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Journal

Nature, 535(7610)

ISSN

0028-0836

Authors

Bäumler, Andreas J

Sperandio, Vanessa

Publication Date

2016-07-07

DOI

10.1038/nature18849

Peer reviewed

HHS Public Access

Author manuscript

Nature. Author manuscript; available in PMC 2016 November 18.

Published in final edited form as:

Nature. ; 535(7610): 85–93. doi:10.1038/nature18849.

Interactions between the microbiota and pathogenic bacteria in the gut

Andreas J. Bäumler[1] and **Vanessa Sperandio**[2,3]

[1]Department of Medical Microbiology and Immunology, University of California, Davis, School of Medicine, Davis, California 95616, USA

[2]Department of Microbiology, University of Texas Southwestern Medical Center, Dallas, Texas 75390-9048, USA

[3]Department of Biochemistry, University of Texas Southwestern Medical Center, Dallas, Texas 75390-9038, USA

Abstract

The microbiome has an important role in human health. Changes in the microbiota can confer resistance to or promote infection by pathogenic bacteria. Antibiotics have a profound impact on the microbiota that alters the nutritional landscape of the gut and can lead to the expansion of pathogenic populations. Pathogenic bacteria exploit microbiota-derived sources of carbon and nitrogen as nutrients and regulatory signals to promote their own growth and virulence. By eliciting inflammation, these bacteria alter the intestinal environment and use unique systems for respiration and metal acquisition to drive their expansion. Unravelling the interactions between the microbiota, the host and pathogenic bacteria will produce strategies for manipulating the microbiota against infectious diseases.

Appreciation of the important role of the microbiota in human health and nutrition has grown steadily in the past decade. Initial studies focused on cataloguing the microbial species that comprise the microbiota and correlating the composition of the microbiota with the health or disease state of the host. The present period of renaissance has resulted in technologies and interdisciplinary research that are conducive to mechanistic studies and, in particular, those that focus on associations between the microbiota, the host and pathogenic bacteria. Exciting research is now starting to unravel how the composition of the microbiota can offer either resistance or assistance to invading pathogenic species. The majority of these studies were conducted in the gastrointestinal tract, in which associations between the host and microbes are of paramount importance. The gut microbiota of each individual is unique at the genus and species levels; however, it is more generally conserved at the phylum level, which is populated most prominently by Bacteroidetes and Firmicutes, followed by Proteobacteria and Actinobacteria. Host genetics, diet and environmental insults such as

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Correspondence should be addressed to V.S. (vanessa.sperandio@utsouthwestern.edu).

The authors declare no competing financial interests.

Readers are welcome to comment on the online version of this paper at go.nature.com/28ix4vg.

treatment with antibiotics alter the microbiota[1–4], which can lead to varying susceptibility to infectious diseases between individuals[5].

The microbiota can promote resistance to colonization by pathogenic species[6–9]. For instance, mice that are treated with antibiotics or that are bred in sterile environments (known as germ-free mice) are more susceptible to enteric pathogenic bacteria such as *Shigella flexneri, Citrobacter rodentium*, *Listeria monocytogenes* and *Salmonella enterica* serovar Typhimurium[10–13]. And some microbiotas can lead to the expansion or enhanced virulence of pathogenic populations[7]. A notable example concerns how differences in the composition of microbiotas determine the susceptibility of the mice to infection with *C. rodentium*: the transplantation of microbiotas from strains of mice that are susceptible to infection induced similar susceptibility in animals that were previously insusceptible, and the transplantation of microbiotas from resistant animals led to resistance to infection in previously susceptible animals[14,15]. Epidemiological surveys reinforce this idea. For example, differential susceptibility to infection with *Campylobacter jejuni* was shown to depend on the species composition of the microbiotas in a study of Swedish adults[16]. Individuals with a higher diversity within their microbiotas, and with an abundance of bacteria from the genera *Dorea* and *Coprococcus*, were significantly recalcitrant to *C. jejuni* infection compared with people who had low-diversity microbiotas and non-abundance of *Dorea* and *Coprococcus*.

The host's diet profoundly affects the composition of the microbiota, with repercussions for the physiology, immunity and susceptibility to infectious diseases of the host[17]. Dietary choices have been shown to affect colonization by enterohaemorrhagic *Escherichia coli* (EHEC) serotype O157:H7 and the severity and length of its resulting disease[18], and supplementation of the diet with phytonutrients promotes the expansion of beneficial Clostridia species that protect mice from colonization by *C. rodentium*[19].

The use of innovative technologies, in combination with more conventional approaches, is driving our understanding of the interactions between the microbiota, the host and pathogenic bacteria. The genetic tractability of several species of bacteria, as well as of their mammalian hosts (such as mice), allows for the mechanistic investigation of these relationships. The investigation of changes in the composition of microbiotas has been driven by next-generation sequencing, which also facilitated the analysis of transcriptomes. The growing power and finesse of metabolomics studies are quickly expanding our knowledge of the impact of both the microbiota and of pathogenic bacteria on the metabolic landscape of the gut. Here, we review advances in our understanding of the complex relationships that determine the severity and outcome of gastrointestinal infections. The majority of the mechanistic studies that investigate these interactions have been conducted in *S*. Typhimurium, EHEC and *Clostridium difficile*: therefore, these pathogenic organisms are covered more extensively than others in this Review.

Antibiotics

Antibiotics revolutionized medicine and were justifiably dubbed 'magic bullets' against bacterial infections. However, conventional antibiotics are generally bacteriostatic or

bactericidal, which means that they indiscriminately kill or prevent the growth of both pathogenic and beneficial microbes. Antibiotics can alter the taxonomic, genomic and functional features of the microbiota, and their effects can be rapid and sometimes everlasting[20]. They can decrease the diversity of the microbiota, which compromises resistance to colonization by incoming pathogenic bacteria[20] — most notably leading to an expansion of *C. difficile* that can cause diarrhoea that leads to potentially fatal colitis[21].

