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Enteric Pathogens Exploit the Microbiota-generated Nutritional Environment of the Gut

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Abstract

Host bacterial associations have a profound impact on health and disease. The human gastrointestinal (GI) tract is inhabited by trillions of commensal bacteria that aid in the digestion of food and vitamin production and play crucial roles in human physiology. Disruption of these relationships and the structure of the bacterial communities that inhabit the gut can contribute to dysbiosis, leading to disease. This fundamental relationship between the host and microbiota relies on chemical signaling and nutrient availability and exchange. GI pathogens compete with the endogenous microbiota for a colonization niche (1, 2). The ability to monitor nutrients and combine this information with the host physiological state is important for the pathogen to precisely program the expression of its virulence repertoire. A major nutrient source is carbon, and although the impact of carbon nutrition on the colonization of the gut by the microbiota has been extensively studied, the extent to which carbon sources affect the regulation of virulence factors by invading pathogens has not been fully defined. The GI pathogen enterohemorrhagic *E. coli* (EHEC) gages sugar sources as an important cue to regulate expression of its virulence genes. EHEC senses whether it is in a gluconeogenic versus a glycolytic environment, as well as fluctuations of fucose levels to fine tune regulation of its virulence repertoire.

DYNAMICS OF INTESTINAL COLONIZATION BY PATHOGENIC BACTERIA

The mammalian gastrointestinal (GI) tract harbors a diverse collection of indigenous bacteria known as the microbiota. The number of bacterial cells within our bodies exceeds the number of our cells by one order of magnitude (3). Homeostasis of the microbiota is maintained by differential nutrient utilization and physical separation from the gut mucosa (4). However, environmental perturbations such as antibiotic treatment, changes in diet, and infection lead to substantial alterations in composition and structure of the microbiota, referred to as *dysbiosis* (5–8).

Efficient use of nutrient sources in the gut has a major impact on colonization by pathogenic bacterial species given that nutrient sources are scarce, and they compete with the exquisitely adapted commensal bacteria for these nutrients. According to Freter's hypothesis, the ability of a pathogen to thrive during intestinal colonization depends on its ability to efficiently utilize nutrient sources and find a suitable niche for colonization (9). Competition for nutrient acquisition between enteric pathogens and the microbiota

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constitutes a protective mechanism against infection and is an important aspect of colonization resistance. Therefore, evolution of new nutrient acquisition mechanisms and metabolic diversification contributes to a pathogen's survival and persistence and is an important determinant of the course of bacterial infections. Two important strategies employed by enteric pathogens are the alternative use of carbon sources (10) and utilization of byproducts of the microbiota's metabolism.

Linking metabolism to the precise coordination of virulence gene expression is a key step in the adaptation of pathogens towards their colonization niches. In this chapter, we will discuss nutrient detection, acquisition, and utilization by enteric pathogens and the barriers against intestinal infection, highlighting the vital role played by the gut microbiota in these processes.

INTESTINAL BARRIERS AGAINST INFECTION

Enteric pathogenic bacteria face a series of barriers to colonizing the GI tract. The human gut is a very complex ecosystem that harbors a high number of commensal bacteria that compete with pathogens for nutrients and space. In addition, the intestinal epithelium is covered by a protective viscous mucus layer that impairs easy bacterial access to the epithelium (10, 11). Suffice it to say, tropism for the mammalian intestine co-evolved with several virulence traits that helped bacterial pathogens cross the aforementioned barriers. Some crucial virulence traits comprise the ability to attach to mucus and cell surface receptors; production of proteases and toxins; expression of flagella to swim across the mucus layer; invasion of epithelial cells; and quorum sensing (12–17). In addition to strict *pro quo* virulence factors, pathogens also require suitable nutrients. Therefore, nutrient-sensing systems play a major role in both early and late phases of infection.

The Intestinal Microbiota

The mammalian GI tract microbiota plays a fundamental role in human health. Ten trillion to 100 trillion microbes inhabit the distal segment of the human gut, with most belonging to the Bacteroidetes (Gram-negative) and Firmicutes (Gram-positive) phyla (3, 18, 19). Metagenomic studies have shown that the species composition of the microbiota is very diverse; nonetheless, there is conservation in the microbial phyla shared by all individuals (20, 21). In addition, the composition of the intestinal microbial community can vary according to the host genetic background (22, 23). The gut microbiota plays many roles in the host homeostasis and has been referred to as the "forgotten organ" (24). The genetic repertoire of this community is referred to as the *microbiome* and gives the human host metabolic capabilities not encoded in our genome (25, 26).

