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The dynamics of gut-associated microbial communities during inflammation

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Our intestine is host to a large microbial community (microbiota) that educates the immune system and confers niche protection. Profiling of the gut-associated microbial community reveals a dominance of obligate anaerobic bacteria in healthy individuals. However, intestinal inflammation is associated with a disturbance of the microbiota—known as dysbiosis—that often includes an increased prevalence of facultative anaerobic bacteria. This group contains potentially harmful bacterial species, the bloom of which can further exacerbate inflammation. Here, we review the mechanisms that generate changes in the microbial community structure during inflammation. One emerging concept is that electron acceptors generated as by-products of the host inflammatory response feed facultative anaerobic bacteria selectively, thereby increasing their prevalence within the community. This new paradigm has broad implications for understanding dysbiosis during gut inflammation and identifies potential targets for intervention strategies.

Keywords: intestinal inflammation; dysbiosis; anaerobic respiration; Enterobacteriaceae

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See the Glossary for abbreviations used in this article.

Introduction

Over 90% of the cells in the human body are microbes, most of which reside in bacterial communities—known collectively as microbiota—that inhabit the large intestine. Advances in high-throughput microbiota sequencing provide a powerful tool for profiling the previously hidden microbial diversity in the gut. For example, such metagenomic analysis shows that the large intestine is host to a diverse bacterial community the structure of which, at the phylum level, is maintained through unknown mechanisms. The bacterial species dominating the microbiota in the large bowel are strict anaerobes, which lack the ability to respire oxygen and rely on fermentation of complex polysaccharides for growth [1]. Towering above all others are obligate anaerobic bacteria belonging to the phyla Bacteroidetes (class Bacteroidia) and Firmicutes (class Clostridia; [2]). This dominance of Bacteroidia and Clostridia is a conserved feature of the large intestine microbiota of both humans and mice [2,3].

However, intestinal inflammation can lead to a microbial imbalance—known as dysbiosis—which is characterized by a marked decrease in the representation of obligate anaerobic bacteria and an increased relative abundance of facultative anaerobic bacteria. For example, acute intestinal inflammation triggered by pathogenic Enterobacteriaceae (class Gammaproteobacteria, phylum Proteobacteria)—such as *Salmonella enterica* or *Citrobacter rodentium*—is accompanied by changes in the bacterial community structure that are marked by an outgrowth of the respective facultative anaerobic pathogen [4–6]. Similarly, a reduction of strictly anaerobic members of the classes Bacteroidia and Clostridia, and a concomitant increase in facultative anaerobic commensal bacteria belonging to the class Gammaproteobacteria (most commonly members of Enterobacteriaceae) or to the class Bacilli (phylum Firmicutes), is seen in individuals with inflammatory bowel disease [7–13]. Likewise, a marked decrease in Bacteroidia and Clostridia and an increased relative abundance of Enterobacteriaceae is also observed in mice when colitis is induced chemically [6] or through genetically engineered immune defects [14]. Although metagenomics provides a powerful lens for viewing these changes in the microbial community structure during conditions of intestinal inflammation, the mechanisms responsible have remained an enigma. Here, we review advances in the understanding of the fundamental principles that govern the phylum-level changes in the structure of host-associated microbial communities in the inflamed gut.

Enterobacteriaceae in the healthy gut

The lumen of the distal gut is a fairly anaerobic environment. The traces of oxygen present in this habitat are readily consumed by facultative anaerobic bacteria—such as Enterobacteriaceae—that constitute a small fraction (approximately 0.1%) of the microbiota [2]. The amount of available oxygen seems to limit the growth of Enterobacteriaceae in this environment, because elevated oxygen levels increase their relative abundance. For example, the ileostomy of small bowel transplant patients provides a portal that allows oxygen to reach the otherwise anaerobic distal ileum. An increase in the relative abundance of Enterobacteriaceae is observed in close proximity to the ileostomy

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Glossary

ABC	ATP-binding cassette
DMSO	dimethyl *S*-oxide
DSS	dextran sulphate sodium
DUOX2	dual function NAD(P)H oxidase 2
ExbB/D	excretion of enterobactin gene B/D
IFN-γ	interferon gamma
IL-22	interleukin 22
iNOS	inducible nitric oxide synthase
LamB	outer membrane receptor for phage λ
MaLE/F/G/K/S	maltose utilization genes E/F/G/K/S
MoaA	molybdopterin synthesis protein A
MPO	myeloperoxidase
NADPH	nicotinamide adenine dinucleotide phosphate
NF-κB	nuclear factor kappa B
SOD	superoxide dismutase
SopE	*Salmonella* outer protein E
Sus	starch utilization system
TonB	phage T1 resistance locus B

and the microbial community returns to its normal composition after its surgical closure [15]. Thus, once the available oxygen is consumed, Enterobacteriaceae are apparently poorly equipped to compete with obligate anaerobic bacteria for high-energy nutrients to support their growth by fermentation.

The dense bacterial communities that inhabit the distal gut compete fiercely for a limited quantity of diet-derived or host mucus-derived carbohydrate available for fermentation [16,17]. Changes in the diet can alter the microbial community structure at the species level, but the dominance persists of obligate anaerobic Clostridia and Bacteroidia over Enterobacteriaceae [18–22]. A comparison of the strategies by which Clostridia, Bacteroidia and Enterobacteriaceae acquire fermentable nutrients illustrates why the latter might be at a disadvantage during anaerobic growth on a limited quantity of carbohydrate (Fig 1).

