Host microbiota influences interactions between hosts and pathogens

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Abstract

Bacterial microbiota have significant effects on host interactions with pathogens in both vertebrate and invertebrate organisms. Here we discuss the direct and indirect impacts of microbiota on defense against pathogens. We found that microbiota have direct effects on host defense against pathogens through interference and niche competition, and by influencing host immune system development and function. The host microbiota also impacts host-pathogen interactions beyond immunity, by influencing physical barriers and physiological responses. In addition, it can influence the establishment of tumorigenic microbes thereby increasing cancer risk. Thus, the relationship between the host and its microbiota has short- and long-term impacts on overall health. Research that aims to identify and characterize the mechanisms that underlie these direct and indirect effects on host health will inform future medical treatments.

Introduction

Communities of microorganisms in defined environments are collectively known as “microbiota” (Marchesi and Ravel 2015; Schwiertz and Rusch 2016). The communities are found ubiquitously in non-host and host environments, and their composition varies according to their surrounding environments (Fraune and Bosch 2007; Chen *et al.* 2018; González-Serrano et al. 2020). Interactions between the microbiota and their host has many consequences, including direct and indirect impacts on host health (Wang *et al*. 2017). Growing evidence suggests that the microbiota plays a significant role in shaping the extent and specificity of the host response to pathogen colonization through microbe-to microbe interactions (Chiu *et al*. 2017), various physiological responses (McDermott and Huffnagle 2014), and promoting immune system development and function (Agüero *et al*. 2016; Pickard *et al*. 2017). Additionally, the microbiota can influence cancer establishment through its effect on tumorigenic pathogens (Rathje et al. 2020).

The importance of the bacterial microbiota has been observed and confirmed through the use of “germ-free” or antibiotic-treated hosts, in combination with culture-dependent and culture-independent techniques (Weinstock 2012). These treated hosts are more susceptible to infection, highlighting the importance of the microbiota for host health (Croswell *et al*. 2009; Jandhyala *et al*. 2015; Kennedy *et al*. 2018; Thackray *et al*. 2018). Understanding the microbiota’s function can provide important information on disease progression and can inform treatment options (Konturek *et al*. 2015; Magnusson *et al*. 2017; Khan *et al*. 2019; Man *et al*. 2019). As part of a graduate-level course focusing on host-pathogen interactions, the motivation for this review is to provide a general introductory understanding of direct and indirect impacts of bacterial microbiota on host interactions with pathogens. We first discuss direct impacts of the bacterial microbiota on pathogens within a host, and then give examples of indirect effects of resident bacteria on the host response to infection. In doing so, we hope to provide an educational resource for undergraduate and graduate students interested in the microbiota’s impact on host-pathogen interactions.

Direct impacts of microbiota on defense against pathogens

This section focuses on the direct mechanisms that the host microbiota use to protect against colonization by exogenous pathogens. These mechanisms may be either active or passive competition. During active competition, bacterial cells damage one another through the production of inhibitory molecules whereas during passive competition, one strain harms another one through resource consumption (Ghoul and Mitri 2016).

Active competition

During active competition, the host microbiota employ a wide range of mechanisms to harm, inhibit and kill off their competitors (Figure 1A). Due to the substantial literature on bacterial weapons (thoroughly reviewed elsewhere (Granato *et al*. 2019)), we are giving a brief overview of mechanisms used by the microbiota that directly affects the pathogen's fitness through the production of inhibitory molecules (Dykes and Hastings 1998; Stubbendieck and Straight 2016; Pickard *et al*. 2017). Inhibitory molecules such as toxins are synthesized compounds that are broadly classified as contact-dependent and contact-independent toxins, according to their delivery mode (García-Bayona and Comstock 2018).

Contact-dependent toxins are delivered directly to the target cell via cell-to-cell contact through a variety of syringe-like protrusions called secretion systems (Chen *et al*. 2019; Coyne and Comstock 2019) (Figure 1A.1). One of the widely studied systems is the Type VI secretion system (T6SS), which is mainly distributed in Proteobacteria and Bacteroidetes (Russell *et al*. 2014; Morton *et al*. 2015; Coyne and Comstock 2019). The presence of a T6SS and its effectors has a major role in the competition between commensal (non-toxigenic) and pathogenic (enterotoxigenic) strains of *Bacteroides fragilis* in the mouse gut (Hecht *et al*. 2016).