C. difficile is a spore-forming bacterium that, on germination, colonizes the large intestine and causes colitis through the action of two toxins: TcdA and TcdB. The majority of *C. difficile* infections are nosocomial, but there has also been an increase in community-acquired infections, mainly due to the ubiquitous presence of *C. difficile* spores. *C. difficile* can colonize the mammalian intestine without causing disease, but one of the most important risk factors for colitis that is mediated by *C. difficile* is the use of antibiotics[21]. The antibiotics-mediated loss of resistance to colonization also allows colonization by *S.* Typhimurium and the development of disease[22]. Both *C. difficile* and *S.* Typhimurium catabolize sialic acid as a source of carbon in the lumen to promote their expansion[23]. They rely on saccharolytic members of the microbiota, such as *Bacteroides thetaiotaomicron*, to make this sugar freely available in the intestinal lumen. Treatment with antibiotics increases the abundance of host-derived free sialic acid as well as enhancing its release into the lumen by *B. thetaiotaomicron*, which promotes the expansion of the two pathogenic bacteria[23]. Antibiotic use also triggers production of the organic acid succinate, another microbiota-derived nutrient that confers a growth advantage to *C. difficile*. It is often present at a low concentration in the microbiotas of conventional mice, but its presence increases on treatment with antibiotics, which promotes a bloom of *C. difficile*[24] (Fig. 1).

Knowledge of how microbiota disruption affects the ability of bona fide or opportunistic pathogenic organisms to infect hosts is still in its infancy. However, two underlying themes converge: microbiota-induced changes in the metabolite landscape of the gut and inflammation.

Utilization of nutrients

Simple dietary sugars are absorbed in the small intestine, which means that they are unavailable as sources of carbon for the microbiota and pathogenic bacteria in the colon. The most abundant members of the microbiota are those that are able to utilize the undigested plant polysaccharides and host glycans that are present in the colon[25].

The gut epithelium is protected by a layer of mucus that is composed of proteins known as mucins that are rich in fucose, galactose, sialic acid, *N*-acetylgalactosamine, *N*-acetylglucosamine and mannose. These sugars are harvested by saccharolytic members of the microbiota, such as Bacteroidales in the gut, which makes them available to species within the microbiota that lack this capability[26]. However, pathogenic bacteria in the gut can also exploit the availability of these sugars to promote their own expansion. Several studies have used *B. thetaiotaomicron* as a model Bacteroides in which to investigate these syntrophic links. Sialic acid is a terminal sugar of some mucosal glycans, and *B. thetaiotaomicron* has sialidase activity but lacks the catabolic pathway for sialic-acid

utilization. The bacterium therefore releases sialic acid to gain access to underlying glycans that it can use as a source of carbon. The sialic acid that *B. thetaiotaomicron* releases from the mucus can be catabolized by both *C. difficile* and *S.* Typhimurium, which provides them with a growth advantage[23]. The ability of the microbiota to use sialic acid therefore depends on the action of *B. thetaiotaomicron*, and mutants that lack sialidase fail to enhance the growth of these two pathogenic bacteria[23].

B. thetaiotaomicron also releases fucose from the mucus. It harbours multiple enzymes that can cleave fucose from host glycans, so its presence results in the high availability of fucose in the lumen of the gut[27–30]. This free fucose can also be used as a source of carbon by *S.* Typhimurium[23]. Importantly, *B. thetaiotaomicron* can promote the fucosylation of mucosal glycans when introduced into monoassociated germ-free mice[31,32].

The microbiota resides in the lumen and the outer mucus layer of the intestine. EHEC, however, aims to achieve a unique niche by closely adhering to the enterocytes of the intestinal epithelium. To achieve its goal, EHEC must successfully compete with the microbiota for nutrients. *B. thetaiotaomicron* does not need to compete with EHEC, however, because it can utilize polysaccharides; EHEC can only utilize monosaccharides and disaccharides[13,33]. EHEC's main competitors are commensal *E. coli*, which preferentially utilizes fucose as a source of carbon when growing in the mammalian intestine[13,33]. To circumvent this competition, EHEC utilizes other sources of sugar, such as galactose, the hexuranates, mannose and ribose, which commensal *E. coli* cannot catabolize optimally[33,34] (Fig. 2).

EHEC uses fucose as a signalling molecule with which to adjust its metabolism and to regulate the expression of its virulence repertoire in the lumen and the outer mucus layer of the colon[35]. It horizontally acquired a pathogenicity island of genes that encode a fucose-sensing signalling-transduction system[35]. This system is unique to EHEC and to *C. rodentium*[35] (which is used extensively in mouse models as a surrogate for the human pathogen EHEC[36]). It is composed of the membrane-bound histidine sensor kinase FusK, which specifically autophosphorylates in response to fucose. FusK then transfers its phosphate to a response regulator called FusR, which is a transcription factor. Phosphorylation activates FusR, which represses the expression of the fucose utilization genes in EHEC, and helps EHEC to avoid the need to compete for this nutrient with commensal *E. coli*[35]. To prevent the unnecessary expenditure of energy by EHEC, FusR represses the genes that encode the EHEC virulence machinery, a syringe-like apparatus known as a type III secretion system (T3SS), which the bacterium uses to adhere itself to enterocytes and highjack the function of these host cells[35]. EHEC therefore uses fucose, a host-derived signal that is made available by the microbiota, to sense the environment of the intestinal lumen and to modulate its own metabolism and virulence.