The Gram-positive Clostridia lack an outer membrane and use glycoside hydrolases to degrade complex carbohydrates. The oligosaccharides generated through this process are transported actively against a concentration gradient across the cytoplasmic membrane by using ABC transporters (Fig 1A; [16]).

The starch utilization system encoded by the *sus* gene cluster of *Bacteroides thetaiotaomicron* is a well-studied glycan acquisition strategy conserved among the Bacteroidia. The outer membrane proteins SusD, SusE and SusF bind starch to the bacterial surface [23]. The outer membrane protein SusG degrades starch into malto-oligosaccharides [24], which are subsequently actively transported against the concentration gradient by the energy-coupled outer membrane import protein SusC [25]. The energy required for transport through this class of outer membrane import proteins is provided by the proton motive force of the cytoplasmic membrane, which is transmitted to the outer membrane through the TonB, ExbB and ExbD proteins (reviewed in [26]). Finally, periplasmic malto-oligosaccharides are further degraded by SusA to glucose, which is actively transported into the cytosol to support growth by fermentation [27]. Sequencing of the *B. thetaiotaomicron* genome revealed the presence of 88 gene clusters related to the *sus* system, suggesting that this general strategy is used for degradation of a multitude of carbohydrate sources in the intestinal lumen (Fig 1B; [28]).

Escherichia coli is a well-studied member of the Enterobacteriaceae commonly present in the large bowel. Malto-oligosaccharides and maltose cross the bacterial outer membrane passively along a concentration gradient through an outer membrane diffusion channel (LamB; [29]). In the periplasm, malto-oligosaccharides are degraded to maltose by the α-amylase MalS [30,31]. Maltose is bound by the periplasmic binding protein MalE [32] and actively transported into the cytosol by an ABC transporter formed by the MalF, MalG and MalK proteins [33–38]. Passive transport through outer membrane diffusion channels is a conserved feature of carbohydrate acquisition within the Enterobacteriaceae (Fig 1C). The only energy-coupled outer membrane import proteins present in Enterobacteriaceae transport low-molecular-weight iron chelators—known as siderophores—and vitamin B12 [26].

The above examples illustrate the main difference between the carbohydrate acquisition strategies of Clostridia, Bacteroidia and Enterobacteriaceae. Both Clostridia and Bacteroidia use glycoside hydrolases to degrade complex carbohydrates, make use of binding proteins to concentrate carbohydrate at their surface and then use an active transport system—that is, an ABC transporter or an energy-coupled outer membrane import protein—to import substrates against a concentration gradient across the first diffusion barrier, be it across the cytoplasmic membrane in Clostridia or across the outer membrane in Bacteroidia (Fig 1A,B). By contrast, a paucity of secreted glycoside hydrolases make Enterobacteriaceae ill-equipped to degrade complex carbohydrate. Instead, Enterobacteriaceae rely on the presence of oligosaccharides that are transported passively across the first diffusion barrier—the outer membrane—through diffusion channels (Fig 1C). Using more effective nutrient-uptake mechanisms is predicted to confer a competitive growth advantage on Clostridia and Bacteroidia over Enterobacteriaceae during anaerobic growth on a limited quantity of carbohydrate. This competitive growth advantage might at least in part explain the dominance of obligate anaerobic Clostridia and Bacteroidia over the facultative anaerobic Enterobacteriaceae in the healthy gut.

From metagenomics to mechanisms

One important consequence of intestinal inflammation is diarrhoea, the flushing action of which limits the availability of fermentable high-energy carbon sources to host mucus-derived carbohydrate. The more effective nutrient-uptake mechanisms used by Clostridia and Bacteroidia are predicted to confer an advantage in this environment. However, intestinal inflammation is associated with phylum-level changes in the microbiota composition, characterized by an increase in facultative anaerobic bacteria [39]. How can inflammation diminish the competitive anaerobic growth advantage of obligate anaerobic bacteria over Enterobacteriaceae?

To answer this question, it is important to understand how inflammation alters the nutritional environment in the distal gut. Intestinal inflammation is accompanied by the release of antimicrobials, a host defence mechanism designed to eradicate microbes from tissue or from close vicinity to the epithelium. Some antimicrobials withhold nutrients by interfering with the microbial acquisition of trace elements, such as iron or zinc [40,41]. For example, on stimulation with IL-22, epithelial cells release the antimicrobial lipocalin 2 into the intestinal lumen [41]. Lipocalin 2 binds to enterobactin, thereby preventing bacteria from using this siderophore for iron acquisition [42–44]. The release of lipocalin 2 can provide a selective advantage for enteric pathogens that have specific

