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Gastrointestinal host-pathogen interaction in the age of microbiome research

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The microbiota is linked to human health by governing susceptibility to infection. However, the interplay between enteric pathogens, the host, and its microbiota is complex, encompassing host cell manipulation by virulence factors, immune responses, and a diverse gut ecosystem. The host represents a foundation species that uses its immune system as a habitat filter to shape the gut microbiota. In turn, the gut microbiota protects against ecosystem invasion by opportunistic pathogens through priority effects that are based on niche modification or niche preemption. Frank pathogens can overcome these priority effects by using their virulence factors to manipulate host-derived habitat filters, thereby constructing new nutrient-niches in the intestinal lumen that support ecosystem invasion. The emerging picture identifies pathogens as ecosystem engineers and suggests that virulence factors are useful tools for identifying host-derived habitat filters that balance the microbiota.

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Introduction

The widespread use of antibiotics, which commenced in the late 1940's, helped to lower the number of infectious disease deaths, the leading cause of mortality throughout human history. In the wake of these wonder drugs, cardiovascular disease and cancer replaced infectious diseases as leading causes of human mortality in high-income countries. However, the mortality burden from antibiotic-resistant bacterial infections is predicted to surpass that of cancer and cardiovascular disease again by the year 2050 [1], a reminder that the medical miracle generated by antibiotics resembles a bubble that is about to burst. Among the most urgent threats to public health are opportunistic infections caused by antibiotic-resistant enteric pathogens, including *Clostridioides difficile* (phylum *Firmicutes*) and carbapenem-resistant *Enterobacteriaceae* (phylum *Proteobacteria*) [2]. These opportunistic pathogens can infect patients whose gut-associated microbial communities (the gut microbiota) are disrupted by exposure to broad-spectrum antibiotics [3–8], which has rekindled interest in understanding the role the gut microbiota plays in bacterial pathogenesis.

The gut microbiota not only repels opportunistic pathogens, but it is also one of the first lines of defense that frank pathogens need to overcome, in order to establish infection [9]. The ménage à trois among the pathogen, the host, and its microbiota remains incompletely understood, in part because of the daunting complexity of this interaction. To approach this intricate problem, we will first consider the emerging framework that conceptualizes the relationship between the microbiota and its host. We will then discuss how the microbiota provides protection against ecosystem invasion by opportunistic pathogens. Finally, we will review recent insights into how virulence factors enable frank pathogens to elicit host responses that facilitate ecosystem invasion and how studying this process, in turn, reveals how the host maintains gut homeostasis. Whereas host-associated microbial communities are found in diverse anatomic locations where they contribute to health and disease, this review will remain focused on the interaction of enteric pathogens with the mammalian gut microbiota.

The blind men and the elephant: emerging concepts in microbiome research

Concepts explaining the relationship between the host and its microbiota are still evolving. One of the earliest metaphors describes the microbiota as an organ-like assembly of microbes that grants benefit to the host [10–14]. The organ metaphor exerts a marked influence on microbiota research. For instance, the concept that the microbial organ contains our 'second genome' provides the rationale for defining the microbiome as the collective genome of our resident microbes [15], a narrow definition that biases microbiome analysis towards cataloguing species names and microbial genes [16]. Furthermore, since organs do not functionally change over time, the organ analogy implies that there are common elements to a healthy microbiome, an assumption guiding efforts to identify microbiome elements that are shared between individuals [17,18]. Moreover, the benefit provided by a microbial organ is proposed to lead to host interactions that are under selection, thereby creating an evolutionary unit, which forms the foundation of the holobiont concept

[19–23]. However, there are also limitations of the organ analogy. For example, the evolutionary view of the holobiont and its microbial organ requires transmission of microbes across generations [24••], which is not consistent with low heritability estimates for the human microbiota [25,26••]. Furthermore, the Human Microbiome Project did not succeed in identifying elements that define a healthy microbiome [16], illustrating that some assumptions arising from the organ metaphor (i.e. the idea that the human microbiome can be understood by increasing the depth of microbiota measurements) have not been validated by experimental research.

The microbiota can also be conceptualized as part of the immune system, because it primes and educates our host defenses [27–32]. Consistent with its role in maintaining immune homeostasis, the microbiota does not trigger marked inflammatory responses during immune education, which distinguishes it from immune training provided by exposure to pathogens [33,34]. Studies on how microbiota-derived metabolites are detected by host cells to alter immune functions is an active branch of microbiome research that is generating seminal mechanistic insights [35–40,41••,42•,43•]. However, immune education does not fully capture all the relationships between the microbiota and its host, because the microbiota provides benefits that go beyond training the immune system [24••]. For example, the gut microbiota completes an important digestive task by providing us with an estimated 6–10% of our energy budget [44,45].