The Mucus Layer: A Source of Protection and Nutrients

The single layer of epithelial cells that separates the luminal contents from the GI mucosa is the target of many pathogenic bacteria (27, 28). Nonetheless, not all bacteria can directly interact with enterocytes. A gel-like mucus layer overlays the intestinal epithelial cells, shielding the colonic epithelium from bacteria (29). The mucus layer is in a dynamic state, being constantly synthesized and secreted by specialized goblet cells and degraded to a large

extent by indigenous intestinal microbes (30, 31). In fact, mucus utilization has recently been proposed as a co-evolved adaptation of gut resident bacteria and their host (32).

The mucus is composed of mucin, antimicrobial peptides, glycoproteins, glycolipids and epithelial cell debris but 50% of it is made of polysaccharides (33). The major structural component of the mucus is mucin, a glyco-protein that has a protein backbone connected to hydrophilic and hygroscopic oligosaccharide side-chains, which form a gel-like tridimensional structure (34). Also, as part of the mucus composition, there are other goblet cell products such as trefoil peptides (TFF), resistin-like molecule β (RELMβ), and Fc-γ binding protein (Fcgbp), antimicrobial peptides (beta-defensin) and lysozymes secreted by the Paneth cells, and IgA secreted by enterocytes (34, 35). Moreover, the microbiota modulates mucin synthesis and secretion (36). O-linked glycans comprise 80% of the total weight of mucins and are a major nutrient source for bacteria (29). In addition, they provide attachment sites for commensal and pathogenic bacteria (29, 37). A diverse collection of 13 monosaccharides is part of the mucus composition: arabinose, fucose, galactose, gluconate, glucuronate, galacturonate, mannose, glucosamine, N-acetyl-glucosamine, galactosamine, N-acetyl-galactosamine, N-acetylneuramic acid, and ribose. All of these sugars are made available to pathogenic bacteria due to host epithelial cell turnover and the polysaccharide-degrading activity of commensal anaerobes. Hence, the mucus layer is an important habitat and source of nutrients for bacterial communities that colonize mucosal surfaces.

The highly glycosylated MUC2 mucin is synthesized by goblet cells in the small and large intestines and is a major component of the mucus layer (33). The intestinal epithelium also expresses membrane-bound mucins: MUC1, MUC3, MUC4, MUC12, MUC13, and MUC17. However, MUC2 is the predominant mucin in human and murine colons (33), and these mucins are constantly being degraded by the action of glycosidases produced by the anaerobic bacteria that dominate the colonic microbiota (38).

The important role of the mucus as a defensive barrier against infection and injury can be illustrated by the differential length and thickness of its structure along the GI tract. The mucus layer is progressively thicker towards the large intestine, the site where the highest numbers of commensal bacteria reside. The mucus is the front line of the innate host defense against pathogenic microbes (34). Alterations of the mucus layer, such as the lack of MUC2, renders mice susceptible to bacterial adhesion to the intestinal epithelium, resulting in disturbances of the intestinal physiology (33). It is noteworthy that deficiency in mucus production causes changes in the normal localization of commensal bacteria in the colon (39). In addition, recent evidence highlights the importance of mucin in the defense against bacterial infection, with MUC2-deficient mice developing severe life-threatening colitis when infected with the murine enteric pathogen *Citrobacter rodentium* (33). MUC2 has highly fucosylated glycans, and MUC17, a membrane-bound mucin expressed in the large intestine, protects against invasion by enteroinvasive *E. coli* (EIEC) (41).

The stratified mucus layer that protects the intestinal epithelium displays a highly complex structure. The mucus layer is spatially divided in two layers: an outer loose mucus layer facing the intestinal lumen and a thick inner mucus layer firmly attached to the epithelial cells (29). Using fluorescent *in situ* hybridization (FISH) staining for bacteria, Johansson et

al elegantly demonstrated that commensal bacteria communities reside in the outer mucus layer, while the inner layer is virtually devoid of bacteria (39).

Gut commensals are not found in close contact with the epithelium lining, in contrast to pathogenic bacteria, which employ virulence factors to reach close proximity to enterocytes. EHEC produces the plasmid-encoded metalloprotease StcE, which targets intestinal mucins, therefore contributing to bacterial penetration towards the colonic epithelium (42). In fact, one of the theories of how commensal bacteria do not cause disease, even when sharing several common features with pathogenic bacteria, is their localization in the GI tract. Commensal bacteria are associated with the outer mucus layer but not at the interface of the intestinal epithelium, and this physical separation is thought to prevent dysbiosis (43). Both pathogenic and commensal bacteria can consume carbohydrates from the mucus as a carbon and energy source (44–47). In addition to being a nutrient source for indigenous and foreign bacteria, the mucus components can also be exploited as cues to trigger production of virulence factors by pathogens. MUC2 triggers virulence expression in *Campylobacter jejuni*, with MUC2 exposure leading *C. jejuni* to express cytolethal distending toxin JlpA, and flagellin (28).