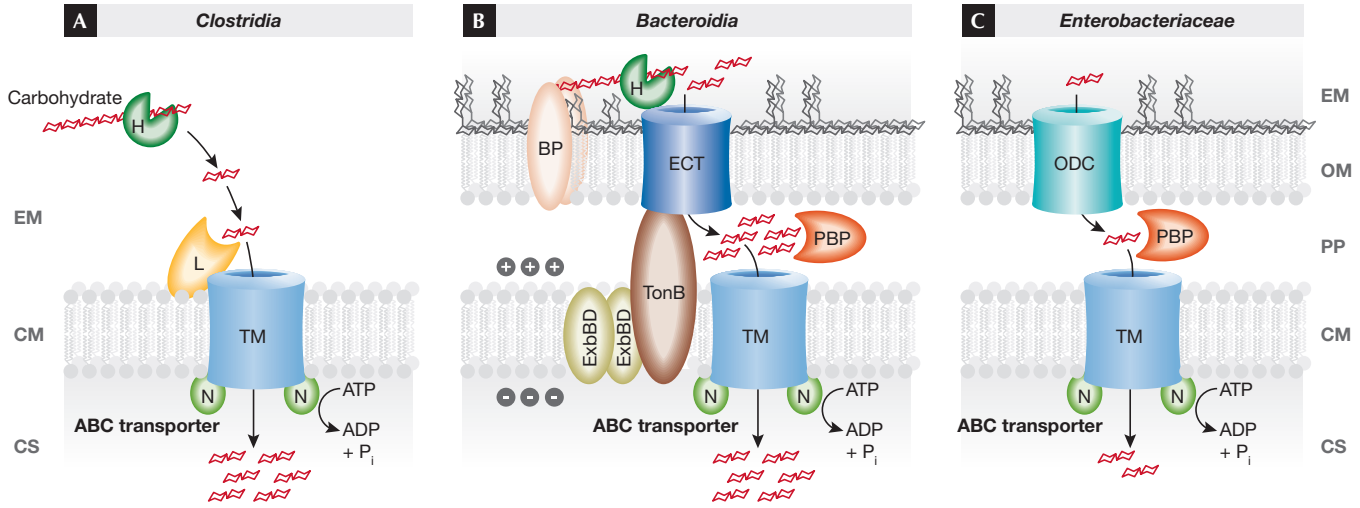

Fig 1 | Carbohydrate acquisition strategies of Clostridia, Bacteroidia and Enterobacteriaceae. The transport of carbohydrate across the cytoplasmic membrane (CM) is typically mediated by ATP-binding cassette (ABC) transporters. These contain either periplasmic binding proteins (PBPs), present in Gram-negative bacteria (shown in panels **B** and **C**), or a membrane-anchored lipoprotein involved in binding (L), present in Gram-positive bacteria (shown in panel **A**), a transmembrane transporter (TM) and membrane-associated nucleotide-binding proteins (Ns) involved in energizing transport by mediating hydrolysis of ATP. Bacteroidia use outer membrane proteins (BPs) to bind carbohydrate, which is degraded into oligosaccharides using glycoside hydrolases (Hs). The resulting oligosaccharides are transported by an energy-coupled outer membrane transporter (ECT) in a process energized through the proton motive force generated by the TonB ExbBD proteins. Enterobacteriaceae rely on outer membrane diffusion channels (ODCs) for passive transport of oligosaccharides across the outer membrane (OM) along a concentration gradient. CS, cytosol; EM, extracellular milieu; ExbBD, excretion of enterobactin gene B/D; PP, periplasmic space; TonB, phage T1 resistance locus B.

lipocalin 2 resistance mechanisms [41]. However, many commensal Enterobacteriaceae are susceptible to lipocalin 2, suggesting that its release is probably not responsible for the phylum-level changes in microbial communities associated with gut inflammation.

A second group of antimicrobials produced during inflammation are reactive oxygen species (ROS) and reactive nitrogen species (RNS). On stimulation with pro-inflammatory cytokines, such as IFN-γ, the intestinal epithelium can produce hydrogen peroxide (H_2O_2) by activating DUOX2 (Fig 2; [45]). In addition, IFN-γ induces expression of the NADPH oxidase 1 (*Nox1*) gene, encoding a second NADPH oxidase of epithelial cells that generates superoxide radicals (O_2^-; [46]). Severe intestinal inflammation can be accompanied by transmigration of neutrophils into the intestinal lumen and subsequent generation of superoxide radicals by the phagocyte NADPH oxidase (PHOX). The generation of superoxide radicals by phagocytes is essential for host defence, as illustrated by the existence of recurrent bacterial infections in individuals with chronic granulomatous disease, an illness caused by PHOX-deficiency [47–49]. Neutrophils also express SOD and MPO, which convert superoxide radicals to hydrogen peroxide and hypochlorite (OCl^-). Furthermore, stimulation with IFN-γ can induce expression of the *Nos2* gene in the intestinal epithelium [50]. The enzyme encoded by *Nos2*, iNOS, catalyses the production of nitric oxide from L-arginine [51]. Phagocytes recruited to the gut mucosa during inflammation are another cellular source of iNOS [52]. Elevated levels of iNOS during inflammation can alter the luminal environment of the large bowel, as indicated by raised nitric oxide concentrations in colonic luminal gas of individuals with inflammatory bowel disease [53–55]. Finally, nitric oxide can react with a superoxide radical, giving rise to peroxynitrite ($ONOO^-$), a potent bactericidal RNS [56,57].