Alternatively, the interaction between microbes and host physiology can be considered through an ecological lens, which suggests that humans should be viewed not as individuals, but as ecosystems [46]. There is a growing appreciation that concepts of community ecology are relevant to microbiota research [47–50]. In ecological terms, the microbiome is defined as the microbiota and its habitat [51] (**Text** Box 1). Traditional ecology examines dynamics of living communities in a nonliving habitat patch. In contrast, the habitat in microbiota research is the host, a living environment that responds dynamically, which requires an expansion of theory to incorporate feedback on the microbiota from mucosal immune responses generated by the host habitat patch [24••,52]. Including the human host in the microbiome definition is of prime importance, because the host could be viewed as a foundation species who shapes microbial communities by modulating fundamental ecosystem processes [53]. But merging immunological concepts with those from ecology and evolution is challenging, because these two disciplines are often physically separated on university campuses and scientists representing each field rarely interact at conferences [52].

In conclusion, the organ concept, the immune system view and the ecosystem framework each capture important aspects of the human microbiome and there is no consensus as to whether one of them is more useful or more accurate than another [24••]. However, each of these competing concepts is based on an incomplete perception, which confines its proponents to a narrow viewpoint. This situation is reminiscent of the one described in John Godfrey Saxe's poem of the blind men and the elephant [54]. Saxe's parable describes how blind men explore what an elephant is like by touching it. As each of them explores a different body part of the animal, each obtains a different perspective and proposes a different metaphor to describe the elephant. For example, the blind man touching the tusks

Box 1 Terms from community ecology applied to the human microbiota

Colonization resistance: ability of the microbiota to prevent engraftment of new microorganisms through niche preemption and/or niche modification.

Foundation species: the human host, who structures the microbiota through host-derived habitat filters and behavior related to diet and habitat choice.

Habitat filters: host-derived resources that select for traits that permit growth and survival in the host, thereby shaping the size, species composition and spatial organization of the microbiota.

Habitat patch: the dynamic host environment in which the microbiota assembles, which is surrounded by host tissue, an unsuitable habitat kept sterile by the immune system.

Historical contingency: the concept that the microbiota composition is determined by the order of species arrival, because resident microbes affect new arrivals through priority effects.

Microbiome: the microbiota and its environment, which is shaped by host habitat filters and host behavior, such as choice of diet.

Niche construction: virulence factor-induced host response that alters host-derived habitat filters to add a new nutrient-niche, thereby supporting engraftment of a new microorganisms.

Niche modification: alteration of the environment that prevents engraftment of microbes by inhibiting their growth or eliminating their nutrient-niche.

Niche preemption: occupation of an ecological position in a community, which provides priority access to growth-limiting resources, thereby preventing engraftment of microorganisms that compete for the same nutrient-niche.

Nutrient-niche: an ecological position defined by growth-limiting resources that support growth of a suitable occupant. The abundance of these growth-limiting resources determine the abundance of the occupant within the microbiota.

Opportunistic pathogen: a microbe associated with disease in immunocompromised members of a host species.

Pathogen (or frank pathogen): a microbe associated with a communicable disease in immunocompetent members of a host species.