Contact-independent toxins are released into the extracellular environment as diffusible molecules (García-Bayona and Comstock 2018) (Figure 1A.2). These toxins range from broad-spectrum peptides and antibiotics (reviewed in (Netzker *et al*. 2018)) to more strain-specific bacteriocins. Bacteriocins are ribosomally-produced proteinaceous toxins that share a similar basic mechanism of action: they diffuse until they reach a target bacterial cell, then bind to an outer-membrane receptor and exploit envelope components for penetration (Chassaing and Cascales 2018). Upon entry, numerous cytotoxic mechanisms are employed: *Enterococcus mundtii* in the gut of the cotton leafworm, *Spodoptera littoralis* secretes mundticin KS, a bacteriocin which kills invading microbial pathogens such as *E. faecalis* and *E. casseliflavus* by

forming pores in the cytoplasmic membrane of the invader (Shao *et al*. 2017). *Bacillus subtilis* strain MMA7, isolated from the marine sponge *Haliclona simulans,* produces antimicrobials that inhibit several microbes, including pathogenic *Candida* species. This activity is associated with the secretion of subtilomycin, a bacteriocin that inhibits cell wall biosynthesis by binding to the precursor lipid II (Phelan *et al*. 2013). The peptidic microcin MccB17 secreted by enterobacteria, kills a wide range of pathogenic bacteria, including *Klebsiella* and *Pseudomonas,* by targeting intracellular enzymes required for DNA/RNA synthesis and structure maintenance (Håvarstein *et al*. 1994; Baquero *et al*. 2019).

Niche competition

In addition to employing direct chemical warfare against competitors, members of the microbiota may also outcompete potential bacterial pathogens within the host by creating a chemically disadvantageous environment or through resource competition (Figure 1B) (Bauer *et al*. 2018). Many members of the microbiota produce secondary bile acids, which create a disadvantageous environment for several pathogens (Ducarmon *et al*. 2019) (Figure 1B.1). Primary bile acids produced by the liver are a feature of the intestinal environment that help breakdown fat and cholesterol initiate defense mechanisms. Some commensal bacteria in the intestine convert primary bile acids into secondary bile acids, which antagonize the establishment and growth of the enteric pathogen *Clostridioides difficile* (Buffie *et al*. 2015; Ridlon *et al.* 2016; Theriot et al. 2016). *Clostridium scindens* is such a commensal bacteria that produces secondary bile acids that inhibit *C. difficile* (Studer *et al*. 2016). *In vivo* experiments have established that this antagonism of *C. difficile* depends on products encoded by the *baiCD* operon of some *C. scindens* strains (Kang *et al*. 2008; Ridlon *et al*. 2016).

Commensal and pathogenic bacteria rely on the host for nutrients and often have efficient systems to acquire these nutrients. Limiting the availability of iron is a common host defensive response to infection. The pathogen *Salmonella typhimurium* secretes a siderophore, salmochelin, a high-affinity iron chelator that scavenges iron and promotes its ability to compete with other gut bacteria (Cukrowska *et al*. 2002; Deriu *et al*. 2013) (Figure 1B.2). The commensal *E. coli* Nissle 1917 is an effective therapeutic for numerous gut-related disorders that prevents colonization of the gut by pathogenic bacteria (Cukrowska *et al*. 2002; Kruis *et al.* 2004). *E. coli* Nissle has several iron uptake systems, the disruption of which abolishes *E. coli* Nissle's ability to outcompete *S. typhimurium* infection and thereby preventing colonization of the host by this pathogen (Deriu *et al*. 2013).