Although the production of RNS and ROS creates a hostile environment in close proximity to the mucosal surface, the generation of these radicals has important side effects. As peroxynitrite, superoxide, hydrogen peroxide and hypochlorite diffuse away from the epithelium, they quickly react with organic sulphides and tertiary amines present in the intestinal lumen to form the respective *S*-oxides (R_2-SO) and *N*-oxides (R_3-N^+-O^-; Fig 2; [58,59]). For example, when dietary contents have been flushed out by diarrhoea, enterocytes released from the tips of villi are the main source of membrane lipids, such as phosphatidylcholine and sphingomyelin, in the intestinal lumen. A nutrient derived from phosphatidylcholine or sphingomyelin is choline. Choline is degraded by the gut microbiota to trimethylamine (TMA; [60])—a compound that can be oxidized by peroxynitrite, superoxide, hydrogen peroxide and hypochlorite to trimethylamine *N*-oxide (TMAO; [58,59]). Alternatively, peroxynitrite can be converted to nitrate (NO_3^-) in a reaction catalysed by carbon dioxide [61]. As a result, nitrate production in the gut lumen is a by-product of chemically induced colitis [62]. Feeding mice the iNOS-inhibitor aminoguanidine hydrochloride prevents nitrate production during colitis [63]. iNOS is responsible for the production of nitrate during inflammation, suggesting that nitrate is host-derived rather than originating from the diet. Ultimately, these processes convert bactericidal RNS and ROS into non-toxic products—that is, *S*-oxides, *N*-oxides and nitrate—the presence of which causes a marked change in the growth conditions encountered in the distal gut.

The lumen of the large bowel is largely devoid of exogenous electron acceptors that would support the growth of bacteria by anaerobic respiration. As a result, fermentation of carbohydrates is the main strategy by which microbial communities in the healthy large intestine support their anaerobic growth. However, the generation of *S*-oxides, *N*-oxides and nitrate as by-products of the

