Published in final edited form as:

Curr Opin Immunol. 2014 August ; 0: 16–22. doi:10.1016/j.coi.2014.03.003.

Commensal bacteria mediated defenses against pathogens

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Abstract

Commensal bacterial communities residing within the intestinal lumen of mammals have evolved to flourish in this microenvironment. To preserve this niche, commensal bacteria act with the host to prevent colonization by invasive pathogens that induce inflammation and disrupt the intestinal niche commensal bacteria rely upon. Thus, it is mutually beneficial to the host and commensal bacteria to inhibit a pathogen's ability to establish an infection. Commensal bacteria express factors that support colonization, maximize nutrient uptake, and produce metabolites that confer a survival advantage over pathogens. Further, commensal bacteria stimulate the host's immune defenses and drive tonic expression of anti-microbial factors. In combination, these mechanisms preserve the niche for commensal bacteria and assist the host in preventing infection.

Keywords

commensal bacteria; intestinal microbiota; bacteria; infection; pathogens; intestinal immune response; colonization resistance

Introduction

The mammalian gastrointestinal tract harbors one of the densest microbial ecosystems found in nature, peaking in the proximal colon at 10^{11} – 10^{12} cells/mL [1]. This microbial community, which is composed of all domains of life, exists in a mutually beneficial relationship with the host. The microbes thrive in relatively stable environmental conditions (pH, temperature, low oxygen tension) while the host exploits enzymes produced by the microbes to break down complex carbohydrates into absorbable small units, thereby increasing the efficiency of energy uptake [2]. Recent studies have started to decipher the

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complex host-microbe relationship, and these commensal bacterial communities have been linked to a wide range of human diseases including obesity, diabetes, cancer, atherosclerosis, atopic disorders, and inflammatory bowel disease [3-5]. The focus of this review is on the role of commensal bacteria in defense against infectious diseases.

The diverse microbiota within the intestine reduces the ability of invasive pathogens to establish an infection, cause damage to host cells and drive disease. Loss of microbiota diversity following antibiotic administration is postulated to increase susceptibility to intestinal pathogens such as *Salmonella typhimurium* and *Clostridium difficile* [6-8]. These experimental studies support clinical experience linking antibiotic use with increased frequencies of opportunistic intestinal infections [9-11]. Reconstituting commensal bacterial communities of the gastrointestinal tract can facilitate the clearance of chronic *C. difficile* infection. A recent study demonstrated in a controlled clinical trial that fecal microbiota transplantation is highly effective at clearing recurrent *C. difficile* infection [12] and restoring the biodiversity of the microbiota [13]. Several studies using mouse models of enteric bacterial infection have demonstrated that transferring commensal bacterial populations can effectively displace antibiotic-resistant pathogens from the intestine [14-18]. This approach of transferring defined commensal bacterial populations into a host to re-establish a diverse microbiota offers an antibiotic-independent approach to combat infection. However, the therapeutic potential of repopulating patients with commensal bacterial communities is currently limited by incomplete characterization of commensal bacterial species and products that antagonize pathogens. The recently described mechanisms of commensal bacteria-driven protection against intestinal pathogens and the countermeasures pathogens employ to establish infection will be discussed below.

Commensal bacteria outcompete pathogens for nutrients

Evolutionary pressures have selected for bacterial communities best adapted at acquiring available nutrients and resources in the intestine. For example, the human commensal bacteria *Bacteroides fragilis* expresses a gene cluster, termed commensal colonization factors, that is essential for penetration of colonic mucus and colonization of intestinal crypts [19]. Other commensal species, such as *Bacteroides thetaiotaomicron*, carry genes that express multiple non-redundant corrinoid transporters, each conferring a survival advantage by enabling uptake of distinct corrinoids (vitamin B₁₂ analogs) that are finitely available in the intestine [20]. Competition amongst the commensal bacterial species deprives potential invasive pathogens of the essential nutrients needed to replicate (Figure 1). This phenomenon, referred to as colonization resistance, is most clearly revealed by germ-free mice, which exhibit increased susceptibility to a wide range of bacterial infections, including *Escherichia coli*, *C. difficile*, *Vibrio cholerae* and *Citrobacter rodentium* [17,21-23]. The murine enteric pathogen *C. rodentium* colonizes the intestinal lumen of germ-free mice, but in the presence of non-pathogenic *E. coli*, *C. rodentium* is outcompeted for available carbohydrates and is eliminated from the intestinal lumen [23]. Only following upregulation of virulence factors that enable attachment to intestinal epithelial cells can *C. rodentium* create its own environmental niche and establish an infection [23]. Perturbation of the microbiota disrupts the intestinal ecosystem and enables pathogens to access resources that would otherwise be consumed by commensal bacteria.