To reach the lining of the epithelium, EHEC and *C. rodentium* produce mucinases[37], which cleave the protein backbone of mucin-type glycoproteins. Expression of these enzymes is increased by metabolites that are produced by *B. thetaiotaomicron*[38]. Because mucus is one of the main sources of sugar in the colon, where EHEC and *C. rodentium* colonize, obliteration of the mucus layer creates a nutrient-poor environment near the epithelium that

is referred to as gluconeogenic. The colonization of mice by *B. thetaiotaomicron* therefore profoundly changes the metabolic landscape of the mouse gut because it raises the levels of organic acids such as succinate[24,38,39]. Moreover, several metabolites that indicate a gluconeogenic environment, such as lactate and glycerate, are also elevated[38]. EHEC and *C. rodentium* sense this gluconeogenic and succinate-rich environment through the transcriptional regulator Cra. On receiving the cue that they have reached the lining of the gut epithelium, these bacteria activate the expression of their T3SSs[38]. EHEC therefore exploits metabolic cues from *B. thetaiotaomicron*, and probably other members of the microbiota, to precisely programme its metabolism and virulence (Fig. 2).

Other pathogenic bacteria can also adjust their gene expression in the presence of microbiota-produced succinate. *C. difficile* induces a pathway that converts succinate to butyrate, which confers a growth advantage *in vivo*[24]. Populations of *C. difficile* mutants that are unable to convert succinate fail to expand in the gut in the presence of *B. thetaiotaomicron*[24].

Several short-chain fatty acids that are produced by the microbiota, are important determinants of interactions between the microbiota and pathogenic bacteria in the gut. The abundance and composition of short-chain fatty acids is distinct in each compartment of the intestine, and the ability to sense these differences might help pathogenic bacteria in niche recognition. The most abundant short-chain fatty acids in the gut are acetate, propionate and butyrate. *S.* Typhimurium preferably colonizes the ileum[40], which generally contains acetate at a concentration of 30 mM. This acetate concentration enhances the expression of the *S.* Typhimurium *Salmonella* pathogenicity island 1 (SPI-1)-encoded T3SS (T3SS-1), which is involved in the bacterium's invasion of the host. Conversely, 70 mM propionate and 20 mM butyrate, concentrations typical of the colon, suppress the expression of the T3SS-1 (ref. 41). Propionate and butyrate seem to affect the T3SS-1 regulatory cascade at various levels. However, the detailed mechanism of this regulation is yet to be unravelled. In EHEC, exposure to the levels of butyrate found in the colon increases the expression of the EHEC T3SS through post-transcriptional activation of the transcriptional regulator Lrp[42]. Exposure to the concentrations of acetate and propionate that are found in the small intestine does not significantly affect the virulence of EHEC.

Diet has a profound effect on the composition of the microbiota and the concentration of short-chain fatty acids in the gut[17]. A diet that is high in fibre results in the enhanced production of butyrate by the gut microbiota. That increases the host's expression of globotriaosylceramide, which is a receptor for the Shiga toxin that is produced by EHEC[18]. Shiga toxin can lead to the development of haemolytic uraemic syndrome (HUS) and is the cause of the morbidity and mortality associated with outbreaks of EHEC[43]. Consequently, animals that are fed a high-fibre diet are more susceptible to Shiga toxin than are those on a low-fibre diet and develop more severe disease[18]. Conversely, increased levels of microbiota-derived acetate protect animals from disease that is caused by the toxin. Certain species of *Bifidobacteria* contribute to higher levels of acetate in the gut, which helps to improve the barrier function of the intestinal epithelium and to prevent Shiga toxin from reaching the bloodstream[44].

Enteric pathogenic bacteria also use other nutrients to successfully overcome the microbiota's resistance to their colonization. Ethanolamine is abundant in the mammalian intestine[45]. It can be used as a source of carbon and of nitrogen by a number of pathogenic species[46], and food-borne bacteria are particularly adept at using it. However, it cannot be metabolized by the majority of commensal species[47]. *S.* Typhimurium, EHEC and *L. monocytogenes* gain a growth advantage in the intestine through their ability to use this compound[45,48,49]. Ethanolamine is also used as a signal by EHEC and *S.* Typhimurium to activate the expression of virulence genes[50,51]. And *S.* Typhimurium uses hydrogen produced by the microbiota as an energy source to enhance its growth during the initial stage of infection[52].

The exploitation of microbiota-derived molecules as both nutrients and signals is crucial for the successful infection of the host by pathogenic bacteria. Although such organisms have clearly developed many strategies through which to circumvent the microbiota's resistance to colonization, and in many cases even employ its help, the microbiota pushes back, which creates an intense competition for resources. The ability of EHEC to colonize the intestine stems from differences in the sources of sugar that are used by EHEC and by commensal *E. coli*. For example, the presence of multiple strains of commensal *E. coli* with overlapping nutritional requirements interferes with the colonization of the mouse intestine by EHEC[53]. This study uses a streptomycin-treated mouse model of EHEC and three distinct commensal strains of *E. coli* to assess differential sugar requirements for the successful colonization of the intestines[53]. EHEC could colonize mice that were pre-colonized with any one of the commensal strains, but it could not colonize mice that were pre-colonized with all three strains[53]. EHEC has evolved to exploit distinct sources of sugar during colonization of the gut. It utilizes catabolic pathways for the hexuronates glucuronate and galacturonate and for sucrose that are not employed by commensal *E. coli* within the gut[33,53]. It can also metabolize several sugars simultaneously. The loss of multiple catabolic pathways has an additive effect on colonization. This phenomenon is not observed in commensal *E. coli*, however, which suggests that *E. coli* uses available sugars in a stepwise fashion[54]. EHEC therefore differs from commensal *E. coli* in metabolic strategy and the use of nutrients for the colonization of the mammalian intestine.

C. rodentium is outcompeted and then cleared from the mouse gut through a bloom in the population of commensal *E. coli*, which competes with *C. rodentium* for monosaccharides for nutrition[13]. By contrast, *C. rodentium* is not cleared by *B. thetaiotaomicron* in germ-free mice that are fed a diet that contains both monosaccharides, which can be used by Enterobacteriacae such as *C. rodentium*, and polysaccharides, which can be used by *Bacteroides*. However, when the mice are switched to a diet that consists only of monosaccharides, *B. thetaiotaomicron* and *C. rodentium* are forced to compete for sugars, and *B. thetaiotaomicron* outcompetes *C. rodentium*[13]. The ability of pathogenic bacteria to successfully compete with commensal species for nutrients is therefore important for their establishment in the gut.