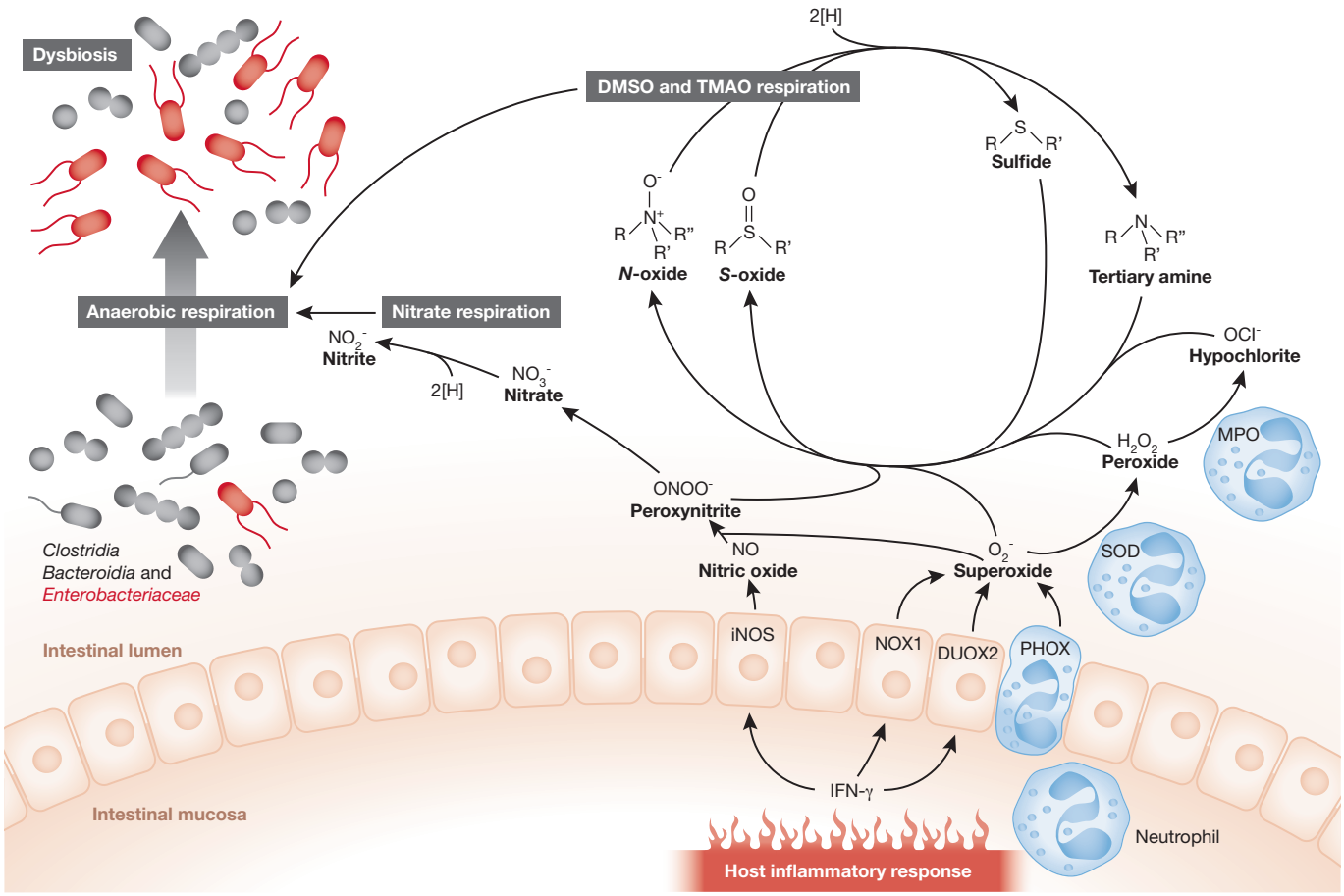

Fig 2 | Phylum-level changes in the microbiota composition on intestinal inflammation. Inflammatory infiltrates—such as neutrophils, and cytokines—such as IFN-γ, are a source of or induce the expression of enzymes (iNOS, NOX1, DUOX2, PHOX, SOD and MPO) that generate antimicrobial radicals, such as superoxide, peroxide, hypochloride, nitric oxide and peroxynitrite. In the intestinal lumen, these radicals react to form harmless oxidation products—such as nitrate, *N*-oxides or *S*-oxides—that serve as electron acceptors. These support the growth of facultative anaerobic bacteria by anaerobic respiration. The resulting outgrowth of facultative anaerobic bacteria gives rise to phylum-level changes in the microbiota composition, including an increased relative abundance of Enterobacteriaceae and a marked decrease in obligate anaerobic Clostridia and Bacteroidia. DMSO, dimethyl *S*-oxide; DUOX2, dual function NAD(P)H oxidase 2; IFN-γ, interferon gamma; iNOS, inducible nitric oxide synthase; MPO, myeloperoxidase; NOX1, NADPH oxidase 1; PHOX, phagocyte NADPH oxidase; SOD, superoxide dismutase; TMAO, trimethylamine N-oxide.

host inflammatory response opens a new alternative for facultative anaerobic microbes to grow in this environment (Fig 2). Enterobacteriaceae can use *S*-oxides, *N*-oxides and nitrate as terminal electron acceptors for anaerobic respiration by expressing DMSO, TMAO and nitrate reductases, respectively [64]. By contrast, Clostridia and Bacteroidia have a primitive electron transport chain and lack the terminal oxidoreductases needed to use the exogenous electron acceptors generated during inflammation [17].

E. coli encodes three nitrate reductases in the *narGHJI*, *narZYWV* and *napFDAGHBC* operons, two DMSO reductases in the *dmsABC* and *ynfFGH* operons, and three TMAO reductases in the *torCAD*, *torYZ* and *yedYZ* operons [65]. Nitrate, DMSO and TMAO reductases, as well as two formate dehydrogenases (FdnG and FdoG), contain molybdenum as a crucial catalyst for electron transfer reactions. The functions of FdnG and FdoG are linked to respiration, because they couple respiratory electron acceptors to the electron donor formate, a fermentation end product present in the large intestine [66,67]. Formate dehydrogenases and respiratory reductases contain molybdenum within a molybdopterin cofactor [68]. MoaA catalyses the first reaction in the biosynthesis of this molybdopterin cofactor [69]. Therefore, an *E. coli moaA* mutant is deficient for several respiratory pathways, including nitrate, DMSO and TMAO respiration. When mice with DSS-induced colitis are challenged with various *E. coli* strains, the *E. coli* wild-type is recovered from the large intestine at a 100-fold higher level than an *E. coli moaA* mutant. By contrast, wild-type and *moaA* mutants are recovered in similar numbers from the non-inflamed intestine of mock-treated control mice [63]. These results suggest that the presence of exogenous electron acceptors in the inflamed gut confers a substantial fitness advantage to *E. coli*—and probably to other commensal Enterobacteriaceae—by supporting their growth through anaerobic respiration. This fitness advantage contributes to a bloom of commensal Enterobacteriaceae, thereby giving rise to the phylum-level changes in the microbiota composition that accompany intestinal inflammation (Fig 2). In other words, one of the mechanisms responsible for dysbiosis in the inflamed gut is that the host response selectively feeds facultative anaerobic bacteria.

Virulence factors change what is on the menu

The enhanced growth of commensal Enterobacteriaceae in the inflamed gut of individuals with inflammatory bowel disease could be viewed as an accident in which, as Louis Pasteur would put it, "chance favours the prepared microbe". However, the fitness advantage conferred by host-derived electron acceptors also represents a potent selective force that is probably responsible for the evolution of pathogenic species within the Enterobacteriaceae (Sidebar A; Fig 3). For example, the genus *Salmonella* comprises a group of pathogens that are closely related to *E. coli*. Since diverging from a common ancestor with *E. coli*, the *Salmonella* lineage acquired several virulence factors through plasmid or phage-mediated horizontal gene transfer [70]. The genes encoding these virulence factors are located on *Salmonella* pathogenicity islands (SPIs), defined as horizontally acquired DNA regions that are absent from the otherwise colinear *E. coli* genome [71]. SPIs that are present in all members of the genus *Salmonella* include SPI1, which encodes the invasion-associated type III secretion system (T3SS-1; [72,73]), SPI4, which encodes a large non-fimbrial adhesin required for epithelial invasion [74] and SPI5, which encodes proteins (known as effectors) that are injected into host cells by the T3SS-1 (Fig 3A; [75]). The genus *Salmonella* can be divided into two species, *S. bongori* (containing 23 serovars) and *S. enterica* (containing 2,587 serovars; [76]). All members of *S. enterica* encode a second type III secretion system (T3SS-2) encoded by SPI2—which is absent from *S. bongori* and *E. coli* [77,78]—and serves to prolong intestinal inflammation [79]. The presence of these pathogenicity islands enables a fraction of the *Salmonella* population to invade the intestinal epithelium (SPI1, SPI4 and SPI5) and survive in macrophages (SPI2), thereby triggering acute intestinal inflammation (gastroenteritis) [75,80–84].