For example, antibiotic-mediated disruption of the microbiota results in elevated levels of free sialic acids, which two distinct intestinal pathogens, *S. typhimurium* and *C. difficile*, catabolize and use as an energy source to expand [24]. Understanding the minimally required biodiversity and bacterial-driven biochemical reactions needed to establish colonization resistance will continue to be an area of intense study as the field defines what comprises a healthy, intact microbiota.

Commensal bacterial-derived products directly inhibit pathogens

Commensal bacteria have evolved defense mechanisms that directly inhibit the ability of pathogens to survive. Most prominent among these mechanisms are a class of peptides called bacteriocins, which are anti-microbial factors produced by bacteria that target a narrow spectrum of competing bacteria. [25]. Bacteriocins, such as Thuricin CD derived from the *Bacillus thuringiensis* DPC 6431, hold the potential to target intestinal bacterial pathogens while minimally impacting the commensal microbial community [26,27].

Metabolic byproducts from commensal bacteria can also deter pathogen growth. Short chain fatty acids (SCFA) produced by commensal bacterial fermentation of ingested complex carbohydrates can inhibit the growth of enteropathogenic bacteria [28]. Commensal species that are high SCFA producers, such as *Bifidobacteria* spp., can mitigate the severity of *S. typhimurium*, enterohaemorrhagic *E. coli* or *C. rodentium* infection ([6,29,30]). *Bifidobacteria*-mediated protection is, in part, attributable to a gene encoding an ATP-binding cassette-type carbohydrate transporter that leads to increased production of acetate, a SCFA, and reduced gut permeability and bacterial translocation [31].

Commensal bacteria harbor a diverse assortment of bacteriophages, viruses that infect bacteria, which, in part, constitute the virobiota of the gut. [32]. The microvirome, the genes derived from the virobiota, encodes a broad range of functions and can influence the relative representation of different bacterial species within the intestine. One such example has been described with a bacteriophage isolated from the commensal species *E. faecalis*, which confers a competitive growth advantage over bacteria not harboring this bacteriophage [33]. The mechanisms employed by bacteriophages to support host bacteria remain largely undefined, yet it is clear from these initial studies that bacteriophages can help shape the bacterial communities in the intestine and in turn the ability of pathogens to establish infection.

Bacteria have also evolved defense mechanisms that involve direct attack against heterologous bacterial species. The bacterial type VI secretion system utilizes a multicomponent organelle to pierce the cell wall of neighboring cells and transport effector proteins into the targeted eukaryotic or prokaryotic cell [34] *Pseudomonas aeruginosa* directly inhibits *V. cholerae* or *Acinetobacter baylyi* growth using its Type VI secretion system, leaving sister cells or non-invasive adjacent bacteria unharmed [35]. *V. cholerae* can counter *P. aeruginosa* by expressing a gene that confers immunity against Type VI effector proteins [36]. Such mechanisms of direct bacterial interaction may contribute to the establishment of stable commensal populations in the competitive microenvironment of the intestine.

Commensal bacterial-derived signals indirectly inhibit pathogens

Commensal bacteria can also support pathogen clearance by activating host defense mechanisms that maintain the physical separation between the microbial world of the intestinal lumen and the host (Figure 1). To accomplish this goal, a complex network of immune and non-immune cells act in concert following detection of microbial molecules by innate immune receptors to upregulate host defenses that limit bacterial dissemination, repair the intestinal epithelial barrier, and maintain intestinal homeostasis [37]. Tonic stimulation of the host's defenses by commensal bacteria benefits the host and resident bacteria by inhibiting expansion of invasive pathogens and preserving the environmental niche for non-invasive commensal species.