The stratification of the mucus layer is important to sustain the symbiotic relationship between the microbiota and the host. It has been demonstrated that the lectin RegIII gamma, produced by Paneth cells of the intestinal epithelium, is important to the physical separation of the microbiota and the intestinal epithelium (4, 48). The physical separation of the intestinal microbiota and the colonic epithelium represents evidence that the mucus layers act as an effective barrier (11).

MICROBIOTA AND NUTRIENT GENERATION IN THE GUT

The consortium of the resident microbes that inhabit the human gut possess an incredible arsenal of glycolytic enzymes that allow the utilization of complex polysaccharides originated from the host itself or its diet (48–50). These polysaccharides are hydrolyzed into monosaccharides, which are subsequently utilized as carbon and energy sources after being released as free sugars in the intestinal lumen (51). Therefore, the metabolic activity of the microbiota is intrinsically related to the generation of the nutritional environment of the gut, which also impacts the survival and persistence of pathogenic bacteria. In this section, we will discuss the role of nutrient acquisition in the gut by pathogenic bacteria and how the microbiota modulates the availability of these nutrient sources.

Commensal Bacteria and Polysaccharide Degradation in the Gut

One of the major roles played by the microbiota is the manipulation of carbohydrate sources in the gut. The members of the microbiota community are fermenters with a broad range of metabolic capacity, being able to digest complex glycan structures originated from the host diet, host structures such as mucus, and cell-associated glycans otherwise not digested by invading microbes (50, 52–54). This plural metabolic capacity allows the microbiota to explore unique niches and survive in the human gut in homeostasis. Hence, the gut microbiota functions as a metabolic organ, helping the human host obtain energy from otherwise indigestible dietary sources (55).

The ability to degrade complex polysaccharides highlights the crucial role displayed by the gut commensals in metabolic pathways in the gut. Most of the knowledge of polysaccharide utilization by the microbiota derives from investigations of the glycophagic symbiont *Bacteroides thetataiotaomicron. B. theta*, a strict anaerobe, is the most abundant resident of the distal small intestine and colon of mice and humans (56, 57). The *B. theta* genome encodes an impressive arsenal of 246 glycolitic enzymes (58). This prominent commensal bacterium can degrade plant- and animal-derived complex glycans into monosaccharides, providing nutrient sources for other commensals, consequently playing a fundamental role in the supply of energy sources in the gut. Pathogenic bacteria exploit this *B. theta*-dependent nutrient availability. Therefore, the polysaccharide-degrading activity of commensal bacteria might affect colonization by pathogens by representing a source of competition but also a nutrient-generating machine capable of supporting pathogens to successfully grow and find a niche in the host. The ability of *Bacteroides sp* to use a diverse range of glycans is dependent on genes in its polysaccharide-utilization loci (PULs) (59).

Mucus utilization by primary fermenters such as *B. theta* involves the production of glycosyl hydrolases secreted into the environment. Consequently, monosaccharides are released from the complex glycan structures into the lumen, where they are accessible to other microbial species. *B. theta* dedicates about 18% of its genome to polysaccharide utilization, the PULs (58). Conversely, gut pathogens are not equally equipped to consume complex host-derived glycans. Although some pathogens, such as EHEC, are able to produce a mucus-degrading protease, StcE, it does not encode glycosidases. This means that gut pathogens rely on the glycosidase activity of commensal bacteria to access and import free monosaccharides for catabolism.

Certain members of the microbiota, such as *Bifido-bacterium sp*, may differ in their effects on nutrient generation to pathogenic bacteria. In contrast with *B. theta*, *B. longum subspecies infantis* (*B.infantis)* typically produces intracellular glycosidases to import complex glycans into the cell for digestion into monosaccharides (60, 61). *B. bifidum*, however, secretes glycosidases similarly to *B. theta* (62). Consequently, the access to free monosaccharides by microbial pathogens during polysaccharide degradation by certain species of *Bifidobacteria* is different, which may have interesting biological implications because *Bifidobacteria* are used as probiotics and may be able to control or prevent enteric infections (63).

A special relationship takes place between *B. theta* and its host. *B. theta* consumption of fucose may have a major impact on enteropathogens able to utilize this sugar as a carbon and energy source (64). *B. theta* induces the expression of fucosylated glycoconjugates by the host intestinal epithelium (65). Then *B. theta* produces fucosidases that harvest fucose from mucosal glycans (66). Fucose is abundant is intestinal glyco-conjugates, and it is usually a terminal α-linked sugar (67, 68). The triggering of intestinal fucosylation by *B. theta* depends on the bacterial density and the production of a *B. theta*-derived signal that remains elusive (69). In addition to using host-derived fucose as a carbon and energy source, *B. theta* also incorporates fucose into a capsular polysaccharide via an O-glycosylation system, which is believed to be important for competitive colonization of the gut (70). Nutrient utilization by *B. theta* can be modulated by diet: a diet rich in plant glycans triggers

expression of genes involved in metabolism of dietary substrates by *B. theta*; conversely, on exposure to a diet devoid of complex glycans, *B. theta* switches its metabolism towards host glycans (50). The metabolic switch that *B. theta* undergoes during a change in diet might affect pathogen access to fucose, which could directly impact the outcome of bacterial infections.