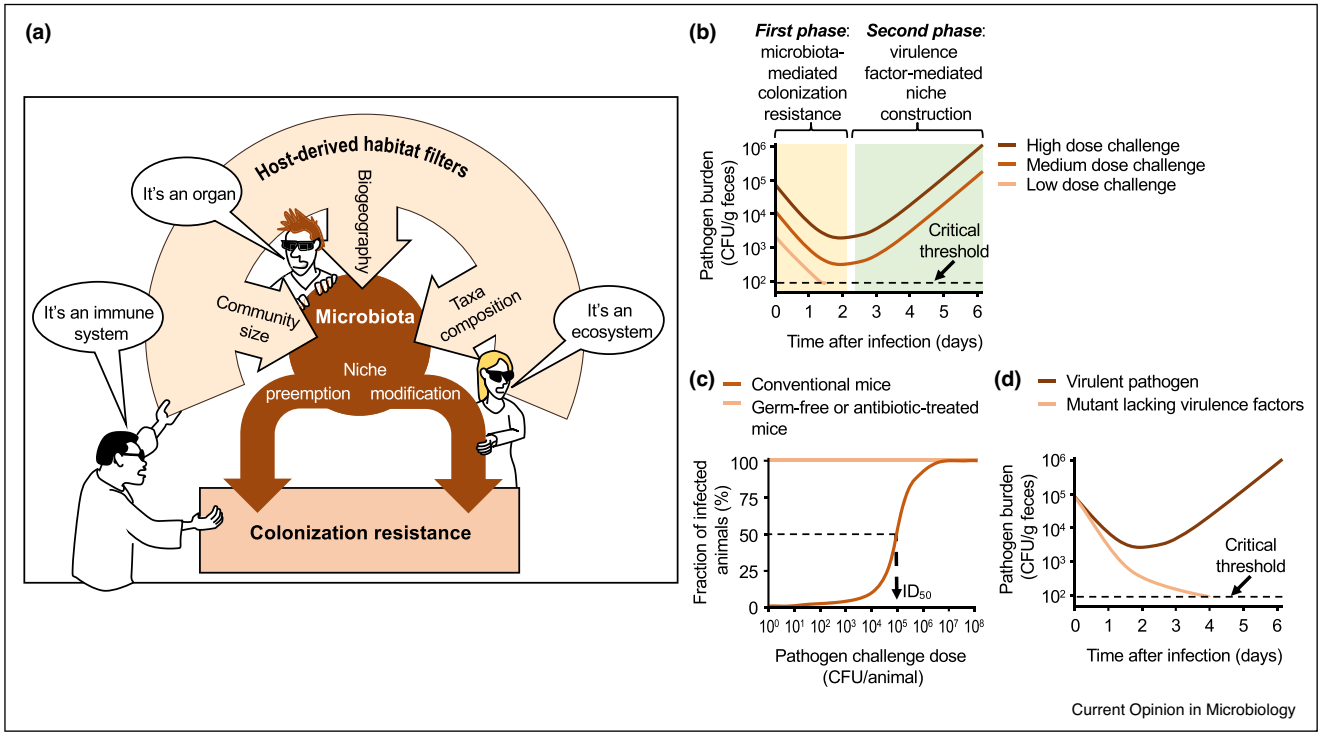
Priority effects: the idea that resident microbes prevent engraftment of new microorganisms through niche preemption and/or niche modification.

Virulence factors: factors that enable pathogens to attach to or enter host cells, inhibit or evade the host's immune responses and obtain nutrients through ecosystem engineering.

concludes the elephant is like a spear, the one feeling the trunk likens it to a snake and the one exploring the ear to a fan. Whereas the subjective experience of each blind man accurately captures an important aspect of the elephant's biology, each of their conclusions is ultimately incomplete and misleading. Similarly, viewing the microbiome from the narrow viewpoint of the organ metaphor, the immune system view or the ecosystem framework generates three different perspectives [24••] (Figure 1a). But each of these narrow viewpoints can illuminate only one facet of the microbiome, thereby ultimately providing an incomplete picture.

Breaking from the confines of metaphors that restrict our vantage point requires embracing a pluralistic approach aimed at integrating multiple scientific points of view into a more complete picture of the microbiome [24••]. The framework of microbiota-nourishing immunity attempts such a synthesis [55]. Microbiota-nourishing immunity is a host-microbe chimera that encompasses the microbiota and host-derived factors that act as 'habitat filters', a collection of resources that shape the size, species composition and spatial organization of microbial communities [9] (Figure 1a). The habitat filters preserve homeostasis by selecting for microbial traits that permit survival and growth in the host, thereby maintaining microbial organ functions, including those unrelated to immunity, such as the digestive tasks performed by the gut microbiota [56,57]. Arguably, the most vital of these microbial organ functions is to repel pathogens, as illustrated by the fact that gnotobiotic animals readily succumb to infection, but remain viable when maintained under germ-free conditions [55]. Thus, a crucial role of microbiota-nourishing immunity is to confer colonization resistance against pathogens, a canonical non-specific immune function that identifies this host-microbe chimera as an integral part of our immune system [9]. Importantly, the mechanisms through which microbiota-nourishing immunity confers colonization resistance are grounded in community ecology [58•]. Thus,

Figure 1



Colonization resistance. **(a)** Different scientific disciplines have explored discrete aspects of the microbiome, thereby generating narrow viewpoints that liken it to an organ, an ecosystem or an immune system. Integrating these viewpoints into a multifaceted conceptual framework suggests that host-derived habitat filters shape the size, species composition and spatial organization (biogeography) of the microbiota to maintain colonization resistance through niche preemption and niche modification. This host-microbe chimera, termed microbiota-nourishing immunity, represents our bodies first line of defense against mucosal pathogens. **(b)** Ecosystem invasion by frank enteric pathogens can be divided into two phases. During the first phase, the gut microbiota confers colonization resistance through niche preemption and niche modification, which leads to a decline in the pathogen load. During the second phase, virulence factors elicit host responses that construct a new nutrient-niche to support pathogen engraftment. **(c)** Challenge of germ-free mice with fewer than 10 bacteria (e.g. *S.* Typhimurium) leads to a successful engraftment of an enteric pathogen. However, in the presence of conventional gut microbiota, colonization resistance markedly increases the challenge dose at which 50% of animals develop intestinal carriage (ID_{50}). **(d)** At a high challenge dose, virulence factors enable frank pathogens to overcome colonization resistance and invade the gut ecosystem, whereas genetic ablation of virulence factors results in their extinction.