Anti-microbial peptides, such as calprotectin, defensins and the RegIII family of proteins are produced by epithelial cells and have direct bactericidal properties [38]. Expression of anti-microbial peptides by intestinal epithelial cells is driven by commensal bacteria [39], and is critical in limiting pathogen expansion [40]. The induction of these antimicrobial peptides by commensal bacteria is best understood with RegIII γ and is a multi-step process that involves several cell types. A subset of intestinal dendritic cells (CD103⁺, CD11b⁺) can detect bacterial-derived signals, leading to secretion of IL-23 and production of IL-22 by neighboring immune cells [41]. IL-22 signals through intestinal epithelial cells and upregulates expression of host defense genes, including RegIII proteins [42]. RegIII proteins bind to peptidoglycans of the bacterial cell wall and form a hexameric pore that permeabilizes the bacterial membrane and kills the bacteria [43]. Resident Lactobacilli spp. can convert tryptophan available in the intestinal lumen into the aryl hydrocarbon receptor (AhR) ligand, indole-3 aldehyde (IAId). IAId stimulates innate lymphoid cells to produce IL-22 in an AhR-dependent manner [44]. This commensal bacteria-driven IL-22/anti-microbial peptide axis is critical in the defense against bacterial pathogens such as *C. rodentium* and vancomycin-resistant *Enterococcus* as well as fungi such as *Candida albicans* [40,44-46]. Genetic ablation of IL-22 or AhR alters the intestinal microbiota and predisposes the host to inflammatory colitis, demonstrating the importance of the commensal bacteria-driven IL-22 axis in promoting intestinal homeostasis [47,48]. Microbiota-mediated stimulation of host pathways and innate immune cells can activate host defenses prior to pathogen encounter, pre-emptively protecting against infection.

Commensal bacteria shape intestinal immune cell function

Commensal bacteria influence the activation and differentiation status of immune cells. This was first described with the commensal species segmented filamentous bacteria (SFB), which enhances CD4⁺ T cell differentiation into T helper 17 (T_H17) cells in the small intestine [49]. Additional microbiota-derived signals induce IL-1 β production in the intestine and further drive CD4⁺ T_H17 cell differentiation as well as support IL-1R⁺ innate lymphoid cells [50,51]. The presence of these cell types in the intestine is important in host defenses against the enteric bacterial infections *C. rodentium* and *S. typhimurium* [45,46,51]. Following pathogen induced epithelial damage, n-formyl peptides from commensal bacteria such as γ -proteobacteria drive neutrophils and inflammatory monocytes into the lumen of the intestine. Here, these immune cells produce reactive oxygen species to aid in the

containment of both pathogen and commensal bacteria while the epithelial layer is repaired [52]. Upon resolution of the infection commensal bacterial-derived signals help restore intestinal homeostasis by eliciting production of prostaglandin E2 from inflammatory monocytes, limiting neutrophil-induced pathology and the opportunity pathogens have to utilize inflammation-induced dysbiosis to expand [53]. Commensal bacteria also shape immune-mediated resistance to *Leishmania major* and influenza virus infection in the skin and lungs, respectively [54-56]. These studies demonstrate that microbiota-induced signals can act at distinct anatomic sites in addition to the intestine to support host defense against infection.

Recent studies indicate that microbiota-induced signals can play a role in driving epigenetic modifications of pathogen response genes. Loss of tonic microbiota-driven stimulation results in increased methylation at the promoter region of immune defense genes, reduced binding of key transcription factors and decreased basal expression of molecules important for defense against viral pathogens [56,57]. Thus microbiota-driven signaling maintains immune response genes in an open, transcriptionally active state that enables a rapid and robust response upon encountering a pathogen. Loss of epigenetic modifiers in intestinal epithelial cells leads to disruption of a healthy and diverse microbiota and diminished expression of immune defense genes [58]. Epigenetic modification of genes involved in host defense by the microbiota facilitates rapid host responses to pathogens.