Primary fermenters differ in their capacity to utilize carbohydrates, which is relevant *in vivo* because dietary changes cause alterations in the community structure of the microbiota, and likely, in the outcome of end-fermented products (71). Polysaccharide degradation has been recognized as a core function encoded within the microbiome (72). The prominent adult gut symbiont, *B. theta* encodes an arsenal of 261 glycosyl hydrolases (73). Other distal gut residents such as *Akkermansia muciniphila* have a mucin-degrading ability that may also lead to release of free monosaccharides that can be utilized by pathogenic enterobacteria (74).

A generation of free monosaccharides in the gut, an end product of extensive polysaccharide degradation by members of the microbiota, is a major modulator of the nutrient environment accessible to invading pathogens. Most pathogenic bacteria do not encode glycosyl hydrolases in their genomes and rely on simple monosaccharides or disaccharides as substrates for growth *in vivo* (32). Therefore, the enzymatic activity of the gut microbiota, to a certain degree, may render the host particularly susceptible to different infections. This concept could be further explored to design customized diets or probiotic interventions aimed at improving pathogen exclusion based on nutrient competition. This concept is exemplified by Deriu et al, who demonstrated that administration of the probiotic *E.coli* Nissle 1917 strain reduced murine colonization by *Salmonella enterica Typhimiurium* (75).

INTERPLAY BETWEEN COMMENSAL AND PATHOGENIC BACTERIA

Given the high content of commensal bacteria residing in the gut, the colonization site of several bacterial pathogens, it is not surprising that a complex relationship might arise from these interactions. Nonetheless, little is known of the mechanisms that govern the crosstalk between pathogens and the microbiota or the impact of the commensal bacteria on pathogenesis and infection outcomes. Elucidation of the processes involved in interactions among host, microbiota, and pathogens is of major importance in the design of novel therapeutic interventions (76–78).

Gut pathogens harbor several traits to maximize proliferation in the lumen, including motility, chemotaxis, and iron-scavenging and nutrient-sensing systems. In addition, pathogenic species can hijack carbohydrate utilization pathways of resident microbes for their own advantage by exploring the end-product of glycosidases produced by anaerobes from the microbiota to obtain monossacharides as carbon sources (38).

Competition for Nutrients and Colonization Resistance

While primary fermenters have a major impact on nutrient generation for bacterial pathogens, commensal bacteria displaying similar nutrient requirements pose a threat against pathogen survival during intestinal infection. By consuming similar carbohydrates,

commensal *E. coli* competes with EHEC O157:H7 for nutrients, leading this pathogen to explore different niches to proliferate in the gut (79–81).

Commensal and pathogenic *E. coli* differs in the types of carbohydrates it preferably utilizes *in vivo* as carbon sources. EHEC can grow on mucus (82). Studies indicate that EHEC can grow *in vitro* on cecal mucus prepared from mice but cannot grow in the luminal content, suggesting that these bacteria colonize the mouse intestine by growing in the mucus layer that overlays the cecal epithelium (43). In addition, other studies provide evidence that carbohydrates derived from the mucus can support the growth of *E. coli* during murine colonization (44–47).

Commensal and pathogenic *E. coli* share their preferences for particular carbon sources they utilize during intestinal colonization, but they also present differences that reflect their spatial segregation inside the human gut. Commensal *E. coli* strains are found in the lumen and attached to the mucus layer, while pathogenic *E. coli* strains are able to cross the mucus layer and reach proximity to the IECs, which also exposes them to nutrients exclusively available at the epithelium interface (44, 83). Among the nutrient sources consumed by both commensal and pathogenic *E. coli* are monossaccharides and disaccharides. The fact that they consume similar carbon sources *in vivo* is the basis of colonization resistance that commensal *E. coli* imposes on pathogenic *E. coli* species.

Colonization of the mammalian intestine by EHEC requires precise coordination of metabolic and virulence factors. The infectious dose of EHEC is remarkably low compared with other enteric pathogens, highlighting important adaptations of EHEC to the human intestine. EHEC must expand its population to high numbers and find a niche in the colon, which is a major challenge considering the immense number of residing commensal bacteria adapted to live in the colon during millions of years of co-evolution with the human host (84).