Interestingly, by using their virulence factors to actively induce intestinal inflammation, *Salmonella* serovars can tip the balance in their favour in competition with the intestinal microbiota [4,5]. For instance, when *S. enterica* serovar Typhimurium (*S.e.* sv typhimurium) induces colitis in a mouse model, the pathogen edges out competing microbes in the gut lumen to become a prominent member of the microbiota. Inactivation of both type III secretion systems renders *S.e.* sv typhimurium unable to trigger intestinal inflammation, thereby markedly reducing its ability to colonize the lumen of the large intestine. However, when mice that develop colitis spontaneously—such as IL-10-deficient mice—are infected with a *S.e.* sv typhimurium mutant lacking both type III secretion systems, an enrichment for the pathogen and a concomitant depletion of Clostridia and Bacteroidia is observed [4]. Although virulence factors are necessary for inducing intestinal inflammation, these data suggest that T3SS-1 and T3SS-2 are not required for securing the growth advantage *S.e.* sv typhimurium gains in the lumen of the inflamed gut.

Which factors confer a growth advantage on *Salmonella* serovars during gastroenteritis? Optimal growth in the environment of the inflamed gut requires resistance to antimicrobial proteins—such as lipocalin 2—which is conferred by the *iroN iroBCDE* gene cluster [41], a DNA region present in all members of *S. enterica* but absent from *S. bongori* [85,86]. Lipocalin 2 resistance might confer an advantage on *S. enterica* during its competition with commensal Enterobacteriaceae, provided that the latter rely on enterobactin for iron acquisition. Rivalry with commensal Enterobacteriaceae probably arises because exogenous electron acceptors—such as nitrate—enhance their growth in the inflamed gut [63]. As *S.e.* sv typhimurium can also use host-derived nitrate to boost its luminal growth [87], the pathogen and commensal Enterobacteriaceae have to compete for this limited resource. However, *Salmonella* serovars have improved their ability to outgrow other gut microbes during inflammation by acquiring additional fitness factors that are absent from *E. coli*. Among these are gene clusters encoding putative DMSO reductases (STM2528-STM2530 and STM4305-STM4308; [88]) and the *ttrSR ttrBCA* gene cluster, which confers the ability to use tetrathionate ($S_4O_6^{2-}$) as an electron acceptor for anaerobic respiration (Fig 3A; [89,90]). The ability to respire tetrathionate has been used since 1923 to enrich for *Salmonella* serovars in biological samples containing competing microbes [91]. However, the fact that tetrathionate is a by-product of the host inflammatory response in the gut was only recently discovered [92]. Hydrogen sulphide (H_2S) and methanethiol (CH_3SH) are fermentation end-products of gut microbes that are converted to thiosulphate ($S_2O_3^{2-}$) by the colonic epithelium to avoid toxicity [93,94]. ROS generated during intestinal inflammation oxidize thiosulphate ($S_2O_3^{2-}$) to tetrathionate ($S_4O_6^{2-}$), thereby boosting luminal growth of *S.e.* sv typhimurium through tetrathionate respiration whilst reducing the relative abundance of Clostridia and Bacteroidia [92]. Importantly, enhanced growth in the intestinal lumen promotes transmission of *S.e.* sv typhimurium by the faecal–oral route [95]. Ultimately, the necessity to spread from an infected to a naive host places virulence factors and fitness factors under selection. Although taxonomists identified tetrathionate respiration empirically as a characteristic that helps distinguish *Salmonella* serovars from close relatives [91], the picture emerging from research is that this function is part of a 'business plan' that defines the genus *Salmonella*.

Sidebar A | In need of answers

(i) Although it seems clear that intestinal inflammation can result in changes in the microbial community structure, relatively little is known about possible consequences of the resulting dysbiosis. Does inflammation select for more harmful bacterial species that can further exacerbate host responses? If so, what are the properties that make them more potent irritants? This area needs to be explored further by using both metagenomic and mechanistic approaches.

(ii) Although an increased relative abundance of facultative anaerobic bacteria is common in individuals with inflammatory bowel disease, not all patients show these changes in their microbiota. Thus, it is tempting to speculate that other mechanisms—in addition to anaerobic respiration—can influence the microbial community structure during intestinal inflammation. These mechanisms remain to be identified.

(iii) How does the quality of the host response alter the nutritional environment in the inflamed gut? This seems to be particularly relevant to understand the evolution of enteric pathogens that use their virulence factors to manipulate host responses. For example, acquisition of the *sopE* gene by horizontal gene transfer enhances the ability of *Salmonella enterica sv* Typhimurium to elicit production of host-derived nitrate, thereby altering the nutritional environment in the intestinal lumen [87]. Deeper insights into how virulence factors change the nutrient availability in the gut are necessary to appreciate how the host response has shaped the evolution of enteric pathogens.

