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Biochemical mechanisms of pathogen restriction by intestinal bacteria

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Abstract

The intestine is a highly complex ecosystem where many bacterial species interact with each other and host cells to influence animal physiology and susceptibility to pathogens. Genomic methods have provided a broad framework for understanding how alterations in microbial communities are associated with host physiology and infection, but the biochemical mechanisms of specific intestinal bacterial species are only emerging. In this review, we focus on recent studies that have characterized the biochemical mechanisms by which intestinal bacteria interact with other bacteria and host pathways to restrict pathogen infection. Understanding the biochemical mechanisms of intestinal microbiota function should provide new opportunities for therapeutic development towards a variety of infectious diseases.

Emerging mechanisms and applications of microbiota-mediated pathogen resistance

The **intestinal microbiota** (see Glossary) is central to host metabolism and immunity [1, 2]. As a result, the microbiome as a whole broadly impacts host physiology and response to intestinal and systemic diseases. The composition of the **intestinal microbiome** is dynamic and is influenced by environmental factors including host diet and exposure to drugs, infection, and probiotics, as well as by genetic factors. Advances in our understanding of specific bacterial genes and molecules have revealed a diversity of inter-bacterial interactions and immuno-modulatory roles for intestinal bacteria that influence pathogen fitness and host response to infection [3]. This review will focus on recent studies of specific intestinal bacterial species, their metabolites and potential biochemical mechanisms (see Table 1 for examples presented in this review). Beyond infection, the intestinal microbiome widely influences host physiology, with specific bacterial factors contributing to diseases including obesity, cardiovascular disease, and diverse neurological disorders. These topics have been recently reviewed elsewhere [4, 5] and will not be discussed in this review.

A molecular understanding of intestinal microbiota-host interactions is of great medical and ecological importance. Insight into microbiota function can help us design targeted therapeutics against a variety of diseases and advance personalized medicine. While the clinical potential of probiotic and other microbiota-based therapies against infectious disease

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is now being explored, available treatments are still coarse. For example, fecal transplantation has recently emerged as an investigational new drug for recurrent *Clostridium difficile* infection [6, 7]. Transitioning to more targeted therapeutics, however, requires a better understanding of the specific mechanisms of individual bacterial species. Beyond the clinical potential, characterizing the intestinal microbiome gives us a generalizable framework for evaluating the ecology of other complex host-microbe interactions, which are ubiquitous on earth.

Inter-bacterial interactions

The intestinal microbiome is shaped by metabolic competition and communication. Inter-bacterial interactions within this community can affect the fitness of key species as well as overall community structure. In this section, we will discuss recently described mechanisms by which intestinal bacteria directly restrict enteric pathogens (Figure 1A). Inhibition of pathogen growth can occur through resource competition as well as through direct bactericidal mechanisms. Alternatively, pathogen infection can be attenuated by modulating specific **virulence** mechanisms.

Metabolic competition

Although microbial community structure at the taxonomic/species level varies greatly across healthy individuals, functional metabolic capacity at the metagenomic level is largely stable [8]. This highlights how metabolic competition and interdependence builds microbial community structure. Within this framework, host genetics and behavior can alter the intestinal environment and shift microbial composition.

Niche competition not only defines microbial community structure, but also serves as a barrier for both enteric pathogens and **pathobionts**. Metabolic competition over resources including diverse carbon sources, trace metals, and vitamins such as B12 [9], shapes the microbiota and likely contributes to **colonization resistance**. Due to metabolic similarity, competition is often greater between related species than unrelated species.