An investigation of the carbohydrate utilization profile of EHEC in the bovine gut has revealed that this strain can catabolize mucus-derived carbohydrates inside the cattle gut and do so more rapidly than resident microbes, including commensal *E. coli* (85). Cattle are the major reservoir of EHEC O157:H7, which has tropism to the recto-anal junction (RAJ) (86). *In vitro* growth competition assays using WT and the EHEC sugar utilization mutant strains Δ*manA*, Δ*nagE*, Δn*anAT,* and Δ*galK*, which are deleted for genes involved in catabolism of six major mucus-derived monosaccharides (galactose, N-acetyl-glucosamine [GlcNAc], N-acetylgalactosamine [GalNAc], fucose, mannose and N-acetyl neuraminic acid [Neu5Ac]), showed that the ability to consume mannose, GlcNAc, Neu5Ac, and galactose is important for EHEC growth, suggesting that metabolism of the aforementioned carbohydrates confers a growth advantage to EHEC in the bovine intestine (85).

In vivo carbon consumption was investigated using the streptomycin-treated mouse model and elucidated many aspects of the competition and nutrient utilization that allows EHEC to successfully colonize the mammalian intestine. Commensal and pathogenic *E. coli* share the ability to consume arabinose, fucose, and N-acetylglucosamine in the mouse intestine. EHEC is able to catabolize galactose, hexuronates, mannose, and ribose, while commensal

E. coli exclusively catabolize gluconate and N-acetyl-neuramic acid (79). These data indicate that differential carbon nutrition in the gut contributes to niche adaptation of EHEC and helps avoid competition with commensal *E. coli*. According to the nutrient-niche hypothesis elaborated by Freter et al, better consumption of a limiting nutrient source than an organism's competitors is imperative for successful colonization of the intestine (87, 89).

Recent evidence indicates that the ability to consume similar carbohydrate sources is an important factor that may influence bacterial infection. Kamada et al reported that competition with members of the gut microbiota for the same nutrients is necessary for pathogen clearance (88). This study represents a great advance on the investigation of the relationship among commensal and pathogenic bacteria, which has also shown that classic virulence traits such as the type 3 secretion system (T3SS), which is known to be required for host cell contact by EHEC, is also important for competition with gut microbiota (88).

EHEC does not significantly compete with *B. theta* for nutrient utilization during growth in mucus but competes with commensal *E. coli* for the same carbon sources during growth in the mammalian intestine (44, 47, 79, 89). One such carbon source is fucose, which is released into the lumen by glycophagic bacteria such as *B. theta* and can be utilized by *E. coli*, which itself cannot hydrolyze complex mucus carbohydrates (44, 47, 79). Because both EHEC and commensal *E. coli* compete for fucose utilization in the lumen, it would be counterproductive for EHEC to invest a lot of resources in the utilization of this carbon source in this compartment, where commensal *E. coli* are present (79, 89). However, EHEC can efficiently use other carbon sources, such as galactose, hexorunates, mannose, and ribose, which are not used by commensal *E. coli* in the intestine (79).

NUTRIENT SENSING IN THE GUT

Differential utilization of limiting nutrients is the basis for the coexistence of members of the gut microbiota. It has also a major impact on bacterial infection, as pathogens explore alternative nutrient sources to avoid competition with commensals. In cases of noncronical infections such as EHEC or EPEC, nutrient competition among commensal and pathogenic bacteria impacts the outcome of infection, leading to resolution of this infection and elimination of the intruder.

Fucose Sensing Regulates Intestinal Colonization by EHEC

EHEC is the causative agent of outbreaks of bloody diarrhea worldwide, with about 5% to 7% of the cases in any given outbreak developing a life-threatening complication known as *hemolytic uremic syndrome* (HUS) (90, 91). EHEC colonizes the human large intestine through the formation of attaching and effacing (AE) lesions on intestinal epithelial cells (92). Most genes necessary for AE lesion formation are clustered in a pathogenicity island (PAI) named the locus of enterocyte effacement (LEE) (93). The LEE region contains five major operons: *LEE1-5* (94–96), which encodes a type III secretion system (TTSS) (12), an adhesin (intimin) (97) and its receptor (Tir) (98), and effector proteins (99–103). The LEE genes and the non–LEE-encoded effector, EspFu, are both required for the formation of AE lesions (104).

Cell-to-cell communication among bacteria in the intestine is a major mechanism that shapes bacteria-host relationships. Pathogenic bacteria such as EHEC can also cross-communicate with the host by detecting mammalian hormones (105). By virtue of its remarkably low infectious dose (50 CFU) (106), successful colonization of the human colon by EHEC relies largely on sensing multiple signals to coordinate the expression of virulence genes. EHEC exploits the autoinducer-3 (AI-3)/ epinephrine (Epi)/norepinephrine (NE) interkingdom signaling cascade to trigger expression of motility and AE lesion genes, two pathogenic traits that are crucial for colonization but are required at different time points during infection (105). The host hormones Epi/NE are specifically sensed by two histidine sensor kinases: QseC and QseE (107, 108). QseE is downstream of QseC in this signaling cascade, given that transcription of QseE is activated through QseC (109). In addition to sensing these host hormones, QseC also senses the bacterial signal AI-3 (110). QseE, however, does not sense AI-3, thereby discriminating between host- and bacterial-derived signals (108). QseC and QseE activate virulence gene expression and pathogenesis *in vitro* and *in vivo* in EHEC (108, 110, 111). On sensing these signals, QseC and QseE autophosphorylation increases, initiating a signaling cascade that promotes virulence gene expression. QseE exclusively phosphorylates the QseF response regulator (112). QseC, however, phosphorylates three response regulators: QseB, QseF, and KdpE.

