Trends in Microbiology

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Review

Using Enteric Pathogens to Probe the Gut Microbiota

Luisella Spiga[1] and Sebastian E. Winter[1,*]

Enteric pathogens have evolved to manipulate the interface between the host and commensal microbial communities, making these pathogenic organisms superb research tools to interrogate the function of the gut microbiota during inflammatory flares. Here, we provide an overview of conceptual insights gained from experimental infection with enteric pathogens, such as *Salmonella enterica* serovar Typhimurium. Metabolic pathways at the host–microbe intersection will be a particular area of focus. A better understanding of the cellular and molecular mechanisms that control host–microbe interactions during episodes of inflammation may aid in the rational design of microbiota-targeting therapies.

Highlights

Enteric pathogens have evolved to manipulate the interface between the gut microbiota and the host to propagate within the host and ensure transmission.

Experimental infection of rodents with enteric pathogens such as *Salmonella enterica* serovar Typhimurium and *Citrobacter rodentium* can be used to interrogate interactions between the host and commensal gut microbes.

During inflammatory flares, the metabolic landscape of the intestinal lumen is altered through the release of reactive oxygen species and molecular oxygen.

Changes in nutrient availability, in particular terminal electron acceptors, allow facilitated fermentation and respiration to occur in the inflamed gut.

The increased availability of terminal electron acceptors during episodes of gut inflammation enables facultative anaerobic bacteria, such as members of the family Enterobacteriaceae, to utilize poorly fermentable carbon sources.

Challenges in Identifying Functional Host–Microbe Interactions

Gut microbial communities participate in a number of beneficial processes such as education of the immune system, digestion, and protection against infection with enteric pathogens. Yet, the permanent presence of microbes within the body poses a significant liability. The gut microbiota represents a reservoir for opportunistic infections, and gut microbiota imbalances are implicated in the development of obesity, cardiovascular disease, and cancer (reviewed in [1–3]). Given the impact of the microbiota on its host, it seems desirable to manipulate microbial functions as a novel way to improve human health. However, these efforts have been hampered by the complexity of the microbial ecosystem, within each host as well as the interpersonal diversity. Many of the molecular mechanisms that dictate the interplay between the host and the gut microbiota are incompletely understood.

Common approaches to studying gut commensal bacteria rely on 16S profiling and metagenomic sequencing, which can be used for global analysis of bacterial communities and to identify populations that correlate with a given phenotype. If single bacterial strains can be isolated, cause-and-effect can be ascertained by analyzing isolates or defined microbial consortia, for example in gnotobiotic mouse models. Although newer technological developments are promising [4], the *de novo* isolation of bacterial strains of interest is tedious and suffers from poor selection methods and unknown growth requirements. These technical hurdles highlight the need for better strategies to investigate functional microbe–microbe and host–microbe interactions in the intestinal tract.

Infection with *S. enterica* serovar Typhimurium Perturbs the Gut Microbial Community Structure

Naturally occurring or experimentally induced disturbances of an ecosystem can be used to reveal functional interactions within the community. One such perturbation of the gut microbiota is the infection with enteric pathogens. Infection of mice with *S. enterica* serovar Typhimurium (*S.* Typhimurium; family Enterobacteriaceae), a common cause of human gastroenteritis, restructures the composition of the commensal microbiota in mouse models [5,6].

[1]Department of Microbiology, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd, Dallas, TX, USA

*Correspondence: Sebastian.Winter@UTSouthwestern.edu (S.E. Winter).

Mucosal inflammation is a key driver of microbiota changes as the pathogen population in the gut lumen expands. *S*. Typhimurium mutants that are unable to induce gut inflammation, for example, by lacking both virulence-associated type III secretion systems, fail to outcompete the commensal microbiota [5,6]. The disease-dependent expansion of enteric pathogens can be viewed as a crucial aspect of the 'business plan', since increased intestinal colonization enhances host transmission success through the fecal–oral route [7].

Intracellular bacterial pathogens have evolved specific mechanisms to manipulate host cells, and these pathogens have been used extensively in the past as tools to decipher basic processes in cell biology [8]. Similarly, enteric pathogens have likely evolved to manipulate the host–microbiota interface. Over the past decade, enteric pathogens have become invaluable tools to investigate host–microbe interactions in the intestinal tract. This review is not intended to be an exhaustive list of newly discovered mechanisms of host–microbe interactions. Rather, these examples highlight how conceptual and technical advances in microbiota research have enlightened our understanding of enteric pathogens. Conversely, research on how enteric pathogens exploit the delicate balance between the host and its microbiota has revealed important concepts of host–microbe interactions in the gastrointestinal (GI) tract.