Enterobacteriaceae is a large family of Gram-negative bacteria that includes **commensal species** as well as several intestinal pathogens including: *Salmonella*, *Shigella*, *Yersinia*, *Citrobacter*, and Enteropathogenic *Escherichia coli*. Indeed, metabolic competition between members of the *Enterobacteriaceae* has been described *in vivo*. For example, competition over monosaccharide use can restrict colonization by *Citrobacter rodentium* in wild-type mice [10]. Colonization of mice with commensal *E. coli* provides greater competition-mediated growth restriction of *C. rodentium* than colonization with *Bacteroides* species, which are able to utilize a more diverse profile of mono- and polysaccharides. However, in an experimentally skewed intestinal environment with monosaccharides as the sole carbon source, colonization of mice with *Bacteroides thetaiotaomicron* can also provide colonization resistance towards *C. rodentium*.

Competition over iron is another well-characterized example of inter-species competition in the intestine. Because iron is an essential and limiting nutrient, many intestinal bacteria produce iron-chelating siderophores to increase iron uptake. Host-secreted antimicrobial proteins, such as lipocalin-2, restrict bacterial growth by binding and inactivating diverse

bacterial siderophores. *Salmonella enterica* can evade lipocalin-2-mediated growth inhibition by producing modified siderophores that cannot be bound by lipocalin-2 [11, 12], improving pathogen fitness [13]. In this context, probiotic *E. coli* Nissle 1917, which expresses four iron uptake systems that are resistant to lipocalin-2, competitively reduces *Salmonella* colonization and subsequent inflammation [14].

Many pathogens generate a distinct niche as a strategy to avoid competition with intestinal bacteria and gain access to nutrients. In a simplistic sense, adhesion to the surface of intestinal epithelial cells, or invasion into host cells, could be viewed as a means to evade competing intestinal bacteria. Intestinal pathogens may also have evolved unique nutrient utilization pathways compared to their commensal counterparts. For example, pathogenic strains of *E. coli* can consume a unique set of intestinal sugars as compared to commensal strains of *E. coli* [15]. Alternatively, pathogen-induced inflammation can disrupt the microbiome, releasing unique metabolites to give pathogens a growth advantage. For example, *Salmonella*-induced intestinal inflammation leads to the generation of tetrathionate by host epithelial cells. Tetrathionate respiration by *Salmonella* confers a growth advantage over other intestinal bacteria that rely on fermentation [16]. In the inflamed intestine, ethanolamine utilization by both *Salmonella* [17] and pathogenic *E. coli* [18] can also enhance their colonization [19]. Inflammation-induced **dysbiosis** can also lead to the outgrowth of specific non-pathogenic species. For example, nitrate released in the inflamed intestine is used in anaerobic respiration by commensal *E. coli* strains [20]. Whether proliferation of nitrate-utilizing *E. coli* species contributes to other pathogenesis mechanisms, however, is unclear.

Antibiotic-induced dysbiosis can also alter nutrient availability to allow the outgrowth of pathogens and pathobionts. For example, antibiotic treatment can alter microbial composition to promote the proliferation of vancomycin-resistant *Enterococci* [21] as well as *C. difficile* [22, 23]. In the case of *C. difficile* infection, antibiotic treatment results in increases in both succinate [23] and sialic acid [22], which are utilized by *C. difficile* to enhance intestinal colonization.

Direct bacterial warfare

Beyond metabolic competition, intestinal bacteria can limit the growth of other bacterial species through the production of antibacterial compounds and inhibitory metabolites as well as through contact-dependent killing. Recent studies have highlighted how microbiota-produced antimicrobials can inhibit intestinal colonization by specific enteric pathogens and pathobionts. For example, bacteriocins are diverse secreted antibacterial peptides that target and lyse related bacterial species [24, 25]. In the intestine, colonization by bacteriocin-producing *Enterococcus faecalis* reduces the numbers of indigenous *E. faecalis*, as well as infection by vancomycin-resistant *E. faecalis* [26]. Microcins produced under iron starvation conditions by probiotic *E. coli* Nissle 1917 reduce intestinal colonization by other commensal *E. coli*, as well as *Salmonella* [25]. The large scale bioinformatic and biochemical mining of the microbiome for other antibacterial molecules has highlighted the potential of microbiota-based antimicrobial compounds as therapeutic leads for new antibiotics [27]. Beyond specific antimicrobial compounds, other bacterial metabolites may

also directly inhibit pathogen growth. For example, *Clostridium scindens* inhibits *C. difficile* growth and pathogenesis in mice [28]. This *in vivo* protection is correlated with the capacity to generate secondary bile acids, which can inhibit *C. difficile* growth *in vitro*.