Interception of signals from the microbiota and the host

The microbiota affects the risks and courses of enteric diseases. *Vibrio cholerae* is a major cause of explosive diarrhoea in which there is extensive disruption of the intestinal population of microbes. Metagenomic studies of the faecal microbiota of people with cholera in Bangladesh show that recovery is characterized by a certain microbiota signature. Reconstitution of this microbiota in germ-free mice restricts the infectivity of *V. cholerae*. Specifically, the presence of *Ruminococcus obeum* can hamper the colonization of the intestines by *V. cholerae* through the production of the furanone signal autoinducer-2, which causes the repression of several *V. cholerae* colonization factors[55].

Another example of the effect of microbiota-derived signals on host colonization is their use by EHEC in the colonization of its ruminal reservoir. EHEC exclusively colonizes the recto–anal junction of adult cattle. Through the sensor protein SdiA, EHEC detects acyl-homoserine lactone signals from the rumen microbiota, which it uses to reprogram itself to survive the acidic pH of the animal's stomachs and to successfully colonize the rectoanal junction[56].

As well as being able to directly detect signals that are derived from the microbiota, pathogenic bacteria can detect host-derived signals that have been modified by the microbiota to modulate their virulence. *V. cholerae* has a type VI secretion system (T6SS), which it uses to kill other bacteria. During its colonization of the intestine, *V. cholerae* comes in contact with the mucosal microbiota, which can affect the composition of bile acids in the intestine. For example, *Bifidobacterium bifidum* negatively regulates the T6SS activity of *V. cholerae* through the metabolic conversion of three bile acids (glycodeoxycholic acid, taurodeoxycholic acid and cholic acid) into the bile acid deoxycholic acid. Deoxycholic acid, but not its unmodified salts, decreases the expression of T6SS genes. This leads to a decrease in the killing of *E. coli* by *V. cholerae* owing to bile-acid conversion by other commensals, which decreases the activity of the T6SS[57].

Another microbiota-modified host signal that is detected by pathogenic bacteria is the neurotransmitter noradrenaline. The gut is highly innervated, and neurotransmitters are important signals in the gastrointestinal tract, where they modulate peristalsis, the flow of blood and the secretion of ions[58]. The microbiota affects the availability of neurotransmitters in the intestinal lumen, as well as their biosynthesis. For example, the microbiota induces biosynthesis of serotonin[59], and microbiota-derived enzymatic activities increase the levels of active noradrenaline in the gut lumen[60]. Noradrenaline is synthesized by the adrenergic neurons of the enteric nervous system[61] and it is inactivated by the host through conjugation with glucuronic acid (to produce a glucuronide). Microbiota-produced enzymes known as glucuronidases then deconjugate glucuronic acid from noradrenaline, which increases the amount of active noradrenaline in the lumen of the intestine[60]. Several pathogenic bacteria of the gut, including EHEC, *S.* Typhimurium and *V. parahaemolyticus*, sense noradrenaline to activate the expression of virulence genes[62–65]. Two adrenergic sensors have been identified in bacteria: the membrane-bound histidine kinases QseC and QseE[66,67]. QseC also detects the microbiota-produced signal autoinducer-3 (refs 64 and 66), so the sensing of

signals from both the host and the microbiota converge at the level of a single receptor, a process known as inter-kingdom signalling.

Inflammation

Although diet and the composition of the microbiota heavily influence the availability of nutrients in the gut, the host also has an important part to play. A crucial driver of changes in the gut environment is the inflammatory response of the host. Intestinal inflammation in people is associated with an imbalance in the microbiota, known as dysbiosis, and is characterized by a reduced diversity of microbes, a reduced abundance of obligate anaerobic bacteria and an expansion of facultative anaerobic bacteria in the phylum Proteobacteria, mostly members of the family Enterobacteriaceae[68–73]. Similar changes in the composition of the gut microbiota are observed in mice with chemically induced colitis[74] and genetically induced colitis[75]. These changes in the structure of the microbiota probably reflect an altered nutritional environment that is created by the inflammatory response of the host.

The availability of nutrients in the large intestine is altered during inflammation through changes in the composition of mucous carbohydrates. Interleukin (IL)-22, a cytokine that is prominently induced in the intestinal mucosa when mice and rhesus macaques are infected with *S.* Typhimurium[76,77], stimulates the epithelial expression of galactoside 2-α-L-fucosyltransferase 2 and enhances the α(1,2)-fucosylation of mucus carbohydrates[78,79]. The gut microbiota can liberate fucose from mucus carbohydrates[23,80], which leads to the induction of genes for fucose utilization in *E. coli*[78]. Similarly, increased fucosylation of glycans is observed during *S.* Typhimurium-induced colitis in mice, which correlates with elevated synthesis of the proteins involved in fucose utilization[81]. Mucus fucosylation that is induced during infection with *C. rodentium* causes changes in the composition of the gut microbiota that help to protect the host from the expansion and epithelial translocation of the pathobiont *Enterococcus faecalis*[79].