Host Release of Antimicrobials and Bacterial Antimicrobial Resistance Mechanisms Are Important Facets of Host–Microbe Interactions in the Inflamed Gut

Epithelial cells constitute the primary barrier against bacterial invasion (Figure 1). The presence of commensal bacteria is sufficient to promote antibacterial responses, including release of C-type lectins [9]. RegIIIγ, an antibacterial C-type lectin that is secreted into the gut lumen, prevents commensal bacteria from residing in close proximity to the intestinal epithelium [10]. Another C-type lectin, RegIIIβ, possesses antibacterial activity against both Gram-positive and some Gram-negative bacteria [9,11]. During *S*. Typhimurium infection, RegIIIβ expression and release is increased [12]; however, the structure of the *S*. Typhimurium lipopolysaccharide makes the pathogen highly resistant to RegIIIβ. Gut commensals such as Bacteroides (class Bacteroidia) are susceptible to RegIIIβ and are killed [12,13] (Figure 1A). As such, changes in the composition of the gut microbiota during *Salmonella* infection are not due merely to an expansion of the pathogen population, and host factors such as RegIIIβ-mediated killing may contribute to gut microbiota dysbiosis. Interestingly, RegIIIβ-deficient mice were more susceptible to *S*. Enteritidis infection [14], suggesting that not all *Salmonella enterica* serovars may exploit host responses in similar ways.

Under homeostatic conditions, the Paneth cell-derived defensins HD5 and HD6 regulate the ratio of Clostridia (phylum Firmicutes) to Bacteroidia (phylum Bacteroidetes) populations in the large intestine [15]. Interestingly, some commensal members of the Bacteroidia class have evolved resistance mechanisms to killing by some antimicrobial peptides. Akin to *Salmonella*, *Bacteroides thetaiotaomicron* chemically modifies the lipid A portion of the lipopolysaccharide to increase resistance to a subset of antimicrobial peptides [16].

The antimicrobial properties of HD6 depend on environmental conditions. HD6 has no discernable antimicrobial properties under standard laboratory conditions, but inhibits growth of a subset of commensal bacteria under reducing, anaerobic conditions [17]. During *S*. Typhimurium infection, HD6 interferes with bacterial invasion by trapping *S*. Typhimurium in nano-nets *in vitro* and in the gut lumen during early stages of infection [18]. Luminal oxygenation and redox potential increases substantially during *S*. Typhimurium infection and during episodes of gut microbiota dysbiosis (for a more detailed discussion see text