In addition to releasing antibacterial compounds into the extracellular space, many Gram-negative bacterial species can directly antagonize neighboring cells by injection of effector proteins using the type-VI secretion system (T6SS). T6SSs are encoded by commensals and pathogens alike, and T6SS-mediated competition is likely prevalent in the intestine. For example, T6SSs are widely encoded amongst the Bacteroidales [29], an order that includes abundant intestinal commensals, and T6SS-encoding *Bacteroides* can target sensitive *Bacteroides* species, presumably to limit competition [30–32]. Intestinal pathogens such as *S. Typhimurium* [33] and *Vibrio cholerae* also encode T6SSs, which may target intestinal bacteria and enhance pathogen colonization. *Ex vivo*, *V. cholerae* uses its T6SS to deliver antimicrobial effectors to intestinal bacteria, such as *E. coli* and *Salmonella* [34], and self-immunity to this secretion system is required for robust intestinal colonization, suggesting that *V. cholera* employs its T6SS *in vivo* [35].

Interference with pathogen virulence

In addition to restricting pathogen colonization and proliferation, intestinal bacteria can also directly affect pathogen virulence mechanisms. Virulence gene expression involves integrating a diversity of environmental cues, some of which can be modified by intestinal bacteria. For example, in response to a local increase in oxygen close to the intestinal epithelial surface, *Shigella flexneri* increases the secretion of effector proteins involved in host cell invasion [36]. This raises the possibility that intestinal bacteria adhered to the mucosa could potentially inhibit *Shigella* invasion by consuming local oxygen.

Intestinal bacteria may also alter the pool of available host-derived nutrients to affect pathogen virulence. For example, fucosidases expressed by *Bacteroides thetaiotaomicron* can release fucose from mucins. Fucose is sensed by Enterohemorrhagic *E. coli* through the FusK/R two-component system, and high fucose leads to a decrease in the expression of virulence genes required for the formation of attaching and effacing lesions [37]. Although *B. thetaiotaomicron* has not been shown to directly affect EHEC virulence, *fusK* EHEC exhibit a growth defect *in vivo*, suggesting that the regulation of virulence through fucose sensing may be relevant to intestinal infection.

Bacterial fermentation in the intestine results in the production of diverse short-chain fatty acids. Short-chain fatty acids not only modulate host immunity but have also been shown to directly modulate virulence gene expression of various pathogens *ex vivo*. For example, short-chain fatty acids can differentially regulate the expression of *Salmonella* virulence genes involved in host cell invasion in a chain-length dependent manner. Specifically, acetate enhances the expression of genes involved in invasion [38], while propionate [39] and butyrate [40] are inhibitory. Other molecules prevalent in the intestinal environment, such as bile acids, have also been shown to modulate virulence gene expression of enteric pathogens *in vitro* [41–43], but the direct biochemical mechanisms of these metabolites on pathogen virulence is not clear.