Another driver of changes in the nutritional environment of the gut is the generation of reactive oxygen species and reactive nitrogen species during inflammation. Pro-inflammatory cytokines such as interferon-γ (IFN-γ) activate dual oxidase 2 in the intestinal epithelium, which produces hydrogen peroxide[82]. Increased expression of *DUOX2*, the gene that encodes dual oxidase 2, in the intestinal mucosa of patients with Crohn's disease and ulcerative colitis correlates with an expansion of Proteobacteria in the gut microbiota[83]. IFN-γ also induces epithelial expression of the gene *Nos2* (ref. 84), which encodes inducible nitric oxide synthase, the enzyme that catalyses the production of nitric oxide from L-arginine[85]. As a result, the concentration of nitric oxide is elevated in gases from the colons of people with inflammatory bowel disease[86–88]. Although reactive oxygen and nitrogen species have antimicrobial activity, these radicals quickly form non-toxic compounds in the lumen of the gut as they diffuse away from the epithelium. For example, when they are generated during inflammation by host enzymes in the intestinal epithelium, these species react to form nitrate[89]. This by-product of inflammation is present at elevated concentrations in the intestines of mice with chemically induced colitis[90] (Fig. 3). Nitrate reductases, enzymes that are broadly conserved among the Enterobacteriaceae, couple the reduction of nitrate to energy-conserving electron transport systems for respiration, a process

termed nitrate respiration. However, the genes that encode them are absent from the genomes of obligate anaerobic Clostridia or Bacteroidia[91]. Nitrate respiration drives the *Nos2*-dependent expansion of commensal *E. coli* in mice with chemically or genetically induced colitis, but not in animals without signs of intestinal inflammation[91]. Respiratory electron acceptors that are generated as a by-product of the host inflammatory response therefore create a niche in the lumen of the intestines that supports the uncontrolled expansion of commensal Enterobacteriaceae rather than of obligate anaerobic bacteria[91]. The resulting bloom in the inflamed intestine is one of the most consistent and robust ecological patterns that has been observed in the gut microbiota[92].

The creation of a niche for respiratory nutrients during inflammation is also an important driver of the strategies that pathogenic bacteria from the family Enterobacteriaceae use to invade the gut ecosystem. In the absence of inflammation or treatment with antibiotics, members of the gut microbiota occupy all available nutrient niches, which makes it very challenging for pathogenic Enterobacteriaceae to enter the community. One solution is for these bacteria to trigger intestinal inflammation, which would coerce the host into creating a fresh niche of respiratory nutrients that is suitable for its expansion — an approach that is used by *S.* Typhimurium[93]. On ingestion, *S.* Typhimurium uses T3SS-1 to invade the intestinal epithelium[94] and T3SS-2 to survive in the tissue of the host[95]. Both of these processes trigger acute intestinal inflammation in cattle and in mouse models of gastroenteritis[96–98] (Fig. 3). The inflammatory response of the host drives the expansion of *S.* Typhimurium in the lumen of the gut[99], which is required for the transmission of this pathogenic species to a new host through the faecal–oral route[100].

Although such expansion allows *S.* Typhimurium to side-step competition with obligate anaerobic Clostridia and Bacteroidia, this strategy forces the bacterium into battle with commensal Enterobacteriaceae over limited resources. For example, *S.* Typhimurium expands in the inflamed gut through nitrate respiration[101,102], which results in rivalry with commensal Enterobacteriaceae that pursue a similar strategy[91]. *S.* Typhimurium can gain an edge in this competition through its ability to utilize a broader range of inflammation-derived electron acceptors than its rivals. A source of one such electron acceptor is sulfate-reducing species of *Desulfovibrio* from the microbiota, which release hydrogen sulfide, a compound that is converted to thiosulfate by the epithelium of the colon to avoid toxicity[103]. Deployment of the virulence factors of pathogenic bacteria leads to the recruitment of neutrophils to the intestinal mucosa, which is the histopathological hallmark of *S.* Typhimurium-induced gastroenteritis[96]. A fraction of these recruited neutrophils migrate into the lumen of the intestine — a diagnostic marker of inflammatory diarrhoea[104]. In the lumen, neutrophils help to protect the mucosa by engulfing bacteria in the vicinity of the epithelium[105], but reactive oxygen species that are generated by the phagocyte-produced NADPH oxidase 2 (also known as cytochrome b-245 heavy chain) convert thiosulfate into tetrathionate, a respiratory electron acceptor that supports the expansion of *S.* Typhimurium in the lumen of the inflamed gut[106] (Fig. 3). Although tetrathionate respiration is a characteristic of *Salmonella* serovars and has been used empirically in their isolation in clinical microbiology laboratories since 1923 (ref. 107), insights into the respiratory nutrient niche that *Salmonella* occupies suggest that this property is part of a strategy to edge out competing commensal Enterobacteriaceae in the inflamed gut[106].

The inflammatory response of the host also ignites competition between commensal and pathogenic Enterobacteriaceae over trace elements such as iron, which is less available during inflammation. IL-22 induces the release of the antimicrobial protein lipocalin-2 (also known as neutrophil gelatinase-associated lipocalin) from the epithelium in mice and rhesus macaques[108,109]. Lipocalin-2 reduces iron availability by binding to enterobactin, a low-molecular-weight iron chelator (or siderophore) that is produced by Enterobacteriaceae[110,111]. To overcome this, *S.* Typhimurium and some commensal *E. coli* secrete a glycosylated derivative of enterobactin, termed salmochelin, which is not bound by lipocalin-2 (ref. 108). By producing salmochelin as well as two further siderophores that are not bound by lipocalin-2, yersiniobactin and aerobactin, the probiotic *E. coli* strain Nissle 1917 can limit the expansion of *S.* Typhimurium in the lumen of the inflamed gut[112]. Conversely, lipocalin-2 secretion by the epithelium generates an environment that enables *S.* Typhimurium to edge out commensal Enterobacteriaceae that depend solely on enterobactin for the acquisition of iron[109] (Fig. 3).

Through its limitation of iron availability, intestinal inflammation also sets the stage for battles between Enterobacteriaceae that use protein-based toxins known as colicins[113] that affect a narrow range of hosts. Iron limitation induces the synthesis of siderophore receptor proteins for the bacterial outer membrane[113], which also commonly serve as receptors for colicins[114–116]. Expression of a siderophore receptor protein termed the colicin I receptor (CirA) confers commensal *E. coli* with sensitivity to colicin Ib produced by *S.* Typhimurium[113]. The respiratory nutrient niche that is generated by the inflammatory response of the host is therefore a battleground on which commensal and pathogenic Enterobacteriaceae struggle for dominance using a diverse arsenal of nutritional and antimicrobial strategies.