The virulence of some pathogenic bacteria depends on the availability of amino acids such as proline (Gough 2010; Lee *et al*. 2014; Christgen and Becker 2019). Studies of gnotobiotic mice suggest that proline concentrations are associated with the establishment of the pathogen *E. coli* O157:H7 (Momose *et al*. 2008b). Commensal *E. coli* isolated from germ-free mice that had been inoculated with human infant faecal dilutions utilize proline at a higher rate than the pathogenic *E. coli* O157:H7, such that resource competition may contribute to the suppression of this pathogen (Momose *et al*. 2008a). Additionally, commensal bacteria such as *B. thetaiotaomicron* which can use both mono- and polysaccharides, have been observed to outcompete the pathogenic bacteria *Citrobacter rodentium* by efficiently using available carbohydrates thus preventing *C. rodentium* from colonization (Kamada *et al*. 2012).

The microbiota influences host immune system development and function

The microbiota also protects its host against pathogens via the immune system. The following section will summarize how the host microbiota shapes the host immune system and how it continues to contribute to immune system function.

The microbiota shapes immune system development and function

The maternal microbiota influences offspring immune responses at multiple levels. Disruption of the maternal gut microbiota during gestation and nursing has consequences on the diversity and composition of offspring's gut microbiota and their T cell and B cell populations (Nyangahu *et al*. 2018). Further, microbes are transmitted from mother to offspring either during birth, nursing, or by other contact. In addition, the infant microbiota is shaped by the acquisition of bacteria delivered with breast milk (Romero *et al*. 2014b,a; Avershina *et al*. 2017; Pannaraj *et al*. 2017). These microbes play a key role in the development of the offspring's immune system (Pannaraj *et al*. 2017; Nyangahu *et al*. 2018). The human vaginal microbiota contains more than 100 species of bacteria, of which *Lactobacillus* spp. are the most dominant taxa in healthy, pregnant women (Romero *et al*. 2014a; Walther-António *et al*. 2014; Avershina *et al*. 2017). Vaginas colonized predominantly by *Lactobacilli* are associated with increased CD45RO + memory and regulatory T lymphocytes, and reduced levels of IL-12 secretion in infant cord blood to protect the infant from pathogenic infection and preterm birth, respectively (Stencel-Gabriel *et al*. 2009). The bacteria from the vagina are thought to migrate to the placenta,

amniotic fluid, and the fetus, via translocation through the choriodecidual space (Goldenberg *et al*. 2008). The vaginal microbiota plays a vital role in influencing neonatal immunity, evidenced in the immunological differences observed between vaginal versus caesarean-delivered infants (Sindram-Trujillo *et al*. 2004; Huurre *et al*. 2008; Schlinzig *et al*. 2017). Mode of delivery can also influence infection susceptibility. Babies born via cesarean section, in comparison to those born vaginally, had a greater abundance of microbial taxa at the first week of life that corresponded to higher incidences of respiratory infections in the child's first year (Reyman *et al*. 2019) (Figure 2A).

Biomolecules, influenced by the mother's microbiota, transferred from mother to offspring also contribute to the infant's immune development. IgG antibodies transferred from the mother through the placenta or breast milk were necessary for induction of ILC3 in the offspring, helping develop the innate immune system (Agüero *et al*. 2016). Furthermore, the commensal microbiota of murine mothers induces production of IgG antibodies that target commensal Enterobacteriaceae species. When transferred to offspring, the pups are protected from infection by pathogenic *E. coli* (Zheng *et al*. 2020). The transfer of microbial products and metabolites from mother to offspring also shapes infant immunity during the early stages of development (Agüero *et al*. 2016).

The impact of the microbiota on host immune system development and function is also found in invertebrates. The bacterial endosymbiont *Wolbachia* naturally infects *Drosophila sp.* and protects the host from harmful RNA viruses (Teixeira *et al*. 2008). Colonization by *Wolbachia* is associated with upregulation of host immune genes (Xi *et al*. 2008). In tsetse flies, three symbiotic bacteria, *Wigglesworthia*, *Sodalis*, and *Wolbachia*, influence host physiology. All three symbionts are transferred to the developing offspring, either through maternal milk gland secretions (Attardo *et al*. 2008), or via the germline (Cheng *et al*. 2019). Tsetse flies that develop in the absence of *Wigglesworthia* are immunocompromised and become susceptible to infections by trypanosomes and *E. coli* K12 (Weiss *et al*. 2011, 2012) (Figure 2B).