microbiota-nourishing immunity integrates ecological concepts and the organ hypothesis into an immunological framework, a scientifically multi-faceted approach that helps assemble a more complete picture of the microbiome. Here we will discuss how this novel conceptual framework can be applied to improve our understanding of ecosystem invasion by enteric pathogens. Our article will remain focused on colonization resistance, and the reader is referred to recent reviews for discussions on digestive functions of the gut microbiota or host control over the microbial ecosystem [56,57,59,60••].

No vacancies: priority effects drive colonization resistance

The infant gastrointestinal tract is likely sterile *in utero* [61]. Upon birth, microbial community assembly proceeds through acquisition of maternal or environmental microorganisms that can coexist because each occupies a different nutrient-niche [62]. Thus, the number of species that can coexist in a community is limited by the number of available nutrient-niches in its habitat patch. One factor influencing the availability of nutrient-niches is the host environment, which acts as a habitat filter [48] (Figure 1a). For instance, human milk oligosaccharides are poorly absorbed in the small intestine and reach the colon, where they direct the gut microbiota composition in breast-fed infants towards a dominance of *Bifidobacteriaceae* (phylum *Actinobacteria*), which consume these nutrients [63–65]. The mucus layer constitutes another host-derived habitat filter. Mucus polysaccharides help to maintain *Bacteroidiaceae* (phylum *Bacteroidetes*) within the gut microbiota, because members of this taxon can sustain themselves on these host-derived nutrients during periods of dietary fiber starvation [66,67••]. A selection for taxa by these host-derived habitat filters might explain why co-speciation with humans and African apes is observed for clades of *Bifidobacteriaceae* and *Bacteroidaceae* [68••].

A second factor influencing the availability of nutrient-niches in the gut is the diet. Shifting from breast-feeding to the introduction of solid food removes human milk oligosaccharides while at the same time introducing dietary fiber composed of complex polysaccharides. Dietary fiber escapes digestion by host enzymes in the small intestine, thus reaching the colon to provide new nutrient-niches for the gut microbiota. The classes *Clostridia* (phylum Firmicutes) and *Bacteroidia* (phylum *Bacteroidetes*) are the taxa with the broadest capacity for dietary fiber degradation compared to other members of the microbiota [69]. Weaning is therefore associated with a marked diet-induced succession during early development, which is characterized by the appearance of new species belonging to the obligate anaerobic *Clostridia* and *Bacteroidia* taxa and a disappearance of milk oligosaccharide-consuming species within the *Bifidobacteriaceae* [70]. As the microbiota matures, the available nutrient-niches become occupied, and community assembly reaches a stable equilibrium state [71]. Although members can still be inserted into or removed from the microbial community, for example, by adding or subtracting nutrient-niches through long-term changes in dietary fiber consumption [67••,72,73••,74••], the overall composition of the microbial community becomes relatively stable against perturbations [71], a phenomenon known as microbiota resistance [75].

Microbiota resistance is based on priority effects that prevent the addition of new microbes to the community, either because a resident microbe modifies its local environment to eliminate niches for other microorganisms (niche modification) or because niche preemption bestows the founding occupant of a nutrient-niche with a fitness advantage over potential competitors [76,77••] (Figure 1a). As a result of priority effects, historical events that govern the initial exposure of neonates to founding occupants of each nutrient-niche play an important role in the outcome of community assembly — this ecological principle is known as historical contingency [58•]. Historical contingency is likely a prominent source of taxonomic diversity in the microbiota composition between individuals [18]. Furthermore, priority effects make it difficult to engraft new microbes whose exclusive nutrient-niche is already occupied or modified [9], which explains the poor engraftment observed for many probiotics [78•]. Notably, the gut microbiota uses these priority effects to prevent ecosystem invasion by microorganisms we encounter through our daily environmental exposure to food and water [9]. This phenomenon, known as colonization resistance, constitutes the main antimicrobial effector function of microbiota-nourishing immunity [55]. Importantly, colonization resistance prevents the growth of opportunistic pathogens. For example, members of the gut microbiota produce secondary bile acids that prevent growth of *C. difficile*, thereby conferring colonization resistance against this opportunistic pathogen through niche modification [79,80••]. However, the opportunistic *C. difficile* can take advantage of an antibiotic-mediated depletion of secondary bile acid-producing bacteria from the gut microbiota [79,80••], which weakens microbiota-nourishing immunity [55]. Since antibiotic treatment permanently removes some microorganisms from the gut microbiota, their corresponding nutrient-niches become vacant, thereby making it possible to engraft new microbes that are suited to fill the respective openings [81]. For instance, reintroducing secondary bile acid-producing bacteria through fecal microbiota transplantation [82,83] or by inoculation with a pure culture [80••] restores niche modification after antibiotic therapy, thereby reinstating colonization resistance against *C. difficile*.