Perspective and the future

The study of the microbiome began more than a century ago. equencing of 16S rRNA genes provided the first insights into the taxonomic composition of microbial communities. Later, sequencing of the complete metagenome of microbial communities provided a more detailed insight into the full genetic capacity of such a community. The use of germ-free animals, either alone or in combination with emerging technologies such as laser-capture microdissection and transcriptomics, enabled mechanistic studies of the associations between the microbiota, the host and pathogenic bacteria[117]. Multi-taxon insertion sequencing now allows researchers to investigate both the assembly and the shared and strain-specific dietary requirements of communities of microbes, and it has also facilitated the informed manipulation of such communities through diet[118]. The development of quantitative imaging technologies has provided insight into the localization of microbes within the gastrointestinal tract, and it has also enabled studies on the proximity of and interactions between microbes[119]. The increasing refinement and power of metabolomics, imaging mass spectrometry and three-dimensional mapping of mass-spectrometry data provide a high-resolution image of the complex chemistry landscape of the interactions between microbes and the host, which sets the stage for manipulating this chemistry to prevent or treat infectious diseases[24,38,120–127]. A marriage of metagenomics and mathematical modelling promises to enhance the precision of microbiome reconstitution,

which has proven successful for tackling *C. difficile* infections in mice[128]. In these exciting times, the expansion of multidisciplinary research is rapidly generating new technologies and mechanistic insights into interactions between the microbiota, the host and pathogenic bacteria (Box 1).

BOX 1

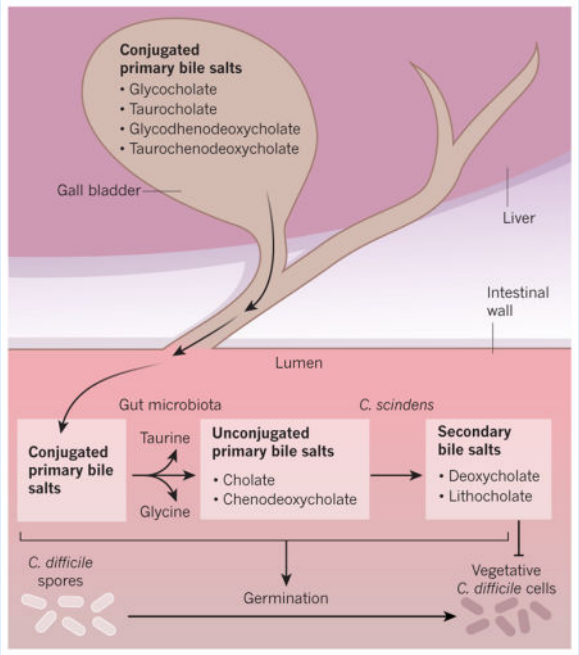
Microbiota interventions as therapeutic strategies to limit pathogen expansion

An imbalance in the gut microbiota might underlie many human diseases but, in most cases, the development of treatment options is still in its infancy. This could be in part because the mechanisms that lead to adverse effects in the host differ for each disease, which means that intervention strategies must be developed for each. The treatment options for antibiotic-induced dysbiosis are perhaps the most advanced, mainly because faecal microbiota transplantation can reverse this imbalance in the gut microbiota[129]. Nonetheless, the mechanisms through which treatment with antibiotics encourages an uncontrolled expansion of the obligate anaerobe *C. difficile* differ markedly from those that stimulate the growth of the facultative anaerobes Enterobacteriaceae, which has implications for the development of precision microbiome interventions.

Mice that are treated with streptomycin have a reduced abundance of members of the class Clostridia[130], which are credited with producing the lion's share of the short-chain fatty acid butyrate in the large intestine[131]. The resulting depletion of short-chain fatty acids drives an expansion of Enterobacteriaceae through mechanisms that are not fully resolved[44,132]. Depletion of Clostridia-derived butyrate affects the metabolism of enterocytes in the colon, which derive most of their energy by butyrate respiration[133]. The depletion of short-chain fatty acids also leads to a contraction in the pool of regulatory T cells in the colonic mucosa[134–136]. These changes in the host physiology increase the inflammatory tone of the mucosa, as indicated by the elevated expression of *Nos2*, the gene that encodes inducible nitric oxide synthase, and contributes to the expansion of commensal *E. coli* through nitrate generation[137]. Although other mechanisms probably contribute to the post-antibiotic expansion of certain populations of bacteria in the gut[126], the transfer of Clostridia, with their capacity for producing short-chain fatty acids, represents the most effective treatment for limiting the growth of *E. coli* in streptomycin-treated mice[138].

By contrast, the post-antibiotic expansion of the *C. difficile* population is driven by a depletion of secondary bile salts. The liver produces the primary bile salts cholate and chenodeoxycholate, which are conjugated to the amino acids taurine (to produce taurocholate and taurochenodeoxycholate) or glycine (to produce glycocholate and glycochenodeoxycholate) and then secreted into the gut. Bile salt hydrolases, enzymes that are produced by many members of the gut microbiota, remove the conjugated amino acid from the primary bile salt. *C. scindens* is one of a limited number of species of bacteria that can actively transport cholate and chenodeoxycholate into its cytosol, where these unconjugated primary bile salts are converted into the secondary bile salts deoxycholate and lithocholate, which are subsequently secreted into the extracellular

environment[139] (Box Fig.). Although both primary and secondary bile salts induce the germination of *C. difficile* spores, only secondary bile salts efficiently prevent the growth of vegetative *C. difficile* cells[140]. By significantly reducing the abundance of species that are capable of producing deoxycholate and lithocholate, treatment with antibiotics causes a depletion of these secondary bile salts and promotes the expansion of vegetative *C. difficile* cells in the large intestine[141,142]. Faecal microbiota transplantation restores the production of secondary bile salts and therefore prevents the expansion of *C. difficile*[143]. Direct supplementation of the diet with secondary bile salts warrants caution because increased concentrations of bile salts have been linked to gastrointestinal cancers[144]. However, inoculation with only the secondary-bile-salt-producing *C. scindens* confers mice with resistance to *C. difficile* expansion following treatment with antibiotics[128]. This remarkable observation opens the door to novel precision microbiome interventions that aim to prevent or treat the colitis that is associated with *C. difficile* infection after antibiotic therapy.