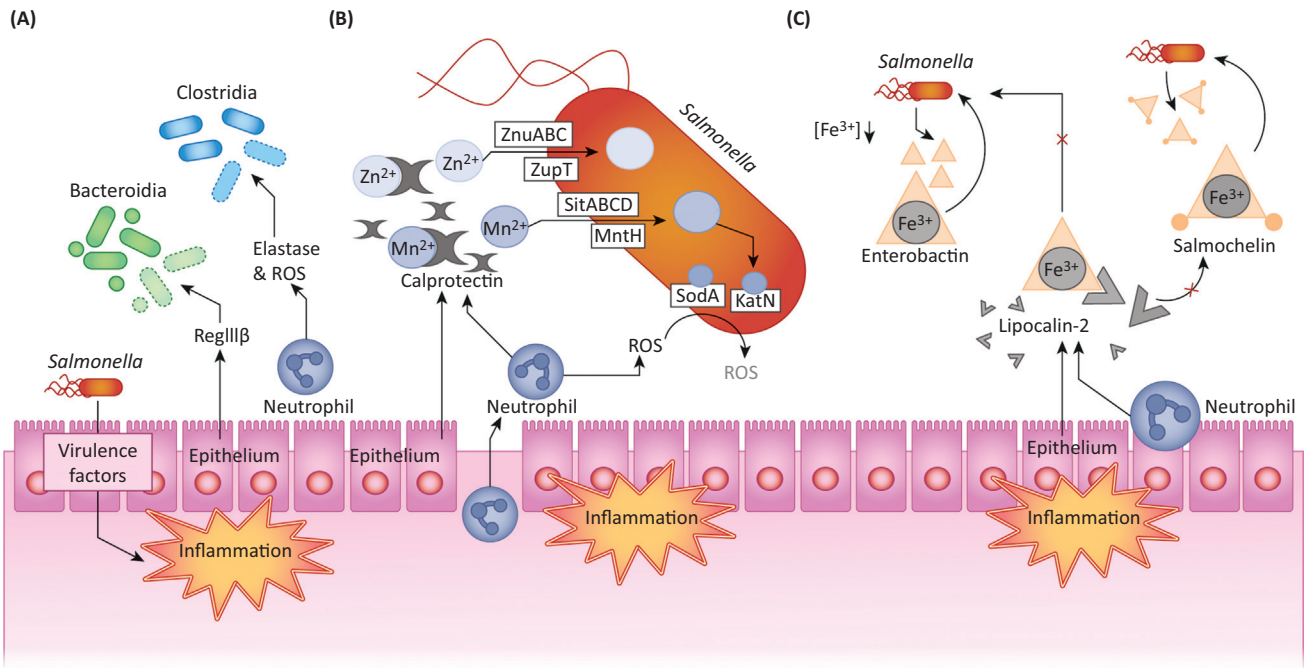

Figure 1. The Effect of Antimicrobials on Commensal Gut Microbes and Subversions of Nutritional Immunity by *Salmonella*. (A) Secretion of the C-type lectin RegIIIβ during *Salmonella* infection removes competing Bacteroidia family members. Release of elastase and reactive oxygen species (ROS) from transmigrating neutrophils kills Clostridia populations. (B) Calprotectin, released by epithelial cells and neutrophils during episodes of inflammation, sequesters zinc and manganese to impede microbial growth. *Salmonella* uses several high-affinity transporters to acquire these micronutrients; zinc-dependent superoxide dismutases and catalases protect *Salmonella* from ROS. (C) Under iron-limiting conditions, *Salmonella* secretes the siderophore enterobactin to facilitate uptake of iron. During gut inflammation, the host produces lipocalin-2 to bind and inactivate enterobactin. *Salmonella* also produces a glucosylated derivative of enterobactin, salmochelin, which is not recognized by lipocalin-2 and enables *Salmonella* to evade lipocalin-2-mediated iron sequestration.

below) [19,20]. The redox-dependence of HD6 activity may serve as a regulatory mechanism to spare commensal microbes during enteric infection. In conclusion, antimicrobial peptides shape the gut microbiota composition, both in the small and the large intestine, with differential susceptibility of bacterial populations (either commensal or enteric pathogen) being a critical determinant in the outcome of this interaction.

Tissue invasion by *Salmonella* triggers a subacute inflammatory response. Infiltrating neutrophils release reactive oxygen species (ROS) and reactive nitrogen species (RNS) to limit systemic spread of the infection [21–23]. Through neutrophil transmigration and local destruction of tissue, antimicrobials enter the gut lumen, thus impacting microbial communities [24]. Neutrophil elastase appears to directly impede growth of commensal gut bacteria, in particular Clostridia populations (families Lachnospiraceae and Ruminococcaceae) [25] (Figure 1A). Gut commensals that do not express catalases and superoxide dismutases, such as Clostridia populations, might be particularly sensitive to inflammatory ROS. Of note, Lachnospiraceae family members are depleted in patients with chronic, noninfectious colitis [26].

Many redox-active enzymes, such as catalases and superoxide dismutases, are metalloenzymes that contain transition elements as a cofactor. To impede bacterial growth, the host reduces the availability of trace metal ions such as iron, zinc, and manganese during inflammatory flares, a process termed nutritional immunity (reviewed in [27]) (Figure 1A,B).

Calprotectin, released from epithelial cells and neutrophils, sequesters zinc and manganese [28,29] (Figure 1B). Unlike most commensal bacteria, *S*. Typhimurium scavenges these micronutrients in the inflamed gut through several high-affinity transporters, including ZnuABC, MntH, SitABCD, and the low-affinity, broad-spectrum metal permease ZupT [28,29] (Figure 1B). Manganese-dependent catalases and superoxide dismutases (KatN and SodA) protect *S*. Typhimurium populations in the inflamed gut lumen against neutrophil-derived ROS [29].

Under iron-limiting conditions, *S*. Typhimurium and commensal Enterobacteriaceae produce the catecholate siderophore enterobactin to sequester iron and to import the iron–siderophore complex in a TonB/ExbB/ExbD-dependent manner [30–33]. The availability of iron in the inflamed gut lumen is limited, and *S*. Typhimurium primarily relies on siderophore-mediated iron acquisition [34] (Figure 1C). Mucosal inflammation is accompanied by the release of lipocalin-2 (neutrophil gelatinase-associated lipocalin) from activated epithelial cells and neutrophils [35,36]. Lipocalin-2 binds enterobactin, thus incapacitating enterobactin-based iron acquisition [37,38]. Unlike many commensal Enterobacteriaceae family members, *S*. Typhimurium produces a glucosyl-modified version of enterobactin termed salmochelin [39]. Lipocalin-2 does not bind to salmochelin [40], allowing *S*. Typhimurium to subvert one arm of nutritional immunity [34]. TonB/ExbB/ExbD-dependent processes also occur in commensal gut bacteria. *In vitro*, *Bacteroides* can utilize enterobactin as an iron source [41]. In the absence of enterobactin sequestration, such as in lipocalin-2-deficient mice, *Alistipes* spp. (phylum Bacteroidetes) populations expand and trigger mucosal host responses [42], indicating that lipocalin is a critical mediator of host–microbe balance in the GI tract. Several nonredundant BtuB-like transporters facilitate the uptake of corrinoids, vitamin B_{12} analogs, in *Bacteroides* spp. [43]. Furthermore, the *sus*-like polysaccharide degradation systems expressed by Bacteroidetes rely on TonB/ExbB/ExbD-dependent transport of degradation products into the bacterial cell (reviewed in [44,45]).