Signal sensing by EHEC is crucial for colonization of the mammalian colon due to the orchestration of multiple virulence pathways that aim to promote intimate attachment of the bacteria to the apical portion of enterocytes (113). Also of major importance is the activation of pathways that allow suitable nutrition during infection. Recently, Pacheco et al (64) demonstrated that EHEC encodes for a two-component system (TCS) named FusKR, in which FusK is the sensor kinase and FusR is the response regulator (64). The FusKR TCS is repressed by the adrenergic-sensing QseBC and QseEF TCSs.

Investigation of the signal triggering *fusKR* transcription indicated that it was a component of the mucus. Using a combination of biochemical and genetic approaches, L-fucose was identified as the signal that activates the FusKR signaling cascade in EHEC. In fact, FusK specifically increases its autophosphorylation in response to fucose (64). FusKR signaling leads to repression of LEE and fucose utilization gene expression, allowing the pathogen to save energy by preventing unnecessary virulence gene expression while crossing the mucus layer and avoiding competition with the commensal *E. coli* for carbon sources, given that commensal *E. coli* preferentially catabolize fucose in the mammalian intestine (64).

In vitro competition assays have demonstrated that the modulation of carbon availability by the prominent gut symbiont *B. theta* alters the effect of fucose utilization by EHEC on the expression of *ler*, the master regulator of the LEE genes. In the presence of free fucose in the media, *B. theta* has no effect on *ler* transcription, but this scenario changed on co-culture of *B. theta* and EHEC on mucin. During growth on mucin, EHEC relies on *B. theta* to access free fucose, and as the result of this relationship, expression of *ler* is reduced (64). Therefore, the interaction between EHEC and the commensal bacterium *B. theta* is able to change the pathogen's virulence due to the nutrient modulatory activity of *B. theta. In vivo* competition assays using the infant rabbit model (114), which is able to reproduce several aspects of EHEC-mediated disease, show that the EHEC *fusK* mutant is attenuated for

virulence, and regulation of *ler* by FusK plays a determinant role on EHEC fitness during intestinal colonization (Fig. 1) (64).

The genes encoding *fusKR* are clustered in a pathogenicity island (OI-20) only found in EPEC O55:H7 (the *E. coli* lineage that gave rise to EHEC O157:H7) (115, 116), EHEC O157:H7, and *C. rodentium*, AE GI pathogens that colonize the colon. EHEC's ancestor, EPEC O55:H7 (116), is the only other serotype of *E. coli* to harbor *fusKR*, suggesting that acquisition of these genes is recent. The recent acquisition of OI-20 on EHEC evolution provided this pathogen with a novel signal transduction system, suggesting that expression of this TCS in mucus facilitates EHEC adaptation to the mammalian intestine.

The modulation of the nutrient supply in the gut by commensal microbes and its effects on bacterial virulence supports the use of probiotic interventions to control bacterial infections. It was demonstrated that the probiotic strain *Lactobacillus casei* consumes fucosyl-α-1,3- N-acetylglucosamine (Fuc-α-1,3-GlcNAc) as a carbon source and releases free L-fucose into the media (117). Given that fucose can repress EHEC virulence, it would be interesting to investigate the effects of a symbiotic approach (i.e., combination of a probiotic strain and a prebiotic diet) on preventing or treating EHEC infections.

Sensing of Glycolytic versus Gluconeogenic Environments

Glycolytic environments inhibit the expression of the LEE genes. Conversely, growth in a gluconeogenic environment activates expression of these genes. Part of this sugar-dependent regulation is achieved through two transcription factors: KdpE and Cra. KdpE and Cra interact to optimally and directly activate expression of the LEE genes in a metabolite-dependent fashion (118). EHEC competes with commensal *E. coli* (the predominant species in the γ-*Proteobacteria*) for the same carbon sources during growth in the mammalian intestine (1, 44, 47, 79, 89). EHEC uses glycolytic substrates for initial growth but is unable to effectively compete for these carbon sources beyond the first few days and begins to utilize gluconeogenic substrates to stay within the intestine (44). Hence, it is advantageous to coordinate expression of the LEE with these environmental conditions. Commensal *E. coli* can be found in the lumen, which is glycolytic due to the abundant sugar sources supplied by the glycophagic microbiota, while the interface with the epithelium is a more gluconeogenic environment. Hence, the KdpE/Cra-dependent activation of the LEE under gluconeogenic conditions ensures that these genes are optimally expressed only at the epithelium interface and not in the lumen (Fig. 1).