The microbiota impacts immune memory

The microbiota can also influence immune memory by facilitating protection of the host during initial and subsequent pathogen invasion. This microbiota-mediated immune response that promotes immune memory, described here as "immune priming," occurs in both invertebrate and vertebrate taxa (Hernández-Martínez *et al*. 2010). In invertebrates, immune priming activates pathways that are induced only when a pathogen is encountered, which then enhances or prolongs the host's immune response to subsequent infections (Hernández-

Martínez *et al.* 2010). The microbiota of *Drosophila sp.* helps prime the immune system against enteric viral infection by activating the extracellular signal kinase (ERK) signaling pathway in the intestinal epithelium. Induction of ERK signaling provides antiviral immunity and requires the contribution of peptidoglycan from the commensal *Acetobacter pomorum* as well as virus recognition initiated signaling from the host (Sansone *et al*. 2015). In *Anopheles gambiae*, the commensal microbiota stimulates hemocyte differentiation upon *Plasmodium* infection(Rodrigues *et al*. 2010) reduces *Plasmodium* survival in subsequent infection cycles (Rodrigues *et al*. 2010). The *A. gambiae* microbiota also activates antimicrobial peptide expression and reactive oxygen species production, which limit *Plasmodium* infection (Dong *et al*. 2009; Rodrigues *et al*. 2010). In some cases, immune priming can induce immune responses that are transferred to the next generation. In insects such as the honeybee, the mother's immune system is able to recognize specific pathogens and subsequently prime the offspring immunity (Salmela *et al*. 2015). Priming can be achieved by the transfer of microbial products to the oocytes through the yolk protein vitellogenin (Salmela *et al*. 2015).

In vertebrates, immune priming improves the response to subsequent pathogen exposure. Gut microbial compounds activate innate immune cells in mammals, subsequently priming them for secondary pathogen encounters (Negi *et al*. 2019). Lipoproteins, flagellin, peptidoglycan, and β-glucan produced by commensal microbes are recognized by immune cells, which stimulate a variety of pathways that contribute to immune memory. This can include the secretion of certain cytokines by inflammasomes (Ifrim *et al*. 2014; Wu *et al*. 2014; Negi *et al*. 2019). The gut microbiota can also prime adaptive immune responses. Hand *et al*. (2012) demonstrated how the mammalian immune system will shift from tolerating commensal gut microbes to producing microbiota-specific T cells during an gastro-intestinal infection, which persist as memory cells that successfully protect the host upon reinfection. The mammalian commensal *B. fragilis* has also been shown to direct development of regulatory T cells, potentially improving the host's resistance to foreign antigens (Round and Mazmanian 2010).

Beyond immunity: additional impacts of the host microbiota on infection

The microbiota broadly influences host biology, and thus impacts pathogen colonization and infection beyond its effects on host immunity. This section discusses how host microbiota

enables or hinders pathogen infection by influencing physical barriers, and how interactions between the host microbiota and pathogens can contribute to diseases like cancer.

Physical barriers to entry are influences by the microbiota

The gut mucus layer provides protection for the host. The gut microbiota influences the state of the mucus layer and indirectly influences pathogen establishment (Figure 3A). These impacts depend on microbe-mediated alteration of mucus lipid levels (Carlson *et al*. 2018) and mucus structure (Sommer *et al*. 2014), as well as mucus production (Wrzosek *et al*. 2013; Martín et al. 2019), maturation (Hayes *et al*. 2018), and degradation (Desai *et al*. 2016). Depleted lipid levels in the mucus layer contribute to motility of pathogens in mucus. Commensals producing lipids reduce the ability of flagellated *E. coli* to move through mucus and thereby reduce *E. coli* persistence (Carlson *et al*. 2018). Certain phyla of pathogenic gut bacteria thrive in mucus layers that are thin or glycosylated, and alter the entire microbiota makeup of the gut (Sommer *et al*. 2014). Gut bacteria such as *Bacteroides thetaiotaomicron, Faecalibacterium prausnitzii*, and *Lactobacillus rhamnosus* restore gut mucosal layers through direct mucus production, and maintaining goblet cell differentiation responsible for producing mucin, preventing pathogen establishment (Wrzosek *et al*. 2013; Martín *et al*. 2019). Colonization of commensals in germ-free mice increases maturation or thickness of the mucus layer, strengthening the physical barrier against pathogens (Hayes *et al*. 2018). Gut bacteria that flourish in low fiber conditions degrade the mucus layer to obtain fiber and thereby allow pathogens such as *Citrobacter rodentium* to colonize (Desai *et al*. 2016). These examples illustrate how the microbiota impacts barriers to pathogen entry in mammalian systems, however, similar impacts occur in invertebrate systems.