In addition to their immediate antimicrobial activity, neutrophil-derived antimicrobials alter the metabolite pool in the gut lumen (Figure 2). Inflammatory ROS, elicited by *S.* Typhimurium, react with endogenous thiosulfate to form tetrathionate [46]. Peroxynitrite, a bactericidal RNS, decomposes to nitrate [47]. Unlike obligate anaerobic bacteria, *S*. Typhimurium can utilize tetrathionate and nitrate for anaerobic respiration. In mouse models, nitrate respiration and tetrathionate reduction support the bloom of *S*. Typhimurium [46,48,49].

Collectively, these studies highlight the role of epithelial and neutrophil-derived antimicrobials in shaping gut microbiota composition and function, through direct killing, micronutrient withholding, and changes to the metabolic landscape. The importance of these host responses is emphasized by the fact that enteric pathogens have evolved distinct mechanisms to circumvent and exploit these host defense mechanisms.

The Oxygen Tension in the Gut Lumen Is Controlled by Epithelial Metabolism, Microbial Respiration, and Chemical Processes

The lumen of the large intestine is considered anoxic under homeostatic conditions, and most constituents of the gut microbiota are obligate anaerobic members of the classes Bacteroidia and Clostridia. In contrast, the submucosal tissue is oxygenated due to its high vascularization. Oxygen-respiring microbes, such as facultative anaerobic Enterobacteriaceae members, are preferentially found in close proximity to the epithelium [50,51]. It was generally assumed that the steep oxygen gradient, extending from the oxygenated tissue to the almost entirely anoxic gut lumen, was a sole function of microbial consumption of oxygen [52]. Newer findings, in particular in mouse models of colitis and enteric infection, suggest that host metabolism plays a