Ethanolamine Utilization

Ethanolamine is a breakdown product of phosphati-dylethanolamine, which is an abundant phospholipid of mammalian and bacterial cell membranes (119–121). Epithelial cell turnover and the gut microbiota are important sources of ethanolamine in the gut, which can be taken up and utilized as a carbon and/or nitrogen source by a number of bacterial species, including pathogenic bacteria such as EHEC and *Salmonella*. Exfoliation of intestinal cells also releases ethanolamine into the intestine (122–125). Ethanolamine is broken down into acetaldehyde and ammonia by the enzyme complex ethanolamine ammonia lyase, encoded

by genes *eutB* and *eutC*. Ammonia is used as a nitrogen source, while acetaldehyde is converted into acetyl-CoA (126).

Ethanolamine consumption supports the growth of EHEC *in vivo* and also confers EHEC a competitive advantage over the indigenous microbiota in the bovine intestine (127). EHEC can utilize ethanolamine as a nitrogen source in the bovine small intestine, in contrast with commensal *E. coli* strains. Ethanolamine metabolism allows EHEC to flourish in the bovine gut (its main reservoir), which contributes to the spreading of EHEC infections (127).

In addition to its role as a nitrogen source, ethanol-amine functions as a signaling molecule that triggers EHEC virulence expression. Kendall et al demonstrated that EHEC senses ethanolamine partially via EutR, a previously known receptor for ethanolamine. On EHEC growth on M9 minimal medium with ethanolamine as the sole nitrogen source, expression of the LEE and Stx increases markedly, indicating that ethanolamine is a signal that triggers EHEC virulence gene expression (123). It was also shown that ethanolamine triggers transcription of the EHEC adrenergic sensors *qseC* and *qseE*, which are involved in cell-to-cell signaling and bacteria-host communication (123). These studies suggest that ethanolamine sensing may contribute to EHEC persistence in the mammalian gut, not only by supporting EHEC growth but also by controlling transcription of major virulence factors. The research conducted by Kendall et al also indicates that EHEC encodes an additional, yet unidentified, ethanolamine sensor (123).

While currently available data leave no doubt of the pivotal role played by ethanolamine during gut colonization by enteric pathogens, little is known of the sensory systems employed in ethanolamine detection. Future research is necessary to unravel the receptors involved in early ethanolamine sensing, which are critical steps in infection.

The Effects of Inflammation

Pathogen-promoted inflammation during enteric infection is now appreciated as a strategy to promote rather than a consequence of bacterial infection. Enteric pathogens such as *C. rodentium* and *Salmonella* can benefit from the inflammatory environment or the overall changes in the bacterial community that result from inflammation. By provoking intestinal inflammation, the murine pathogen *C. rodentium* reduces the overall number of commensal bacteria in the microbiota, which gives the pathogen a colonization advantage (76, 128). Although later stages of inflammation result in pathogen clearance from the gut, inflammation in the early stages of infection helps *C. rodentium* replicate and increase its population when competing with commensal microbes.

Destruction of intestinal integrity by inflammation promotes *Salmonella Typhimurium* persistence in the gut. Inflammation triggered by *Salmonella* releases a new electron acceptor, tetrathionate, which allows *Salmonella* to outcompete the gut microbiota and proliferate in the gut lumen (129). In addition, tetrathionate allows *Salmonella Typhimurium* to use ethanolamine as a carbon source in the inflamed intestine (124). The *eutC* mutant, which cannot grow anaerobically on ethanol-amine as a carbon source, was outcompeted by the wild-type (WT) strain only in the presence of tetrathionate, indicating that ethanolamine utilization and tetrathionate respiration likely occur concomitantly. Ethanol-amine levels

present in colons of mice infected and uninfected were similar, indicating that ethanolamine consumption in the inflamed intestine was not due to release of ethanolamine due to epithelial cell destruction caused by inflammation. Interestingly, the growth advantage conferred by the ability to consume ethanol-amine *in vivo* relies on the ability to respire tetrathionate (124). In the absence of the electron acceptor tetra-thionate, respiration of ethanolamine does not support *Salmonella* growth in the mouse intestine. Therefore, the inflammatory response orchestrated by *Salmonella* during infection creates a nutritional environment that supports its replication in the gut lumen.