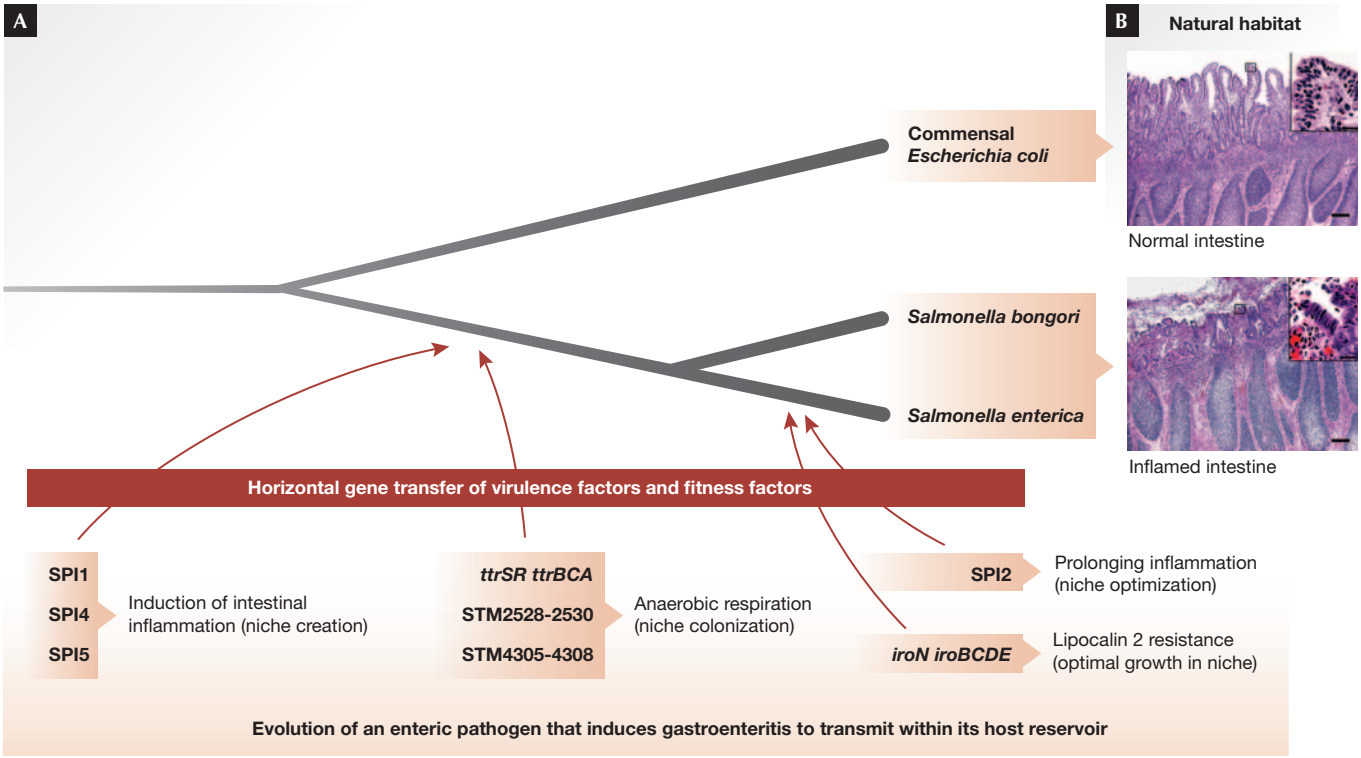

Fig 3 | Selective forces driving the evolution of pathogenic Enterobacteriaceae species. (**A**) The genus *Salmonella* comprises enteric pathogens that are closely related to commensal *Escherichia coli*, with whom they have a common ancestor as indicated by the schematic drawing of their phylogenetic tree (not to scale). The timing of horizontal gene transfer events introducing virulence factors or fitness factors is indicated. Acquisition of the indicated DNA regions conferred the ability to induce inflammation, benefit from the resulting host response and enhance their transmission. (**B**) The images show the natural habitat of commensal *E. coli* (normal intestine, top panel) and of pathogenic *Salmonella* species (inflamed intestine, bottom panel). The images of calf intestine were reproduced from [112] with permission. SPI1/2/4/5; Salmonella pathogenicity island 1/2/4/5.

These findings suggest that an important initial event in the evolution of the *Salmonella* lineage was the acquisition of virulence factors the deployment of which induces intestinal inflammation, thereby creating a new niche in the host (Fig 3B). At the same time, the *Salmonella* lineage acquired fitness factors that enabled these pathogens to occupy this new niche to ensure transmission (Fig 3A). Evolution is still at work to fine-tune this 'business plan' by incorporating new virulence factors to instigate subtle alterations in host responses that generate exogenous electron acceptors. These processes probably contribute to the rise of new epidemic clones, as illustrated by an analysis of the prophage-encoded T3SS-1 effector protein SopE [87].