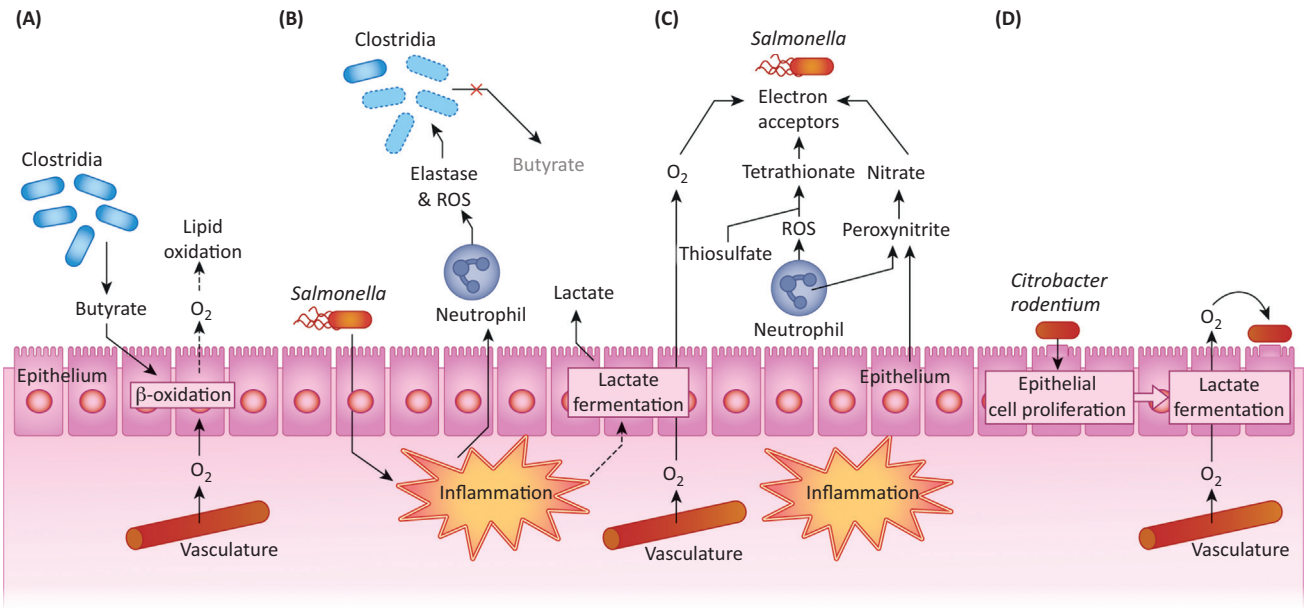

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Figure 2. Changes in the Availability of Terminal Electron Acceptors during Infection with Enteric Pathogens. (A) Under homeostatic conditions, mature colonocytes perform β-oxidation of short-chain fatty acids (SCFAs), such as butyrate. This metabolism depletes oxygen at the epithelial interface. Small amounts of oxygen entering the gut lumen are consumed by microbial activities (not pictured) and oxidation of lipids. (B) During inflammatory flares, for example, *Salmonella* infection, butyrate-producing Clostridia populations are depleted. In the absence of butyrate, colonocytes perform lactate fermentation and oxygen leaks from the vasculature into the gut lumen. (C) Neutrophil-derived reactive oxygen species (ROS) oxidize endogenous thiosulfate to tetrathionate. The reactive nitrogen species (RNS) peroxynitrite decays to nitrate. Oxygen, tetrathionate, and nitrate serve as terminal electron acceptors for *Salmonella*. (D) *Citrobacter rodentium* causes attaching/effacing lesions and crypt hyperplasia in the large intestine. Undifferentiated epithelial cells do primarily perform lactate fermentation, resulting in the release of oxygen into the gut lumen which, in turn, supports pathogen growth.

critical role in controlling luminal oxygenation and that purely chemical processes, such as the oxidation of unsaturated lipids and phospholipids contribute to local oxygen depletion in the absence of a microbiota [52] (Figure 2A).

Under homeostatic conditions, microbial degradation of dietary and host-derived glycans leads to the production of large quantities of short-chain fatty acids (SCFAs), such as acetate, propionate, and butyrate (reviewed in [53]). The host uses SCFAs as cues of a normally functioning microbiota to guide immune responses and to support its own metabolism [54,55]. In colonocytes, microbial butyrate activates the nuclear receptor peroxisome proliferator-activated receptor gamma (PPARγ) to initiate β-oxidation of SCFAs and other carbon sources [56] (Figure 2A). Under normal conditions, consumption of oxygen in mature colonocytes is so vigorous that the epithelium permanently remains in a hypoxic state, thus limiting oxygen diffusion into the gut lumen [57]. In contrast, undifferentiated epithelial cells are not responsive to butyrate, do not utilize butyrate as a carbon and energy source, and do not exhibit the physiological hypoxia that is characteristic of mature colonocytes [54,55,57].

Oxygen Leakage, a Result of Epithelial Dysfunction, Supports Pathogen Growth and Impedes Commensal Bacteria

Enteric pathogens exploit this dichotomy of epithelial metabolism. The host inflammatory response elicited by *S.* Typhimurium alters gut microbial communities [5]. Butyrate-producing Clostridia are diminished and butyrate levels dwindle [19]. In the absence of butyrate, epithelial

cell metabolism shifts to glycolysis, and epithelial consumption of oxygen ceases [54,55,57] (Figure 2B). The oxygen seeping into the gut lumen supports aerobic respiration in *S*. Typhimurium [19] (Figure 2C).

Citrobacter rodentium, a member in the family Enterobacteriaceae, causes transmissible colonic hyperplasia in mice. The pathological features of this type of colitis, attaching and effacing lesions, are reminiscent of human enterohaemorrhagic *Escherichia coli* infection, making *C. rodentium* a model for the human disease. *C. rodentium* expresses a type three secretion system to translocate effector proteins into epithelial cells. In addition to mediating attaching and effacing lesions, a subset of these effectors (EspH, EspF, and Map) is required for the induction of crypt hyperplasia. Excessive proliferation of undifferentiated epithelial cells as part of the pathogen-induced hyperplasia allows oxygen to leak from the vasculature into the gut lumen [58] (Figure 2D). Adherent *C. rodentium* populations respire aerobically using the high-affinity terminal oxidase CydAB [58].

Increasing oxygen levels have a profound impact on obligate commensal bacteria. Low-level oxygen exposure and the ROS produced during inflammation impede obligate anaerobic bacteria. Critical enzymes in the central metabolism of *B. thetaiotaomicron* are inactivated by oxidation of iron sulfur clusters, thus slowing the growth of these obligate anaerobes [59,60]. Taken together, these studies exemplify the profound role of oxygen in governing bacterial metabolism and thus controlling microbial community structures in the gut.