Beyond metabolite regulation of virulence mechanisms, quorum sensing is a widespread form of bacterial “communication” that regulates diverse processes, including pathogen virulence. In quorum sensing, bacteria use secreted small molecules to regulate gene expression in a density-dependent manner. Bacteria can integrate intra-species and inter-species quorum sensing signals to optimize gene expression based on local environment. In the intestine, the extent of quorum sensing-mediated communication is unclear; but intestinal delivery of the inter-species quorum sensing molecule autoinducer-2 (AI-2) alters microbiota composition after antibiotic treatment, suggesting that intestinal bacteria can broadly respond to quorum sensing pathways *in vivo* [44]. Pathogen sensing of another inter-species quorum sensing molecule class, N-acyl homoserine lactones (AHLs), has been proposed as a possible mechanism by which intestinal bacteria could modulate virulence. Both EHEC and *Salmonella* encode the transcription factor SdiA, which is required for AHL recognition, but lack the machinery to synthesize AHLs [45, 46]. In the case of EHEC, AHLs repress the expression of virulence genes involved in the formation of AE lesions, but enhance the expression of acid-resistance genes [47]. Consistent with this, SdiA is required for EHEC colonization of the cow rumen, but has no effect on intestinal colonization. Curiously, AHLs are largely absent in the intestine [48, 49], making the relevance of AHL-mediated quorum sensing in the intestine unclear. Host epithelial cells may also engage bacterial quorum sensing pathways to affect intestinal bacteria, as epithelial cells have been shown to produce a partially functional AI-2 mimic *in vitro* [50].

Host modulation

In addition to inter-bacterial interactions that influence pathogen fitness and overall microbiota composition, intestinal bacteria can also directly affect host susceptibility to infection by regulating epithelial barrier function as well as innate and adaptive immunity (Figure 1B). Modulation of epithelial barrier function can limit pathogen access to relevant host cell types, while activation of other innate and adaptive immune mechanisms can result in direct killing of microbes or pathogen tolerance locally or systemically.

Recognition of microbial-associated molecular patterns (MAMPs) by both intestinal epithelial cells and lymphoid cells is required for proper immune system development and response to microbial pathogens [51]. MAMPs are conserved microbial factors, such as bacterial cell-surface components, that elicit a host immune response upon recognition by pattern recognition receptors (PRRs). Toll-like receptors, Nod-like receptors, and C-type lectin receptors all respond to MAMPs derived from commensal bacteria and pathogens alike, leading to a variety of downstream innate and adaptive immune responses. The basal immune responses elicited by these receptors in response to intestinal bacteria help establish and maintain immune system homeostasis [52]. Bacterial surface components such as lipopolysaccharide, lipoteichoic acid, peptidoglycan, capsular polysaccharides, flagellin, and other surface proteins can all be recognized by PRRs to modulate intestinal immunity [53].

In mammals, innate immune recognition of MAMPs is a balance between mechanisms of tolerance versus immune activation. Although the intestine is full of indigenous commensal bacteria, hyper-activation of PRRs may be prevented by controlling the localization [54] and abundance of receptors, as well as by specific negative regulators of downstream pathways.

Host immune responses may also be tailored to pathogenic versus commensal bacteria by recognition of species-specific molecules. Downstream responses to MAMP recognition include the modulation of epithelial barrier function, as well as the priming and regulation of other innate and adaptive immune mechanisms. In this section, we highlight recent mechanistic studies that reveal how probiotic or commensal bacteria enhance epithelial barrier function and host immunity to pathogens.

Activation of epithelial barrier function and other innate immune mechanisms

One important downstream response of MAMP recognition is the modulation of epithelial barrier function [55]. Secreted mucins, antimicrobial proteins, and secretory IgA (sIgA), all serve to exclude bacteria from a sterile zone maintained close to the surface of intestinal epithelial cells [56]. Bacterial transgression of the epithelial barrier can lead to pro-inflammatory responses that influence the pathogenesis of diverse chronic diseases including HIV/AIDS [57] and inflammatory bowel disease (IBD) [58]. Not only does the epithelial barrier repel bacteria, but it also reduces permeability to other metabolites that could aggravate systemic extra-intestinal inflammatory responses.

Recognition of intestinal bacterial MAMPs by both intestinal epithelial and innate immune cell lineages helps to maintain epithelial barrier function. For example, administration of lipopolysaccharide and peptidoglycan can restore the mucous barrier in germ-free mice, and protect against chemically-induced colitis [59]. Intestinal bacteria can enhance barrier function by inducing the production of RegIII γ , IgA, and other antimicrobial proteins [60–65]. Although several antimicrobial proteins can be produced in an epithelial cell-intrinsic manner, some, such as RegIII γ , require concomitant innate lymphoid cell-derived IL-22 signaling for full expression [66].