Commensal bacteria regulate inflammation to limit pathogen expansion

Microbiota-driven activation of host defenses must be carefully regulated to prevent rampant inflammation that would alter the intestinal microenvironment and harm both commensal bacteria and their host. Therefore, many commensal bacteria elicit immunoregulatory activities within the host that also limit pathogen expansion. For example, commensal *Bifidobacteria breve* express an exopolysaccharide that elicits an immunoregulatory response, enabling their colonization of the intestine and impeding colonization of *C. rodentium* [29]. A class of commensal Clostridia species promotes expansion of colonic regulatory T (T_{reg}) cells, which limit inflammation and maintain intestinal homeostasis, thereby preserving colonization resistance [59]. The anti-inflammatory properties of microbiota-derived SCFA are, in part, attributed to augmentation of the colonic T_{reg} cell population [60-62]. SCFA inhibit histone deacetylase expression and increase histone acetylation in the promoter region of *foxp3* locus, skewing differentiation of CD4⁺ T cells toward a regulatory T cell lineage. [60-62]. SCFA derived from commensal bacteria enhance T_{reg} cell suppressive activities that help maintain a diverse commensal microbial community.

Mechanisms utilized by pathogens to outcompete the commensal bacteria

Pathogens can use microbiota or host-derived products to gain a competitive advantage in the intestine. Host-driven inflammation alters the intestinal microenvironment and creates conditions favorable for select bacterial species, and the resulting dysbiosis can be exploited by opportunistic pathogens [63][64]. Inflammatory by-products produced by the host, including reactive oxygen species, nitrates, elastases, and ethanolamines all can enhance the growth of pathogenic bacteria. [63,65-67]. Nutrients released by commensal bacteria can

also trigger pathogen expansion. Enterohemorrhagic *E. coli* upregulates virulence genes that aid in establishing an infection following detection of fucose, which is released after enzymatic break down of mucins by *Bacteroides thetaiotaomicron* [68]. *Samonella* can use hydrogen, a byproduct of commensal bacteria metabolizing carbohydrates into SCFA, by expressing a hydrogenase enzyme. Hydrogen is used by *Samonella* as an electron source during respiration and enables the pathogen to colonize the intestine [69-71]. Different classes of intestinal pathogens, such as enteric viruses, utilize microbiota-derived signals to establish infection of the host [72,73]. Enteric viruses can establish infections by attaching to commensal bacteria surface polysaccharides, which increases the virion's stability and promotes viral transmission [74].

6. Concluding Remarks

Commensal bacteria of the intestine provide an important but often imperfect line of defense against infection. Marked differences in microbiota composition between healthy individuals, as demonstrated by the Human Microbiome Project [75], suggest that variation in the representation of distinct commensal bacterial taxa may, at least in part, explain variation in susceptibility to a wide range of intestinal pathogens. As the identities of protective commensal bacterial species are revealed and their mechanisms of protection defined, it is becoming increasingly realistic to speculate that the human microbiota can be modified to optimize resistance to pathogens while reducing the risk of deleterious inflammatory diseases. Progress in this area will depend on the meticulous characterization of the heretofore mysterious commensal intestinal microbes and extensive study of their impact on the innate and adaptive components of the mucosal immune system.

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Abbreviations used

SCFA	short chain fatty acids
SFB	Segmented Filamentous Bacteria
VRE	vancomycin resistant enterococcus
ILC	innate lymphoid cells
AhR	aryl hydrocarbon receptor

Highlights

- Commensal bacteria directly inhibit invasive pathogen colonization.
- The microbiota indirectly inhibit pathogens by stimulating host defense mechanisms.
- Pathogens exploit host and commensal bacteria-derived products to grow.
- Manipulating the microbiota is an effective therapeutic option against infection.

Figure 1.

Commensal bacteria-mediated mechanisms of protection against intestinal pathogens. (1) Commensal bacterial communities consume nutrients and energy sources, depriving pathogens of their niche. (2) Microbiota-derived metabolic byproducts directly inhibit pathogens. (3) Commensal bacteria stimulate host immune cells and drive basal expression of host defense factors.