A Respiration-Driven Oxidative Metabolism Allows for the Utilization of Poorly Fermentable Carbon and Energy Sources

Low-molecular-weight metabolites are predominantly absorbed in the small intestine, making host-inaccessible complex polysaccharides the major carbon and energy source for bacteria in the large intestine. The ability to degrade a variety of complex polysaccharides correlates with the relative abundance of commensal microbes, suggesting that access to complex polysaccharides is a driving force in the assembly of the community under homeostatic conditions [61,62]. Members of the classes Bacteroidia and Clostridia encode a plethora of secreted glycoside hydrolases, facilitating the catabolism of structurally diverse polysaccharides [61]. Fermentative pathways to generate energy predominate in the anoxic gut. The emergence of electron acceptors with a positive reduction potential, such as oxygen and nitrate, as byproducts of the host's inflammatory RNS and ROS metabolism has several consequences on the metabolism of facultative anaerobic bacteria. From a bioenergetics standpoint, anaerobic respiration is more efficient than fermentation as it allows for recycling of reduction equivalents (as NADH, NADPH, or $FADH_2$) and electron transfer processes at the plasma membrane [63]. In the most simplistic scenario, only the latter occurs and an electron acceptor with a very positive reduction potential is coupled with an electron donating reaction in an electron transport chain to generate proton motive force and ATP. For example, commensal Enterobacteriaceae utilize formate as the electron donor in conjunction with aerobic respiration [64]. The ability to recycle redox equivalents also reconfigures the central intermediary metabolism. In the absence of respiration, the bacterial TCA cycle is bifurcated into an oxidative and reductive branch [65–67]. Balancing of redox equivalents is achieved by transferring electrons onto an internal, carbon-based electron acceptor such as fumarate. The end product of fumarate reduction, succinate, is excreted. In a respiratory metabolism, the TCA cycle operates in a cyclical fashion, while generating NADH as an electron donor for the electron transport chain. The presence of electron acceptors in the inflamed gut changes expression of TCA cycle enzymes in *S*. Typhimurium (Figure 3A) [68]. The oxidative TCA cycle in *S*. Typhimurium facilitates the utilization of poorly fermentable compounds (Figure 3B,D), such as succinate