The bactericidal mechanisms of intestinally-localized antimicrobial proteins are diverse and include both non-enzymatic membrane disruption and enzymatic cell wall hydrolysis [67]. For example, alpha-defensins and cathelicidins are broad-spectrum antimicrobials that form membrane pores to kill bacteria, while RegIII C-type lectins bind peptidoglycan prior to pore formation and therefore preferentially target Gram-positive bacteria [68]. In contrast, lysozyme and secretory phospholipase A2 enzymatically hydrolyze peptidoglycan and phospholipids respectively to disrupt bacterial membranes.

Microbiota-mediated modulation of key metabolites and MAMPs may contribute to epithelial barrier function and pathogen restriction. Indeed, microbiota-associated metabolites such as taurine, histamine, and spermine have been shown to modulate NLRP6 inflammasome signaling, epithelial IL-18 secretion and the production of antimicrobial peptides. The activation of inflammasome signaling by microbial metabolites is important for preventing gut dysbiosis and intestinal inflammation [69]. The intestinal bacteria *Enterococcus faecium* has been shown to remodel MAMPs to enhance epithelial barrier function and pathogen restriction. *E. faecium* secretes an NlpC/p60-type peptidoglycan hydrolase, SagA, that is sufficient to protect *C. elegans* and mice from pathogens such as *S. Typhimurium* and *C. difficile* [70, 71]. Based on studies *in vitro* and in *C. elegans*, SagA was found to remodel peptidoglycan and generate muramyl-peptide fragments to improve host immunity against pathogens. In mice, SagA enhances host epithelial barrier function through

the induction of mucins and antimicrobial peptides, most strikingly *RegIIIγ*, and protection is dependent on pattern recognition genes *MyD88* and *Nod2*. Interestingly, *Lactobacillus rhamnosus*, another probiotic bacteria, secretes two peptidoglycan hydrolases, p75 and p40, that enhance epithelial cell proliferation [72–74]. Whether these and other secreted peptidoglycan hydrolases can also induce host responses by remodeling MAMPs has yet to be determined.

The regulation of tight junctions and epithelial cell turnover by intestinal bacteria and bacterial metabolites also serves to enhance epithelial barrier integrity. For example, indole produced by intestinal bacteria enhances the expression of tight junction proteins, and reduces chemically-induced colitis in mice [75, 76]. Indole may signal via the aryl hydrocarbon receptor (AHR) in intestinal epithelial cells to maintain epithelial barrier integrity [77], similar to dietary indolyl-metabolite-induced AHR signaling in intraepithelial lymphocytes [78] and innate lymphoid cells [79]. Dietary acetate as well as acetate produced by different species of *Bifidobacteria* has been shown to provide strong protection against EHEC pathogenesis [80]. Acetate is produced as a result of fructose catabolism, and protective *Bifidobacteria* species encode unique carbohydrate transporters, including a fructose transporter. Heterologous expression of this transporter in non-protective *Bifidobacteria* species confers a protective phenotype against EHEC that is correlated with an increase in acetate production. In mice and in epithelial cell culture, acetate prevents epithelial cell apoptosis as well as Shiga toxin translocation to inhibit EHEC pathogenesis [81, 82].