The T3SS-1 induces host responses by injecting effector proteins into host cells [81]. SPI1 and SPI5 encode effector proteins that are conserved among *Salmonella* serotypes, whereas other effector proteins are encoded by prophages and have a limited distribution. One of these prophage-encoded T3SS-1 effector proteins is SopE [96]. On injection into host cells, SopE activates small Rho GTPases to induce NF-κB-dependent gene expression [97], which leads to a modest increase in the severity of intestinal inflammation in animal models [98]. Remarkably, deployment of SopE significantly enhances the production of iNOS in the intestinal mucosa, thereby increasing luminal growth of *S.e.* sv typhimurium by nitrate respiration [87]. SopE is encoded by a prophage present in only a few *S.e.* sv typhimurium clones, which caused an epidemic among cattle and humans in Europe during the 1970s and '80s [99]. Collectively, these data suggest that phage-mediated horizontal transfer of the *sopE* gene confers a nitrate respiration-dependent fitness advantage that might have contributed to the emergence of an epidemic *S.e.* sv typhimurium clone.

Side-stepping the competition

The findings reviewed above point to anaerobic respiration as one of the fundamental principles that governs the phylum-level changes in the composition of gut-associated microbial communities during inflammation. But how does anaerobic respiration enable Enterobacteriaceae to outcompete Clostridia and Bacteroidia, which can thrive on a limited quantity of carbohydrate? One possibility is that anaerobic respiration is more efficient for energy production than fermentation. Although this might be true for electron acceptors with a high standard redox potential, such as the nitrate–nitrite redox couple ($E^{\circ} = 433$ mV; [100]), this explanation seems less convincing for the tetrathionate–thiosulphate redox couple, which has a relatively low standard redox potential ($E^{\circ} = 170$ mV; [101]). A second possibility is that exogenous electron acceptors enable Enterobacteriaceae to use carbon sources that cannot be fermented. The removal of gut contents during diarrhoea limits nutrients to mucus-derived carbohydrate and nutrients derived from the release of enterocytes from the tips of villi.

Membranes of enterocytes contain phosphatidylethanolamine as their most abundant phospholipid [102]. Ethanolamine—a non-fermentable substrate present in the intestinal contents of calves at a concentration of approximately 2 mM [103]—is a nutrient derived from phosphatidylethanolamine. *S.e.* sv typhimurium requires tetrathionate for anaerobic growth on ethanolamine as a sole carbon source *in vitro* [104]. In the lumen of the inflamed intestine, the ability to consume ethanolamine bestows a marked growth advantage on *S.e.* sv typhimurium, which depends on the ability of the pathogen to perform tetrathionate respiration [105]. These data suggest that anaerobic respiration enables *S.e.* sv typhimurium to consume an abundant simple substrate, ethanolamine, which is provided by the host but cannot be readily fermented by competing obligate anaerobic bacteria. Thus, a significant benefit of anaerobic respiration is the ability of *S.e.* sv typhimurium to side-step nutritional competition with Clostridia and Bacteroidia, thereby fostering its own growth in the gut.

Mechanistic insights guide future research efforts

The mechanistic insights discussed above suggest that the phylum-level changes in gut-associated microbial communities are a consequence rather than a cause of intestinal inflammation. However, one possible consequence of this dysbiosis is an exacerbation of pre-existing inflammatory conditions (Sidebar A). The presence of gut microbes is a prerequisite for the development of chronic intestinal inflammation in genetically predisposed mice [106,107], and studies suggest that a bloom of Enterobacteriaceae can be associated with enhanced intestinal inflammation. For example, in a mouse model of inflammatory bowel disease, changes in the microbial community structure characterized by an increased luminal abundance of Enterobacteriaceae can be transferred to other animals, resulting in an exacerbation of intestinal inflammation [14,108]. Adherent-invasive *E. coli* (AIEC) are isolated more commonly from the intestinal mucosa of individuals with Crohn's disease than from healthy controls [109,110]. AIEC colonize and exacerbate gut inflammation in mice with DSS-injured colon [111]. Thus, the mechanisms leading to dysbiosis might also select for intestinal colonization with more harmful members of the Enterobacteriaceae—such as AIEC—thereby exacerbating inflammation and interfering with its resolution.

Anaerobic respiration emerges as a potential target for new intervention strategies aimed at restoring a normal microbial community structure. A formal proof of principle for this intervention strategy is the fact that the iNOS-inhibitor aminoguanidine hydrochloride can blunt nitrate respiration-dependent growth of *E. coli* in mice with DSS-induced colitis [63]. This approach would have to be broadened to block the use of multiple respiratory electron acceptors, thereby ending the bloom of Enterobacteriaceae in the distal gut by essentially suffocating these facultative anaerobic bacteria. Exploring this approach for the treatment of intestinal inflammatory disorders represents an exciting direction for future research.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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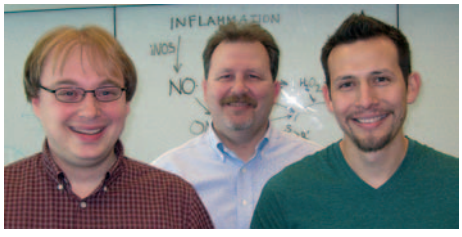

[Left to right] Sebastian E. Winter, Andreas J. Bäumler & Christopher A. Lopez