In summary, the ecological forces that underlie the phenomenon of microbiota resistance are also responsible for preventing engraftment with opportunistic enteric

pathogens and the resulting colonization resistance represents an important health benefit provided by microbiota-nourishing immunity [9] (Figure 1a). Ecosystem invasion by opportunistic enteric pathogens requires a weakening of microbiota-nourishing immunity. In contrast, frank enteric pathogens are characterized by their ability to overcome intact defenses to establish infection.

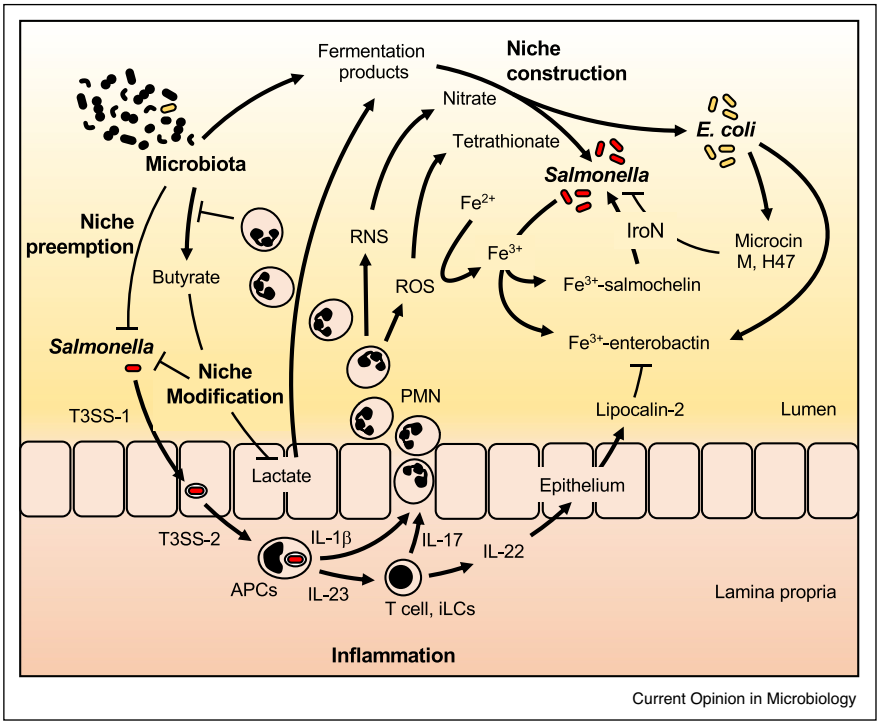
Mechanisms and consequences of ecosystem engineering by enteric pathogens

Invasion of the gut ecosystem by frank enteric pathogens can be divided into two phases. Upon entry, the pathogen encounters an ecosystem in which all available nutrient-niches are occupied. A lack of available nutrients (niche preemption) and/or niche modification by the gut microbiota limit the pathogen's ability to grow, thereby resulting in an initial decline in pathogen burden in lumen and feces. The first phase of ecosystem invasion represents a perilous situation for the pathogen, because the initial decline in its burden can lead to an extinction when the challenge dose is low (Figure 1b). This mechanism enables the gut microbiota to confer colonization resistance against a low-dose pathogen challenge. The magnitude of this protection is substantial, as illustrated by comparing the challenge dose required for infection of a vertebrate host in the presence or absence of an intact microbiota (Figure 1c). In case of *Salmonella enterica* serovar (*S.*) Typhimurium (family *Enterobacteriaceae*), a challenge dose of more than 10^5 bacteria is required to establish fecal carriage in 50% of conventional mice (implantation dose, or ID_{50}), whereas germ-free mice or antibiotic-treated mice can become colonized after challenge with fewer than 10 bacteria [84,85,86•].