Acknowledgments

Work in the V.S. laboratory is supported by US National Institutes of Health (NIH) grants AI053067, AI077613, AI05135 and AI114511. Work in the A.J.B. laboratory is supported by US Department of Agriculture grant 2015-67015-22930 and NIH grants AI044170, AI096528, AI112445, AI114922 and AI117940. The contents of this Review are solely the responsibility of the authors and do not necessarily represent the official views of the NIH National Institute of Allergy and Infectious Diseases.

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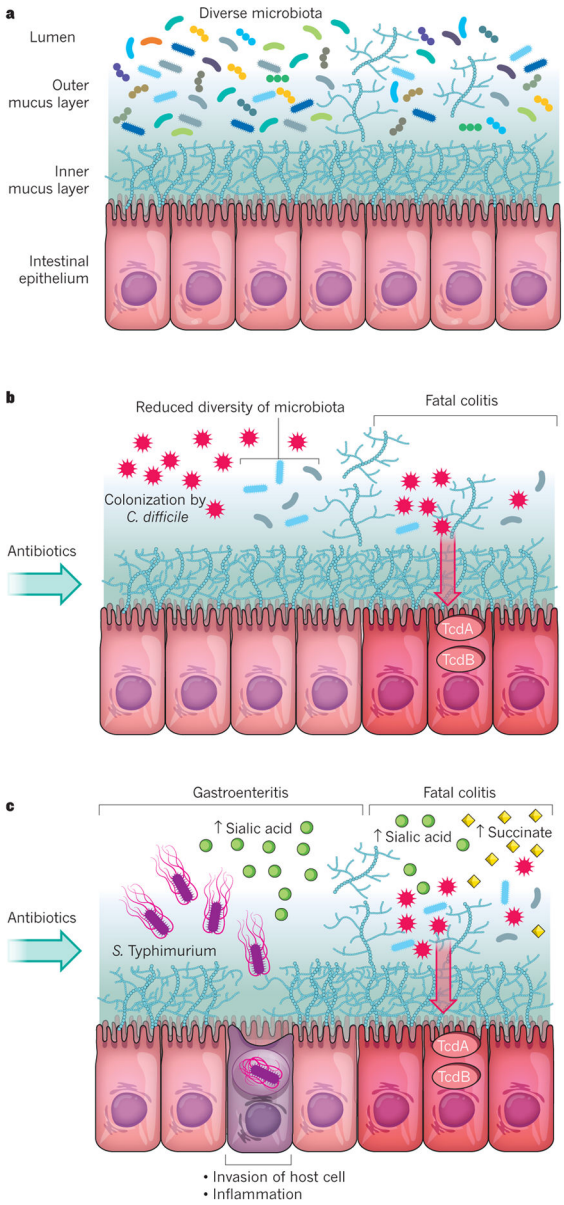

Figure 1. The impact of antibiotics on the microbiota and the expansion of enteric pathogens

a, A diverse and non-disturbed microbiota confers resistance to colonization by enteric pathogens in the intestinal epithelium. **b**, Treatment with antibiotics decreases the diversity of the microbiota and leads to expansion of the *C. difficile* population. Toxins that are released from *C. difficile* (TcdA and TcdB) enter and damage the cells of the epithelium, which leads to inflammation (colitis) and cell death. **c**, Treatment with antibiotics also leads to an increase in the levels of free sialic acid (from the host) and succinate (from the microbiota) in the lumen of the intestine. Elevated sialic acid promotes the expansion of the *S.* Typhimurium population, which can lead to inflammation (gastroenteritis) if the bacterium invades the cells of the intestinal epithelium. Elevated levels of sialic acid and succinate further promote the expansion of the *C. difficile* population and the development of colitis and cell death.

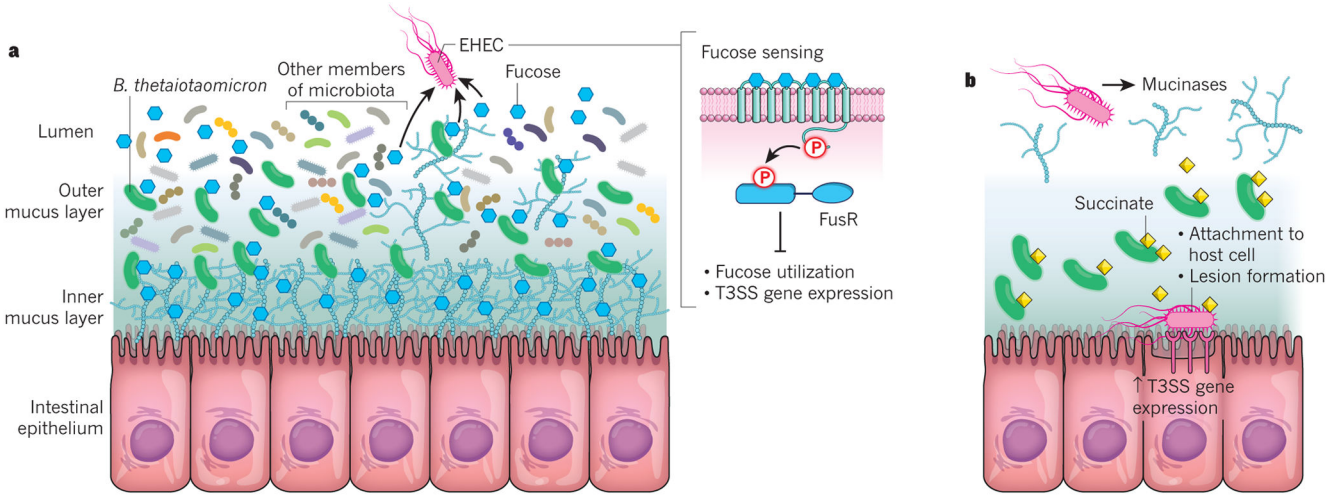

Figure 2. Modulation of enterohaemorrhagic E. coli virulence through nutrients provided by the microbiota