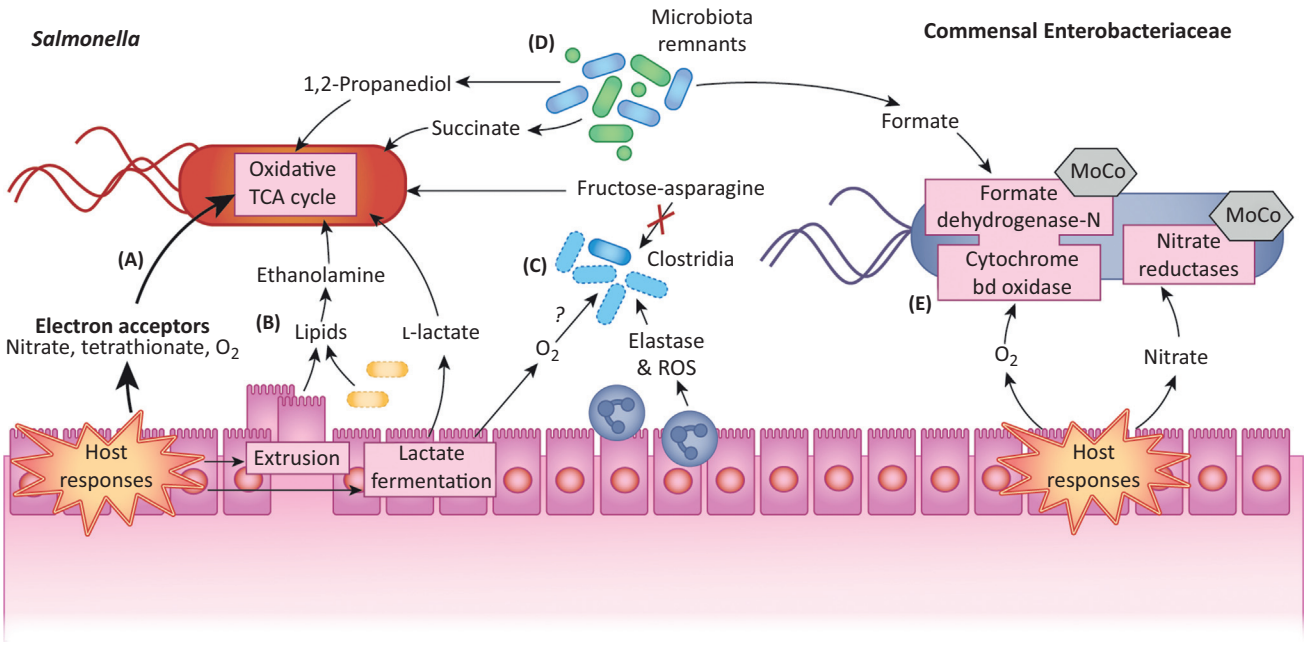

Figure 3. Members of the Family Enterobacteriaceae Utilize a Variety of Carbon and Energy Sources through an Oxidative Central Metabolism. (A) Host-derived terminal electron acceptors induce an oxidative central metabolism in *Salmonella*. (B) Infected epithelial cells are extruded as part of an innate host defense. Ethanolamine, derived from host membrane lipids, is utilized by *Salmonella*. The end product of colonocyte lactate fermentation, L-lactate, is consumed by the pathogen. (C) Commensal Clostridia degrade dietary fructose-asparagine. The inflammatory responses elicited by *Salmonella* kill Clostridia, thus removing a nutritional competitor for the pathogen. (D) Fermentation end products produced by remnants of the obligate anaerobic microbiota can be utilized by *Salmonella* through its oxidative central metabolism. (E) During inflammatory flares, commensal members of the Enterobacteriaceae perform a respiratory metabolism. Oxygen respiration is coupled to the utilization of microbiota-derived formate in a simple electron transport chain. Host-derived nitrate facilitates nitrate respiration in conjunction with an unknown electron donor. Nitrate reductases and formate dehydrogenase contain a molybdenum cofactor (MoCo).

and 1,2-propanediol (Figure 3D), which are common fermentation products of *Bacteroides* polysaccharide degradation [68,69]. Notably, the availability of these fermentation end-products does not profoundly change with the onset of inflammation. Rather, the emergence of electron acceptors is sufficient to allow degradation of these fermentation products. Similarly, ethanolamine utilization contributes to gut colonization of *Salmonella* in a murine colitis model [70] (Figure 3B). In this model, ethanolamine levels do not increase with the onset of inflammation; however, tetrathionate respiration is required for *Salmonella* to catabolize ethanolamine *in vitro* [71] and *in vivo* [70]. Potential sources of ethanolamine include phospholipids from the diet, host cells, or gut bacteria. For example, *Salmonella*-infected epithelial cells are extruded into the gut lumen as an epithelial defense mechanism [72] and contribute to free phosphatidylethanolamine levels. A significant portion of bacterial cells within gut microbial communities are damaged or dying, which may also contribute to the local phosphatidylethanolamine pool [73].

The metabolic versatility of *S.* Typhimurium in conjunction with its inflammation-adapted lifecycle can be used as a tool to investigate the fate of small metabolites in the gut. Fructose-asparagine was identified in a forward genetic screen as a compound metabolized by *S.* Typhimurium during infection of the murine gut [74]. Fructose-asparagine is commonly found in heat-treated human food and is formed in a Maillard reaction by heating complex mixtures of amino acids and sugars [75]. *In vitro*, a number of commensal gut bacteria,

including *Clostridium* spp. can grow on fructose-asparagine [76], suggesting that diverse bacterial populations could access the fructose-asparagine pool. Curiously, the host inflammatory response elicited by *S*. Typhimurium perturbs microbial gut communities. Fructose-asparagine-degrading commensal populations are depleted, thus removing nutritional competition for the enteric pathogen [77] (Figure 3C).

In addition to microbial-derived carbon sources, the host as a potential source of metabolites should be considered. Fecal lactate concentrations, in particular L-lactate, are increased in inflammatory bowel disease patients [78]. This change in metabolite levels during inflammatory episodes was thought to occur as a consequence of altered gut microbiota metabolism and a breakdown of syntrophic relationships between lactate-producing and lactate-consuming bacterial populations [79]. In mouse models of *S*. Typhimurium, lactate levels are elevated and the pathogen consumes L-lactate [80]. In this rodent model, the majority of lactate is not of microbial origin, but is produced by the host [80]. PPARγ-dependent changes in epithelial metabolism result in the production of L-lactate, the product of fermentative glycolysis. The lactate secreted into the gut lumen is consumed by *S*. Typhimurium through a respiratory metabolism [80]. As such, it is tempting to speculate that changes in host metabolism might contribute to the increased lactate levels observed in noninfectious inflammatory diarrheal disease.