The peritrophic matrix secreted by insects is analogous to the mucosal layer in the gut of mammals. This matrix is composed primarily of chitin and has several functions, including physical protection of the midgut epithelium from food particles and digestive enzymes, as well as protection from pathogens (Kato *et al*. 2008; Rodgers *et al*. 2017). The synthesis and function of the matrix is influenced by microbiota present within the midgut. The peritrophic matrix of blood-feeding insects, such as mosquitoes, plays an important role in limiting pathogen entry into the midgut epithelium (Cirimotich *et al*. 2011; Wang *et al*. 2012; Rodgers *et al*. 2017). For example, the susceptibility of the mosquito *Anopheles gambiae* to the human malaria parasite, *Plasmodium falciparum* is greatly influenced by the gut microbiota (Figure 3). Microbe-free mosquitoes are more susceptible to *Plasmodium* than mosquitoes with bacteria present in addition to *P. falciparum* (Dong *et al*. 2009).

The microbiota impacts physiological responses that affect pathogen susceptibility

The microbiota is an essential component of host physiology (Vuong *et al*. 2017). The composition of the microbiota, in turn, is altered by the host's physiological responses to external stimuli (Dickson *et al*. 2014). The microbiota influences core neurological and physiological processes, including neurogenesis, synaptic plasticity, neurotransmitter signaling and neuroinflammation (Vuong *et al.* 2017). Exposure of the host to stress can alter the composition of the microbiota. Stress influences the gut microbiota composition via several mechanisms. For example, catecholamines and other neuroendocrine hormones directly modulate microbial growth and are secreted by cells in the GI tract in response to stress (Vuong *et al*. 2017). In addition, stress-induced changes in signaling via the vagus nerve and enteric nervous system alter GI motility and reduce digestive activity which likely impacts the gut microbiota by interacting with physical forces within the GI tract.

Increases in intestinal epithelial permeability increases infection risk (Fukui 2016). Altered signaling along the vagus nerve and enteric nervous system can result in increased GI permeability and intestinal damage, and alters the resident microbiota (Karl *et al*. 2018). Clinical and experimental data suggest a relationship between intestinal hyper-permeability and the inflammatory changes that present in several diseases (Fukui 2016).

Impact of microbiota on cancer development caused by tumorigenic pathogens

The host microbiota can influence cancer establishment by influencing the establishment and behavior of tumorigenic pathogens. For example, changes in the vaginal microbiota are linked to HPV-derived cancer: women with greater variability in bacterial species within their vaginal microbiota (particularly those with greater proportions of non-*Lactobacillus* in their flora) have an increased risk of cancer and inflammation (Łaniewski *et al*. 2019; So *et al*. 2020). *Lactobacillus* produces lactic acid which lowers the pH of the vagina, thereby reducing the risk of dysbiosis. Vaginal microbiota with more Gammaproteobacteria (Kwasniewski *et al*. 2018) or *Prevotella, Gardnerella,* and *Atopobium* (So *et al*. 2020) are associated with greater vaginal inflammation and upregulated cancer biomarkers, suggesting a greater risk of lesions progressing to a cancerous state (Łaniewski *et al*. 2019).