In addition to directly killing extracellular pathogens or limiting their invasion into host cells, intestinal bacteria can also modulate cell-intrinsic microbial clearance mechanisms. Notably autophagy, the intracellular recycling pathway, has emerged as an important part of intestinal barrier function and innate immunity [83]. Epithelial cell-intrinsic autophagy of invasive enteric pathogens, such as *Salmonella* and *E. faecalis*, is activated by PRR signaling and serves to limit pathogen spread to distal tissues [84]. The autophagy pathway in Paneth cells also plays a role in intestinal immune homeostasis, through the regulation of inflammation. Hypomorphisms in ATG16L1, a major component of the autophagosome, are associated with Crohn's disease, and decreased expression in mice results in Paneth cell abnormalities including defective granule exocytosis [85] and an increased propensity for inflammation [86]. Whether specific intestinal bacterial species or factors can modulate autophagy to restrict pathogens remains an intriguing possibility.

Tuning adaptive immunity

While the epithelial barrier controls host immunity by regulating pathogen proliferation and/or access to host tissues, the microbiome can also directly regulate adaptive immune responses, which can facilitate antigen-specific clearance of pathogens and/or alter systemic inflammatory responses. In this section, we highlight studies where specific intestinal bacterial species and/or factors directly modulate adaptive immune mechanisms.

Antibodies provide important antigen-specific protection against potential pathogens. In the gut, the induction of secreted IgA antibodies by intestinal bacteria may facilitate the clearance of pathobionts and enteric pathogens. For example, recent studies using IgA-SEQ

have shown that high levels of IgA binding identify pathobionts that can drive intestinal disease [87]. Interestingly, SCFAs produced by the gut microbiota have also been recently suggested to metabolically enhance B cell responses and facilitate homeostatic and pathogen-specific antibody responses [88]. These studies highlight the importance of the intestinal microbiome in stimulating B cell and antibody responses.

T_H17 cells and T_{Reg} cells are important T cell subsets that mediate pro-inflammatory and anti-inflammatory responses in the intestine respectively. Specific intestinal bacteria can skew the ratio of these two cell types, which influences immune system maturation and can differentially affect host response to bacterial pathogens, as well as systemic inflammatory diseases. For example, adhesion of segmented filamentous bacteria (SFB) to intestinal epithelial cells induces the differentiation of T_H17 cells, which leads to the production of pro-inflammatory cytokines, antimicrobial peptides, and secretion of IgA that cumulatively inhibit *C. rodentium* infection [89]. SFB appears to induce antigen-specific T_H17 cell responses to mediate these effects [90, 91]. Adhesion may also be the molecular cue sensed by host epithelial cells, as other adherent microbes, including EHEC, *C. rodentium*, and *Candida albicans*, can induce similar Th17 cell responses [92]. Alternatively, ATP secreted by intestinal bacteria is recognized by purinergic receptors on intestinal epithelial cells and immune cells, which also drives the differentiation of T_H17 cells [93]. Intestinal bacteria can also induce the proliferation of anti-inflammatory T_{Reg} cells. For example, *Bacteroides fragilis* capsular polysaccharide A (PSA) stimulation of dendritic cells promotes the development of anti-inflammatory T_{Reg} cells, which protect against both chemically- and bacterially-induced colitis [94–98]. Beyond the intestine, SFB and *B. fragilis*/PSA have opposing effects on inflammation and host outcomes in an experimental autoimmune encephalomyelitis mouse model [99]. Interestingly, although the epithelial barrier restricts most intestinal bacteria to the lumen, certain commensal species naturally reside in innate lymphoid tissues and colonize dendritic cells to elicit anti-inflammatory IL-10 cytokine production [100]. Bacteria-derived short-chain fatty acids have been shown to exert anti-inflammatory effects particularly by promoting the differentiation of T_{Reg} cells. G-protein coupled receptors on the surface of epithelial cells and other immune cells can bind to acetate, propionate, and/or butyrate [101] leading to the induction of anti-inflammatory cytokines and T_{Reg} cells [102, 103]. Inhibition of histone deacetylases by butyrate results in an increase in histone acetylation, which can also lead to an anti-inflammatory response in T_{Reg} cells [104, 105] as well as macrophages [106].