Changes in Nutrient Availability and Energy Metabolism Contribute to Dysbiosis during Noninfectious Colitis

Anaerobic respiratory processes shape the gut microbiota composition in settings other than infectious colitis. Inflammatory bowel disease, a chronic inflammatory diarrheal disease, is accompanied by an alteration of the microbiota composition. One common feature of this inflammation-associated dysbiosis in patients, and in rodent models, is the expansion of commensal members of the family Enterobacteriaceae [81,82]. Mirroring the respiration-driven bloom of pathogenic members of the family Enterobacteriaceae, *Salmonella* and *Citrobacter*, introduction of oxygen into the intestinal lumen by surgical means increases the abundance of commensal Enterobacteriaceae [83]. The gut microbiota of ileostomy patients shifted to a dysbiotic state with a high representation of facultative anaerobic Enterobacteriaceae, with closure of the surgical opening reversing this dysbiotic alteration [83]. It appears that the lack of respiratory electron acceptors, in particular molecular oxygen, is a limiting factor for the size of the Enterobacteriaceae population in the human intestinal tract. Based on these findings, and the observation that enteric pathogens bloom through respiratory processes, it was hypothesized that changes in the availability of oxygen and possibly other electron acceptors may be a driver of microbiota changes in inflammatory bowel disease [84]. This 'oxygen hypothesis' has been experimentally tested in mouse models of noninfectious colitis. In murine models of acute colitis and antibiotic-induced dysbiosis, butyrate production is decreased and epithelial cells rely predominantly on glycolysis and lactate fermentation [56,57]. Consistent with these observations in animal models, fecal butyrate levels are decreased in inflammatory bowel disease patients, and the mucosa actively secretes lactate into the gut lumen [85,86]. In the absence of epithelial oxygen consumption, oxygen is thought to leak into the gut lumen. Aerobic respiration through cytochrome bd oxidases contributes to the bloom of commensal Enterobacteriaceae in mouse models of colitis, suggesting that the gut lumen forms a microaerobic environment during inflammatory conditions [56,64,87] (Figure 3E).

The availability of oxygen respiration allows commensal Enterobacteriaceae to catabolize formate, a microbial fermentation product, in a simple electron transport chain [64]. A second, independent metabolic pathway used by commensal Enterobacteriaceae during inflammatory

flares is the respiration of ROS-generated nitrate [64] (Figure 3E). Enterobacterial nitrate reductases and formate dehydrogenases harbor a molybdopterin cofactor in their active site [88]. Replacement of molybdenum with tungsten allows for selective inhibition of enterobacterial molybdoenzymes [88–90]. Pharmacological inhibition of nitrate respiration and formate oxidation with tungsten curbs the inflammation-associated growth of naïve Enterobacteriaceae populations in mouse models of acute colitis [91]. Preventing the bloom of Enterobacteriaceae decreased mucosal inflammation, implying that the expansion of Enterobacteriaceae population during inflammation-associated dysbiosis is not a mere bystander effect but indeed aggravates host responses [91].

Concluding Remarks and Future Perspective

Over the past years, numerous studies have documented how the gut microbiota is differentially involved in numerous homeostatic and disease-driving host processes. The microbiota holds great potential for the development of novel intervention strategies that target disease-driving gut microbes, with the ultimate goal of promoting homeostasis. Since enteric bacterial pathogens have evolved to benefit from perturbations of host–commensal relationships, we infer that these pathogens present unique tools to interrogate host–microbe interactions in the intestinal tract. In particular, experimental infection of mice with *Salmonella* and *C. rodentium* has been productive in establishing mechanistic insights. The availability of genetics for these pathogens, representative commensal bacteria, and the murine host allows for epistasis-type experiments to investigate intersecting pathways. While this review has focused on metabolic interactions, other aspects of microbial physiology must be considered for a more comprehensive understanding (see Outstanding Questions). For example, it is somewhat unclear how increased availability of ROS and oxygen during inflammatory flares impacts obligate anaerobic communities and how these bacteria coordinate gene expression to alleviate stress. It is also unclear whether other enteric pathogens, such as *Shigella* spp. and *Yersinia enterocolitica*, bloom in the intestinal lumen to enhance transmission success, and which pathways contribute to this phenomenon. Future experimental work in these systems may reveal novel information on host–microbe interactions in the intestinal tract.

Outstanding Questions

Do enteric pathogens other than *Salmonella* and *Citrobacter*, such as *Shigella* spp. and *Y. enterocolitica*, exploit host responses to proliferate in the inflamed gut lumen? If so, are the mechanisms driving these blooms conserved or distinct?

How do changes in nutrient availability and oxygen concentration impact obligate anaerobic microbial communities during inflammation?

How do gut microbes coordinate stress responses to ensure survival during perturbations?

Should the field consider moving from an organism-centric view of the gut microbiota to a metabolism-centric approach?

How detailed does our mechanistic understanding of host–microbe interactions have to be to enable the rational design of novel, gut microbiota-based intervention strategies?

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