At high challenge doses, the initial pathogen burden is sufficient to avoid extinction during the first phase of ecosystem invasion, thus enabling the pathogen to enter the second phase characterized by pathogen growth in the intestinal lumen (Figure 1b). Pathogen growth in the gut lumen during the second phase of ecosystem invasion is driven by virulence factors [87]. In support of this idea, genetic ablation of virulence factors renders pathogens unable to expand in the intestinal lumen and feces, even after a high-dose challenge, thereby resulting in their extinction (Figure 1d). Surprisingly, virulence factors that are critical for ecosystem invasion frequently target host cells, rather than the microbiota itself. For instance, the murine pathogen *Citrobacter rodentium* (family *Enterobacteriaceae*) uses a type III secretion systems (T3SS) to inject proteins into epithelial cells to induce the formation of attaching and effacing lesions that enable bacteria to intimately attach to the epithelial surface [88], which results in epithelial injury. T3SS-mediated epithelial injury triggers epithelial repair responses that culminate in colonic crypt hyperplasia [89,90], a host response required for *C. rodentium* expansion in the intestinal lumen during the second phase of ecosystem invasion [91,92•]. However, T3SS-dependent pathogen growth is not required for intestinal *C. rodentium* growth in the absence of the microbiota [91], thus supporting the idea that the T3SS enables the pathogen to overcome colonization resistance. Similarly, *S.* Typhimurium encodes two T3SSs that inject proteins into the cytosol of host cells to mediate epithelial invasion (T3SS-1) [93] or bacterial dissemination within host tissue (T3SS-2) [94] (Figure 2). Host-pathogen interactions mediated by T3SS-1 and T3SS-2 ultimately trigger acute intestinal inflammation [95]. In turn, this inflammatory response is required for *S.* Typhimurium expansion in the intestinal lumen during the second phase of ecosystem invasion [96], thereby promoting transmission by the fecal oral route [97,98]. Genetic ablation of T3SS-1 and T3SS-2 renders *S.* Typhimurium unable to invade the gut ecosystem of conventional mice, but does not impair the pathogen's ability to colonize the intestine of germ-free mice [96], which illustrates that the pathogen uses its virulence factors to overcome colonization resistance mediated by the gut microbiota.

Framing the outcome of gut colonization by frank pathogens as an ecological problem highlights the importance of niche opportunities as determinants of success in ecosystem invasion [48]. This view suggests that during the second phase of ecosystem invasion, virulence factors elicit host responses to construct new nutrient-niches that are subsequently occupied by the pathogen. Consistent with this idea, migration of phagocytes into the intestinal lumen during acute intestinal inflammation is associated with an antimicrobial respiratory burst [99]. However, as reactive oxygen species and reactive nitrogen species diffuse into the intestinal lumen, they react to form non-toxic oxidation products, such as tetrathionate or nitrate, which serve as electron acceptors for anaerobic respiration [100–102] (Figure 2). This mechanism enables *S.* Typhimurium and *Yersinia enterocolitica* (family *Enterobacteriaceae*) to construct new nutrient-niches in the intestinal lumen [100,103,104], because anaerobic respiration enables pathogen growth on microbiota-derived fermentation products, such as ethanolamine or 1,2-propanediol, that the microbiota themselves are unable to utilize [105,106]. Similarly, T3SS-mediated intimate attachment by *C. rodentium* triggers epithelial repair responses, thereby constructing a new luminal nutrient-niche for the pathogen [92•]. The metabolism of terminally differentiated epithelial cells in the colon is polarized towards high oxygen consumption through mitochondrial oxidative phosphorylation [107], which renders the mucosal surface hypoxic [108]. Epithelial hypoxia is one of the habitat filters of microbiota-nourishing immunity that helps to maintain anaerobiosis in the intestinal lumen, thus shaping the colonic microbiota to be dominated by obligate anaerobic *Clostridia* and *Bacteroidia* [55]. *C. rodentium*-induced colonic crypt hyperplasia is characterized by an excessive division of undifferentiated epithelial cells [109], which exhibit low mitochondrial activity and low-oxygen