In addition to modulating B and T cell functions, intestinal bacteria may directly regulate antigen presentation to tune adaptive immune responses. Of course, peptide and protein antigens from intestinal bacteria can be processed and presented by classical MHC class I and II proteins to generate bacteria-specific T cell responses that are important for tolerance or immune activation [107, 108]. However, bacteria-specific metabolites can also directly alter antigen presentation pathways. For example, *B. fragilis* produces the glycosphingolipid α -galactosylceramide [109], which can be presented by CD1d proteins and inhibit the development of colonic invariant natural killer T (iNKT) cells to reduce intestinal inflammation in mice [110]. Mucosal-associated invariant T (MAIT) cells, another type of innate T cell, are activated by MHC-I related (MR1) presentation of riboflavin (vitamin B2) metabolites [111], which are uniquely bacterial in origin and thus potentially generate an

adaptive immune response to enteric pathogens [112]. More recently, the discovery of microbiota-derived lysosomal protease inhibitors (pyrazinones and dihydropyrazinones) [113] suggests that intestinal bacteria can affect antigen presentation by interfering with proteases that degrade proteins into antigenic peptides. These studies highlight how intestinal bacteria may directly affect adaptive immune responses through modulating B cells, T cells, and antigen-presenting cells, which will likely expand to other lymphocytes (i.e. innate lymphoid cells and others).

Concluding Remarks

Recent studies have elucidated diverse biochemical mechanisms employed by the intestinal microbiome to influence host susceptibility to infection. Within the intestine, commensal bacteria can directly antagonize enteric pathogens and pathobionts through metabolic competition, the production of antibacterial compounds, and interference with virulence gene expression. Immune sensing of the microbiome is required for proper immune development, and provides a mechanism for specific intestinal bacteria to shape immune function. MAMPs as well as specific antigens from intestinal bacteria are recognized by intestinal epithelial cells and gut-associated lymphoid tissues to regulate immune system maturation. Regulation of the downstream inflammatory response and epithelial barrier function are two general strategies by which intestinal bacteria can influence host physiology both within and beyond the intestine. With marked progress in the field, questions regarding the challenges, applications, and broader evolutionary and ecological insights of microbiome research have emerged (see Outstanding Questions box).

Reductionist models have helped winnow the bacterial factors involved in modulating microbiota composition and host physiology that might have been otherwise obscured. Nevertheless, the overall relevance and relative impact of many bacterial factors remains to be determined in the context of more complex natural systems. Integrating metagenomic and experimental findings in a systems biology approach may help clarify the dynamic roles of bacterial species that exhibit diverse functions in different model systems.

While germ-free and **gnotobiotic** mouse models continue to be foundational to microbiome research, the utility of non-mammalian model hosts for studying the intestinal microbiome has recently come into focus. Model organisms such as *C. elegans*, *D. melanogaster*, and *D. rerio* offer many experimental advantages and can provide a complementary understanding of the evolutionarily conserved or convergent factors that influence intestinal symbioses.

From a therapeutic perspective, modulation of the microbiome through clinical interventions such as probiotics, drugs, and diet can have far-reaching effects on host health. Beyond this, the microbiome is a rich reservoir of medically and societally important molecules. Recent genomic and chemical approaches to identify and functionally profile small molecules [114, 115] and regulatory enzymes [116] from the microbiome provide an opportunity to build upon our understanding of the vast molecular interactions occurring in the intestine.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Glossary

colonization resistance

any of the mechanisms (either direct or indirect) by which intestinal bacteria limit the colonization of harmful microorganisms

commensal bacteria

colloquially used to refer to any non-harmful symbiotic bacteria, although many species may be beneficial in certain contexts

dysbiosis

any microbial imbalance

gnotobiotic

a condition in which all microorganisms are defined.

intestinal microbiota

all the microorganisms that inhabit the intestine

intestinal microbiome

the full complement of genetic material, metabolites and other molecules that occupy the intestinal ecosystem

niche competition

competition that arises between two species that fill the same ecological role in a given environment

pathobiont

a potentially pathogenic organism that under normal circumstances exists as a commensal

pathogen

a microorganism that causes disease

virulence

the severity with which a pathogen or pathogenic substance causes disease

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Figure 1.