Nutrient Competition

Kamada et al (88) have demonstrated that the combined effects of virulence gene expression and competition with the microbiota are both crucial for pathogen clearance using the murine pathogen *C. rodentium*. *C. rodentium* is a natural mouse pathogen that causes colonic hyperplasia, and similarly to EHEC and EPEC, forms AE lesions on IECs. *C. rodentium* has been extensively used as a model for EHEC and EPEC infections (88). During infection of conventional mice, *C. rodentium* requires expression of the LEE to compete with indigenous microbes, while LEE expression is not necessary for *C. rodentium* colonization of germ-free mice. It was also shown that virulence gene expression (LEE) was triggered early but was reduced during late stages of infection, causing relocation of *C. rodentium* from the epithelium to the gut lumen, where the pathogen was exposed to commensal bacteria and had to compete for similar carbon sources for luminal growth. This shows that virulence and metabolism act in concert during bacterial infection, and both nutrient utilization and production of virulence traits are required to establish a successful colonization by pathogenic bacteria. A closer look at the nutrient competition between *C. rodentium* and commensal *E. coli* and *B. tetha* indicated that *E. coli* can outcompete *C. rodentium* due its ability to grow on monosaccharides, while *B. theta* does not outcompete *C. rodentium* because it can grow on polysaccharides. This work demonstrated that competition for similar nutrient sources is an important determinant of the outcome of bacterial infections of the mammalian intestine, reinforces Freter's concept, and raises the possibility that shifting the commensal microbiota towards nutrient competition with pathogens may be an alternative to fight bacterial infections.

A recent study shows that EHEC colonization could be prevented by the probiotic strains *E. coli* Nissle 1917 and *E. coli* HS, based on the ability of these combined commensal strains to compete for the carbohydrate niches occupied by EHEC to colonize the mammalian gut. EHEC utilizes arabinose, galactose, and gluconate, carbohydrates also consumed by *E. coli* Nissle 1917 and *E. coli* HS. EHEC also competes with *E. coli* HS for ribose and N-acetylglucosamine, while it competes with *E. coli* Nissle 1917 for mannose (81).

CONCLUSIONS

Nutrient scavenging by pathogenic bacteria from microbiota-derived products is an emerging theme in bacterial pathogenesis. Dietary changes causing shifts in gut microbial populations are well established, although the consequences regarding infection by pathogenic agents are mostly unknown. The use of probiotic strains to reinforce colonization

resistance may be a better alternative to treatment of infections for which antibiotic treatment is not advisable, such as EHEC and *Salmonella*. Future investigations on the relationships between indigenous microbiota members and pathogenic microorganisms are crucial for the development of new effective preventive and curative strategies for enteric infections.

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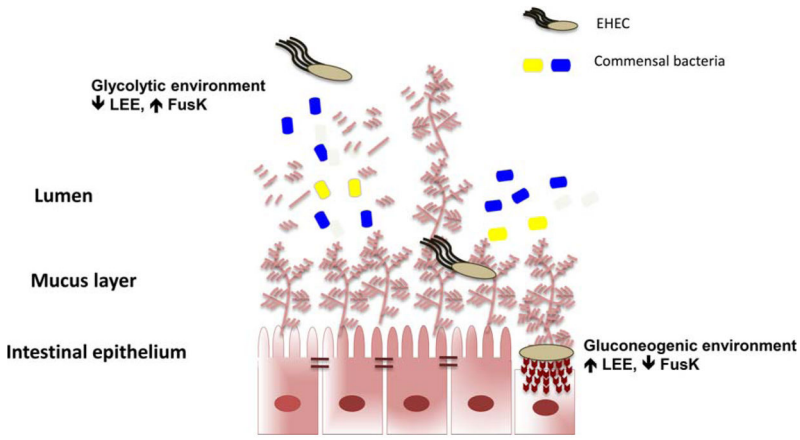

FIGURE 1.

Nutritional cues regulate the locus of enterocyte effacement (LEE) gene expression in enterohemorrhagic *E. coli* (EHEC). Glycophagic members of the microbiota such as *B. theta* make fucose from mucin accessible to EHEC, and EHEC interprets this information to recognize that it is in the lumen, where expression of its LEE-encoded type III secretion system (TTSS) is onerous and not advantageous. Using yet another nutrient-based environmental cue, EHEC also times LEE expression through recognition of glycolytic and gluconeogenic environments. The lumen is more glycolytic due to predominant glycophagic members of the microbiota degrading complex polysaccharides into monosaccharides that can be readily utilized by nonglycophagic bacterial species such as *E. coli* and *C. rodentium*. In contrast, the tight mucus layer between the lumen and the epithelial interface in the gastrointestinal (GI) tract is devoid of microbiota; it is known as a “zone of clearance.” At the epithelial interface, the environment is regarded as gluconeogenic. Hence, the coupling of LEE regulation to optimal expression under gluconeogenic and low-fucose conditions mirrors the interface with the epithelial layer environment in the GI tract, ensuring that EHEC will express only LEE at optimal levels to promote attaching and effacing lesion formation at the epithelial interface. doi:10.1128 /microbiolspec.MBP-0001-2014.f1