging, subsequent step. With this concept in mind, a recent study demonstrated the feasibility of collecting, characterizing, and storing the fecal microbiota of patients before hematopoietic stem cell transplantation (which is often associated with marked antibiotic-mediated destruction of the intestinal microbiota), and then successfully reimplanting the patient's own microbiota following stem cell transplant (Taur et al., 2018). For patients undergoing complex medical procedures associated with microbiota loss, reconstitution of the microbiota with the patient's own commensal microbes represents an approach that may reduce the incidence of subsequent infections.

As previously highlighted, the intestinal microbiota is an ecosystem (Costello et al., 2012). Members of ecosystems establish relationships that range from symbiotic to commensal to competitive. Characteristics of the occupied space, such as temperature, moisture, pH, and osmolarity, can have enormous impacts on which species flourish, struggle, or become extinct. Ecosystem inhabitants modify the spaces they occupy to varying extents. In some circumstances, the very existence of the physical space depends on its inhabitants, as is the case with the microbial ecosystem contained within the intestine of humans and other mammals. Thus, competitive interactions between intestinal inhabitants are likely tempered by the need to maintain the health of their host. In the gut, optimal support of the host requires an array of bacterial species that serve digestive, metabolic, developmental, and immune-activating functions. From the perspective of a commensal bacterial species that lives in the gut lumen, vanquishing a competing species and conquering its niche may seem like a predominating evolutionary strategy, but the associated loss of microbial diversity would reduce the health of the host and thus damage or even eliminate the environment. Deeper and more complete understanding of the complex relationships between commensal bacterial species, mammalian hosts, and invasive pathogens is likely to lead to clinically important approaches to improve human health and resistance to infection.

Acknowledgments

This work was supported by grants from the National Institutes of Health (P30 CA008748, P01 CA023766, R01 AI095706, R01 AI42135, and U01 AI124275) and a grant from Cycle for Survival to the Memorial Sloan Kettering Center for Microbes, Inflammation, and Cancer.

E.G. Pamer has received speaker honoraria from Bristol-Myers Squibb, Celgene, Seres Therapeutics, MedImmune, Novartis, and Ferring Pharmaceuticals, is an inventor on patent application #WPO2015179437A1, entitled "Methods and compositions for reducing *Clostridium difficile* infection" and #WO2017091753A1, entitled "Methods and compositions for reducing vancomycin-resistant enterococci infection or colonization," and holds patents that receive royalties from Seres Therapeutics.

Author contributions: E.G. Pamer and J.W. Keith contributed equally to the writing of this review.

Submitted: 1 March 2018
Revised: 31 May 2018
Accepted: 11 September 2018

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