Table 1

Biochemical mechanisms by which intestinal bacterial species and factors affect host susceptibility to enteric pathogens.

Bacterial strain/species	Bacterial factor/gene	Proposed biochemical mechanism(s)	Activity <i>in vivo</i>	Ref(s)
<i>Inter-bacterial interactions (see Figure 1A)</i>				
Multiple species	Multiple types of bacteriocins	Bacteriocidal activity varies depending on type	<i>In vivo</i> bacteriocidal activity inferred from competition experiments in a variety of models.	[25, 26, 117, 118]
Multiple species	T6SS	Injection of antibacterial effector proteins into target cells	<i>In vivo</i> activity inferred from competition experiments.	[29–35]
<i>Clostridium scindens</i>	Bile acid metabolism	Generates secondary bile acids, which inhibit <i>C. difficile</i> growth	<i>C. scindens</i> inhibits <i>C. difficile</i> pathogenesis in mice.	[28]
<i>Microbiota-mediated host modulation (see Figure 1B)</i>				
Multiple species	taurine, histamine, and spermine	Modulation of NLRP6 inflammasome signaling with resultant increases (for taurine) or decreases (for histamine and spermine) in epithelial IL-18 secretion and antimicrobial peptide production	Taurine treatment alleviates DSS-induced colitis, while histamine exacerbates DSS-colitis in mice.	[69].
<i>Enterococcus faecium</i>	SagA	SagA-generates unique peptidoglycan fragments <i>in vitro</i> and in bacteria	Enhances epithelial barrier function. SagA inhibits <i>Salmonella</i> and <i>C. difficile</i> pathogenesis in mice.	[70, 71]
Multiple species	indole	Ligand for the aryl hydrocarbon receptor.	Enhances epithelial barrier function. Dietary indole protects mice from chemically-induced colitis	[75–79]
<i>Bifidobacterium</i> /Multiple species	Acetate production	Likely through mechanisms described below for SCFAs	Reduces epithelial cell apoptosis and inflammatory responses with correlated inhibitory effects on EHEC pathogenesis.	[80–82].
SFB	SFB-specific antigen and adhesion	Adhesion to epithelial cells and induction of SFB-antigen specific TCR	Induces T _{H17} cell differentiation and pro-inflammatory responses with associated consequences in a variety of models.	[89–92, 119–121]
Multiple species	ATP	Ligand for purinergic receptors in many cell types.	Induces T _{H17} cell differentiation and pro-inflammatory responses with associated consequences in a variety of models.	[93, 122]
<i>Bacteroides fragilis</i>	PSA	PSA is proposed bind and activate TLR-2	Induces T _{reg} cell differentiation and anti-inflammatory responses with associated consequences in a variety of models.	[94–98]
Multiple species	SCFAs; eg. propionate and butyrate	Ligands for GPR43 and/or inhibitors of histone deacetylase.	Enhances epithelial integrity/intestinal homeostasis and anti-inflammatory	[88, 101–105, 123, 124]

Bacterial strain/species	Bacterial factor/gene	Proposed biochemical mechanism(s)	Activity <i>in vivo</i>	Ref(s)
			responses with associated consequences in a variety of models.	
Bacteroides spp.	α -galactosyl ceramide	inhibition of iNKT development.	Reduces inflammation in an oxalozone colitis model.	[109, 110]
Multiple species	riboflavin metabolites	Activation of MATT cells by MIR1 presentation	Unknown effects on enteric pathogens <i>in vivo</i>	[111] [112]
Multiple species	pyrazinones and dihydropyrazinones	lysosomal protease inhibitors that target host cathepsins	Unknown effects on enteric pathogens <i>in vivo</i>	[113]