Figure 2

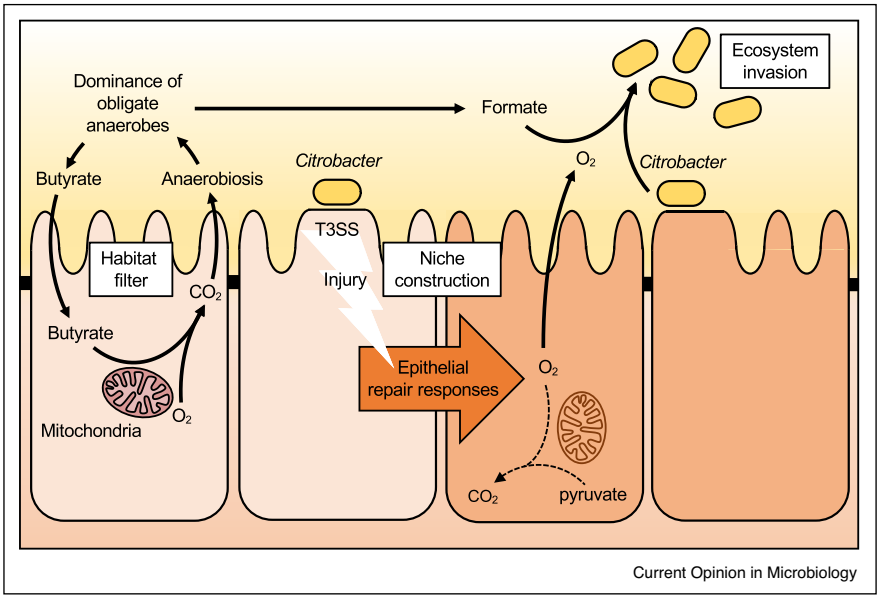


A look at ecosystem engineering by *Salmonella*. The colonic gut microbiota confers colonization resistance against *S.* Typhimurium (*Salmonella*) through niche preemption mediated by commensal *Enterobacteriaceae* and niche modification by *Clostridia*-derived short-chain fatty acids, such as butyrate [86•,98,126••]. *S.* Typhimurium uses its virulence factors (T3SS-1 and T3SS-2) to invade and survive in tissue, which triggers the release of cytokines (IL-1β, IL-17, IL-22 and IL-23) by antigen presenting cells (APCs), T cells and innate lymphoid cells (iLCs) [143–145]. The consequent recruitment and epithelial transmigration of neutrophils (PMN) initiates ecosystem engineering. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) produced in the intestinal lumen by the phagocyte respiratory burst react to form respiratory electron acceptors, such as nitrate and tetrathionate, which contribute to niche construction. The migration of neutrophils into the intestinal lumen also lowers butyrate levels, which in turn increases the epithelial release of lactate. Construction of a new nutrient niche by epithelial release of lactate and the generation of nitrate and tetrathionate supports pathogen growth, but competition might arise with commensal *Escherichia coli* for a growth-limiting resource, iron. During *S.* Typhimurium-induced inflammation, soluble ferrous iron (Fe^{2+}) is oxidized to insoluble ferric iron (Fe^{3+}) and IL-22-induced epithelial release of the antimicrobial protein lipocalin-2 prevents enterobactin-mediated ferric iron uptake. *S.* Typhimurium overcomes these host defenses by secreting salmochelin, a siderophore that is not blocked by lipocalin-2. In turn, some *E. coli* strains produce microcins (M and H47) that are internalized by the outer membrane salmochelin receptor (IroN), thereby impeding *S.* Typhimurium growth in its newly constructed niche.

consumption [110]. The resulting accumulation of undifferentiated cells at the mucosal surface increases epithelial oxygenation, thereby disrupting anaerobiosis in the lumen and driving an expansion of *C. rodentium* in an microaerobic nutrient-niche in which the pathogen consumes microbiota-derived formate [92•] (Figure 3). Thus, by increasing epithelial oxygenation, the *C. rodentium* T3SS recalibrates an important host-derived habitat filter to construct a new nutrient-niche.

Electron acceptors are not the only growth factors tickled out of the host by virulence factors: they can also stimulate epithelial release of carbon sources. *Vibrio cholerae* (family *Vibrionaceae*, phylum *Proteobacteria*) is a facultative anaerobic pathogen that uses its main virulence factor, cholera toxin (CTX), to invade the small intestinal ecosystem [111••]. CTX activates adenylyl cyclase in epithelial cells, thereby increasing intracellular 3′,5′-cyclic AMP levels [112]. The resulting activation of cAMP-dependent protein kinase (PKA) triggers changes in host cell metabolism that result in elevated epithelial L-lactate release, thereby inducing luminal expression of *V. cholerae* genes mediating lactate utilization [111••]. Similarly, *S.* Typhimurium induces an epithelial release of lactate, but through a different mechanism. One of the consequences of neutrophil migration into the intestinal lumen during *S.* Typhimurium-induced colitis is a depletion of *Clostridia* [113,114], the main producers of the short-chain fatty acid butyrate [115,116]. *S.* Typhimurium-induced colitis is therefore associated with a depletion of microbiota-derived butyrate [98], a metabolite that inhibits growth of the pathogen through niche-modification [117,118•] and polarizes the epithelial energy metabolism towards oxidative phosphorylation [107]. In the absence of

Figure 3



Citrobacter virulence factors target a host habitat filter. The energy metabolism of differentiated epithelial cells at the colonic surface is characterized by high oxygen consumption in the mitochondria. The resulting epithelial hypoxia helps maintain anaerobiosis, which constitutes a host habitat filter that shapes the microbiota to be dominated by obligate anaerobic bacteria. Intimate attachment mediated by *C. rodentium* (*Citrobacter*) virulence factors (T3SS) causes epithelial injury, thereby triggering excessive epithelial repair responses that lead to an accumulation of undifferentiated epithelial cells with low mitochondrial oxygen consumption. The consequent increase in epithelial oxygenation impairs the host habitat filter, thereby constructing an aerobic nutrient-niche that supports ecosystem invasion by the pathogen by using oxygen to consume microbiota-derived fermentation products, such as formate.