External environmental factors can cause commensal bacteria to contribute to cancer development. *Helicobacter pylori* is a vertically transmitted bacterium found in the gut of more than half of the human population (Ohno and Satoh-Takayama 2020). Depending on diet, essential micronutrients, and other gastrointestinal bacteria, *H. pylori* can cause gastric cancer

and other gastric diseases (Amieva and Peek 2016; Yang *et al*. 2019). This is due to the inflammatory nature of *H. pylori* infections (Ferreira *et al*. 2018), and its ability to activate host inflammation-related genes (Aviles-Jimenez *et al*. 2014). Commensal bacteria can similarly lead to the development of cancer in early-branched metazoans. *Hydra oligactis* are more likely to develop tumors when their microbiomes contain *Turneriella sp.*, a bacteria not typically found within the *Hydra* microbiota. However, *Turneriella* sp. does not cause tumorigenesis alone; tumorigenesis caused by *Turneriella sp*. requires the common commensal *Pseudomonas sp*. (Rathje *et al*. 2020).

Conclusions and Outlook

The microbiota is integral to overall host health. Commensal bacteria protect the host against pathogens, through both direct and indirect effects on pathogens. Direct effects include pathogens being outcompeted by commensal bacteria, often via the production of antimicrobial molecules. Indirect effects include impacts on the host immune system. Microbiota and microbial metabolites transferred from mother to offspring are pivotal for the immune system development of the offspring, with consequences for their susceptibility to respiratory and gastrointestinal diseases. The microbiota also helps prime the host immune system; commensal microbes produce microbial products which are recognized by immune cells, and contribute to immune memory by providing immunity against pathogenic infection. Additional indirect effects include modifications of host physiology and physical barriers that increase disease risk. This review underscores the importance of the microbiota and how it influences host health. It is the interplay between host, its microbiota, and pathogens that determines the course of infection, colonization, and pathogenesis.

Most studies on the impact of the microbiota on host health study model organisms, with much of the work focusing on murine and select invertebrate hosts. Similarly, studies have largely focused on bacterial members of the microbiota, while other members such as viruses, fungi, and protozoa are less explored. Future studies should broaden the taxonomic scope of study systems to determine the extent of variation in host-microbiota interactions influencing disease risk due to effects on pathogens. While it is clear that interplay between the host, its microbiota, and pathogens influence infection outcomes, the mechanisms underlying these interactions are not well described in many systems. Investigating these mechanisms will be crucial for our understanding of how pathogens enter, establish, and persist within their hosts.

342 Insights from this research will help to develop treatment and management strategies for
343 human, livestock, and wildlife disease.

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Figure legends

Figure 1. The host's microbiota can directly inhibit colonization by pathogenic bacteria through active and passive competition.

In active competition (**A**), the microbiota employs mechanisms such as: production of toxins delivered directly to the target bacterial pathogens via cell-to-cell contact through secretion systems (**A.1**), and production of toxins as diffusible molecules to target pathogens (**A.2**). In passive competition (**B**), the microbiota inhibits pathogens by: creating a disadvantageous environment (**B.1**), and competing for resources (**B.2**).

Figure 2. Influence of microbiota on immune system development and function.

(A) Infants born vaginally had distinct microbial community compositions from infants born by caesarian-section, and the microbiome composition at one week of life correlated to the number of respiratory infections that occurred in the child's first year (Reyman et al. 2019). Microbial taxa that corresponded to a greater quantity of respiratory infections were more abundant in infants born by cesarean-section (Reyman et al. 2019). The mechanistic link between microbial community composition and frequency of respiratory infections has not been identified, as indicated by the dashed arrows.

(B) Tsetse flies with *Wigglesworthia* (symbiotic bacteria) were found to have a higher immunity to trypanosomes than those without the symbiotic bacteria (Wang et al. 2009). The presence of *Wigglesworthia* triggers the production of a pathogen recognition protein (PGRP-LB) in the host that displays anti-trypanosomal activity and also maintains the tsetse-*Wigglesworthia* symbiosis via host immune suppression (Wang and Aksoy 2012).

Figure 3. **Roles of the microbiome on physical barriers in mammals and invertebrates, highlighting the balance needed to maintain homeostasis**.

The panel on the left describes influences of the microbiota on the gut mucus layer, which allows pathogens to persist, versus a microbiota that is able to defend the mucus layer against pathogens. The panel on the right illustrates the similar idea that the microbiota influences the peritrophic matrix which indirectly affects pathogen ability to persist.

Figures available upon request (please email corresponding authors kmichel@ksu.edu and tgplatt@ksu.edu)