a, The microbiota resides in the lumen and outer mucus layer of the intestine. The saccharolytic bacterium *Bacteroides thetaiotaomicron* is a prominent member of the microbiota. It can release fucose from the mucus and makes the sugar available to other bacteria. When EHEC senses fucose through the FusKR signalling system, it represses both its use of the sugar and the expression of genes that encode the T3SS, a protein-translocation apparatus that enables the bacterium to secrete effector proteins into host cells. This repression prevents EHEC from competing for fucose with commensal *E. coli* and from expending energy unnecessarily on T3SS expression. **b**, Metabolites that are provided by *B. thetaiotaomicron*, such as succinate, lead to an increase in the expression by EHEC of the enzyme mucinase, which obliterates the mucus layers of the intestine. EHEC is then able to reach the intestinal epithelium. *B. thetaiotaomicron* then begins to secrete succinate and other metabolites that are required for gluconeogenesis into the now nutrient-poor environment. The compounds are sensed by EHEC, which upregulates its expression of the T3SS to enable the bacterium to attach to the epithelial cells of the host intestine and form lesions that cause diarrhoea.

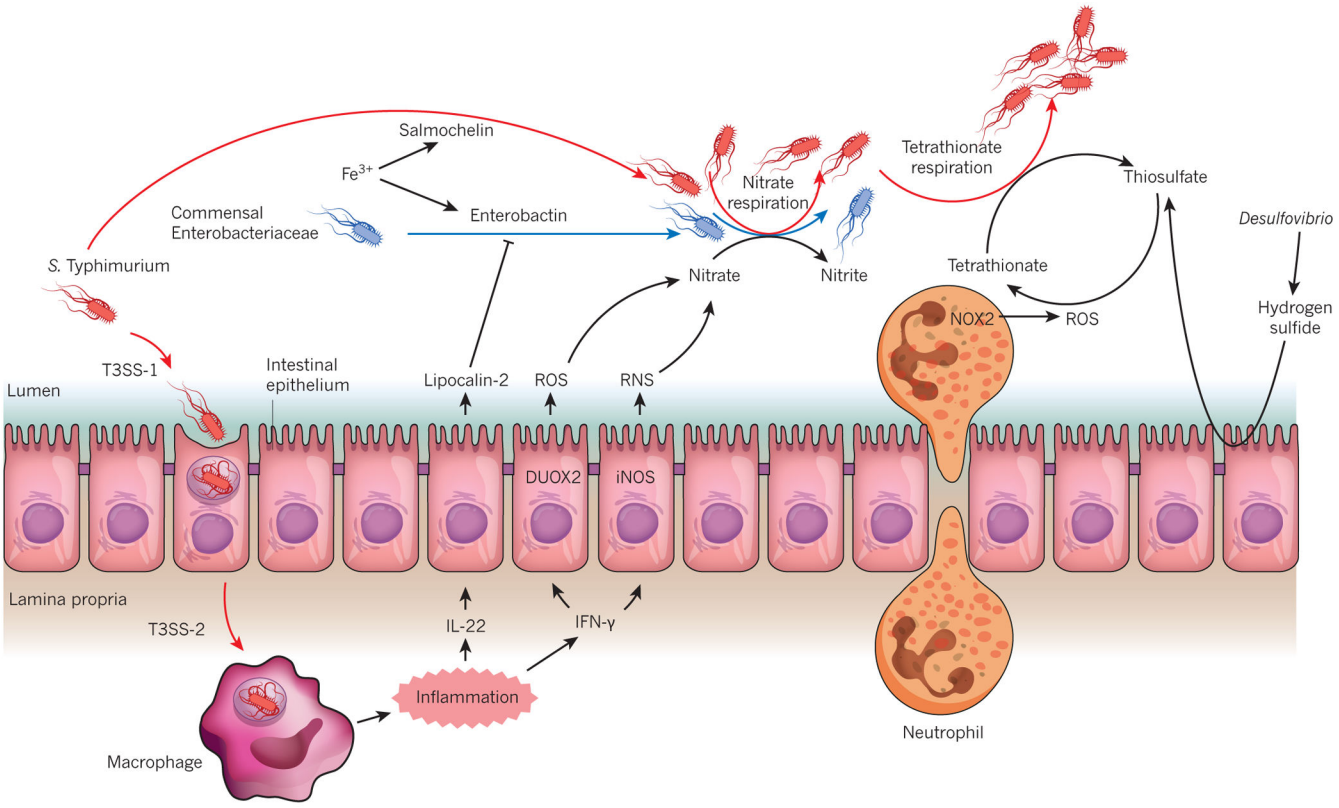

Figure 3. The effect of intestinal inflammation on nutrient availability

S. Typhimurium uses its virulence factors (T3SS-1 and T3SS-2) to trigger intestinal inflammation. Cytokines that are released during inflammation, such as IL-22 and IFN-γ, trigger the release of antimicrobial molecules lipocalin-2, reactive oxygen species (ROS) and reactive nitrogen species (RNS) from the intestinal epithelium. Lipocalin-2 can block the growth of commensal Enterobacteriaceae that rely on the siderophore enterobactin for the acquisition of iron (Fe^{3+}). It does not bind to the *S.* Typhimurium siderophone salmochelin, however, which confers the bacterium with resistance to its effects on growth. RNS and ROS react to form nitrate, which drives the growth of Enterobacteriaceae through nitrate respiration. Microbiota-derived hydrogen sulfide is converted to thiosulfate by colonic epithelial cells. Neutrophils that migrate into the lumen of the intestine during inflammation generate ROS that convert endogenous sulfur compounds (thiosulfate) into an electron acceptor (tetrathionate) that further boosts the growth of *S.* Typhimurium through tetrathionate respiration.