butyrate, the epithelium obtains energy through the Warburg metabolism [119,120], thereby inducing an epithelial release of L-lactate [121••,122]. Host-derived lactate serves as a carbon source that supports respiratory growth of *S.* Typhimurium [121••,122] and alleviates growth inhibition by butyrate [123], thereby enabling *S.* Typhimurium to overcome *Clostridia*-mediated niche modification (Figure 2). These examples illustrate that pathogens can act as ecosystem engineers by altering epithelial energy metabolism to drive release of epithelial-derived metabolites such as L-lactate, and in doing so, they can construct new nutrient-niches in the gut lumen.

Notably, virulence factor-mediated niche construction also provides ecological opportunities for commensal bacteria. For example, the generation of nitrate during acute intestinal inflammation stimulates growth of *S.* Typhimurium [103,124], but it can also drive an expansion of commensal *Escherichia coli* (family *Enterobacteriaceae*)[101] or opportunistic *Klebsiella oxytoca* (family *Enterobacteriaceae*) [125]. Thus, niche construction generates competition between pathogens and commensals [86•,126••]. A resource that becomes growth-limiting during enteric infection is iron and many of the skirmishes fought between a pathogen and its commensal competitors relate to the need to acquire this micronutrient. In the healthy gut iron is readily available in its soluble ferrous form (Fe^{2+}), but during conditions of intestinal inflammation the trace element is oxidized to its insoluble ferric form (Fe^{3+}), which necessitates expression of Fe^{3+} uptake systems to support growth [127,128]. Enterobactin, a low-molecular weight Fe^{3+} chelator (siderophore) is the most effective Fe^{3+} uptake system produced by *Enterobacteriaceae* [129]. However, intestinal inflammation triggered by *S.* Typhimurium virulence factors induces production of interleukin-22 (IL-22) in the intestinal mucosa, a cytokine that acts on epithelial cells to induce the luminal release of lipocalin-2 [130], an antimicrobial protein that sequesters enterobactin [131–133] (Figure 2). *S.* Typhimurium can overcome this iron-withholding mechanism by synthesizing a glycosylated derivative of enterobactin, termed salmochelin [134], which is no longer sequestered by lipocalin-2 [135,136]. Salmochelin synthesis provides a luminal fitness advantage for *S.* Typhimurium in the inflamed intestine [130] and enables the pathogen to successfully compete with commensal *E. coli* strains that lack this Fe^{3+} uptake system [137]. However, the probiotic *E. coli* strain Nissle 1917 produces microcins M and H47, two secreted antimicrobial peptides that are conjugated to salmochelin [138]. *S.* Typhimurium internalizes these microcins through its outer membrane salmochelin receptor IroN and this Trojan horse mechanism limits growth of the pathogen [139••].

Thus ecosystem engineering by virulence factors creates an arena in which pathogens and commensals use their antimicrobial weaponry to fight for dominance [140].

Conclusions

Viewing bacterial pathogenesis through the lens of microbiota-nourishing immunity [60••] provides perspective for the concept that pathogens act as ecosystem engineers by using their virulence factors to manipulate host habitat filters, thereby constructing new nutrient-niches that support their invasion of the gut ecosystem. In contrast, commensal bacteria do not trigger host responses to create a new nutrient niche and thus cannot enter the ecosystem when their respective niche is occupied by a member of the resident microbial community, which explains why the microbiota remains resistant to change [141] despite our daily environmental exposure to microbes. This resistance of the gut microbiota to change also explains why probiotics poorly engraft. However, we would argue that poor engraftment is a desirable trait for probiotics, because ecosystem invasion requires manipulation of host-derived habitat filters, a process that triggers disease, identifying microbes using this strategy as pathogens.

The emerging picture suggests that bacterial virulence factors are excellent tools for identifying host habitat filters that maintain gut homeostasis by shaping the microbiota composition (Figure 1a) [142••]. Thus, mechanistic studies on mucosal pathogens can provide fundamental insights into microbiome research and should be pursued in parallel to data-driven approaches to advance microbiota science. Whereas this review remained focused on the gut microbiota, virulence factors of pathogens that colonize mucosal surfaces outside the gastrointestinal tract could shine light on host control mechanisms that balance polymicrobial communities at these locations, which provides countless opportunities for investigators studying microbial pathogenesis to contribute to microbiome research.

Conflict of interest statement

Nothing declared.

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Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

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