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Review

Immune-Microbiota Interplay and Colonization Resistance in Infection

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Commensal microbial communities inhabit biological niches in the mammalian host, where they impact the host's physiology through induction of "colonization resistance" against infections by a multitude of molecular mechanisms. These colonization-regulating activities involve microbe-microbe and microbe-host interactions, which induce, through utilization of complex bacterial networks, competition over nutrients, inhibition by antimicrobial peptides, stimulation of the host immune system, and promotion of mucus and intestinal epithelial barrier integrity. Distinct virulent pathogens overcome this colonization resistance and host immunity as part of a hostile takeover of the host niche, leading to clinically overt infection. The following review provides a mechanistic overview of the role of commensal microbes in modulating colonization resistance and pathogenic infections and means by which infectious agents may overcome such inhibition. Last, we outline evidence, unknowns, and challenges in developing strategies to harness this knowledge to treat infections by microbiota transfer, phage therapy, or supplementation by rationally defined bacterial consortia.

The human body is inherently metagenomic in that it not only consists of its eukaryotic genome but also integrates the genomes (microbiome) of a myriad of microbes colonizing its surfaces (microbiota), including bacteria, archaea, fungi, protozoa, and viruses (Ley et al., 2008). The metazoan host and its microbiome have coevolved, and a rapidly growing body of research shows that commensal microbial communities interact with almost any aspect of host physiology in health and disease. The microbiome in the gut is most extensively studied, as it surpasses other body habitats in microbial biomass by more than an order of magnitude and is separated from the near-sterile host by only a single layer of lining epithelial cells (Sender et al., 2016). The deeper understanding of the microbiome's diversity and mechanisms at the host-microbiome interface sheds new light on infectious diseases as interactions between species within the context of these complex ecosystems. It is estimated that more than 90% of pathogens infect the human host through its mucosal surfaces (Brandtzaeg, 2010). A crucial function of mucosal microbiotas in humans is, therefore, to provide resistance to infectious diseases (McKenney and Pamer, 2015). To fulfill this purpose, four classes of mechanisms of the microbiota can be delineated in relation to infectious pathogens: direct inhibition, barrier maintenance, immune modulation, and bacterial metabolism (McKenney and Pamer, 2015). Collectively, these mechanisms contribute to "colonization resistance" (Lawley and Walker, 2013). The fact that the commensal microbiota protects against invading pathogens has been known since the 1950s, when Bohnhoff and colleagues discovered that antibiotic treatment resulted in a 100,000-fold decrease in the dose of *Salmonella enterica* serovar Typhimurium required to achieve infection in mice (Bohnhoff et al., 1955-1956). We are not aware of any host-intrinsic immune function conferring a similar magnitude of protection. This connection has since been confirmed for various pathogens by multiple studies in antibiotic-treated and germ-free (GF) animals (Fonseca et al., 2015; Khosravi et al., 2014; Roxas et al., 2010; van der Waaij et al., 1971) and in antibiotic-treated humans (Leffler and Lamont, 2015). Our current understanding of colonization resistance entails detailed insights into a multitude of sophisticated mechanisms. Here, we review existing evidence on interactions between the commensal microbiota, host immunity, and infectious pathogens. Moreover, we outline major research challenges and prospects for application in the prevention and treatment of infectious diseases.

Microbiota-Pathogen Interactions during Infections

Competition over Nutrients

Microbes replicate rapidly and subsist in densely occupied biological niches. Nutrient availability within these micro-habitats is limited; therefore, microbes compete for resources such as amino acids (Figure 1) (Momose et al., 2008), sugars (Curtis et al., 2014; Leatham et al., 2009), iron (Cassat and Skaar, 2013; Deriu et al., 2013), zinc (Cerasi et al., 2013; Gielda and DiRita, 2012), oxygen (Litvak et al., 2019; Marteyn et al., 2010), and anaerobic electron acceptors (Herp et al., 2019). A higher microbial diversity implies more microbes utilizing a more versatile pool of metabolites, posing a challenge for any bacterium to thrive. Any perturbation resulting in a loss of microbial load or diversity destabilizes the microbial ecosystem, creating an opportunity for strains with increased fitness to proliferate. Moreover, it predisposes the host to infections by either endogenous pathobionts or exogenous pathogens (Fonseca et al., 2015; Khosravi et al., 2014; Sprinz et al., 1961; Voravuthikunchai and Lee, 1987; van der Waaij et al., 1971; Zachar and Savage, 1979).

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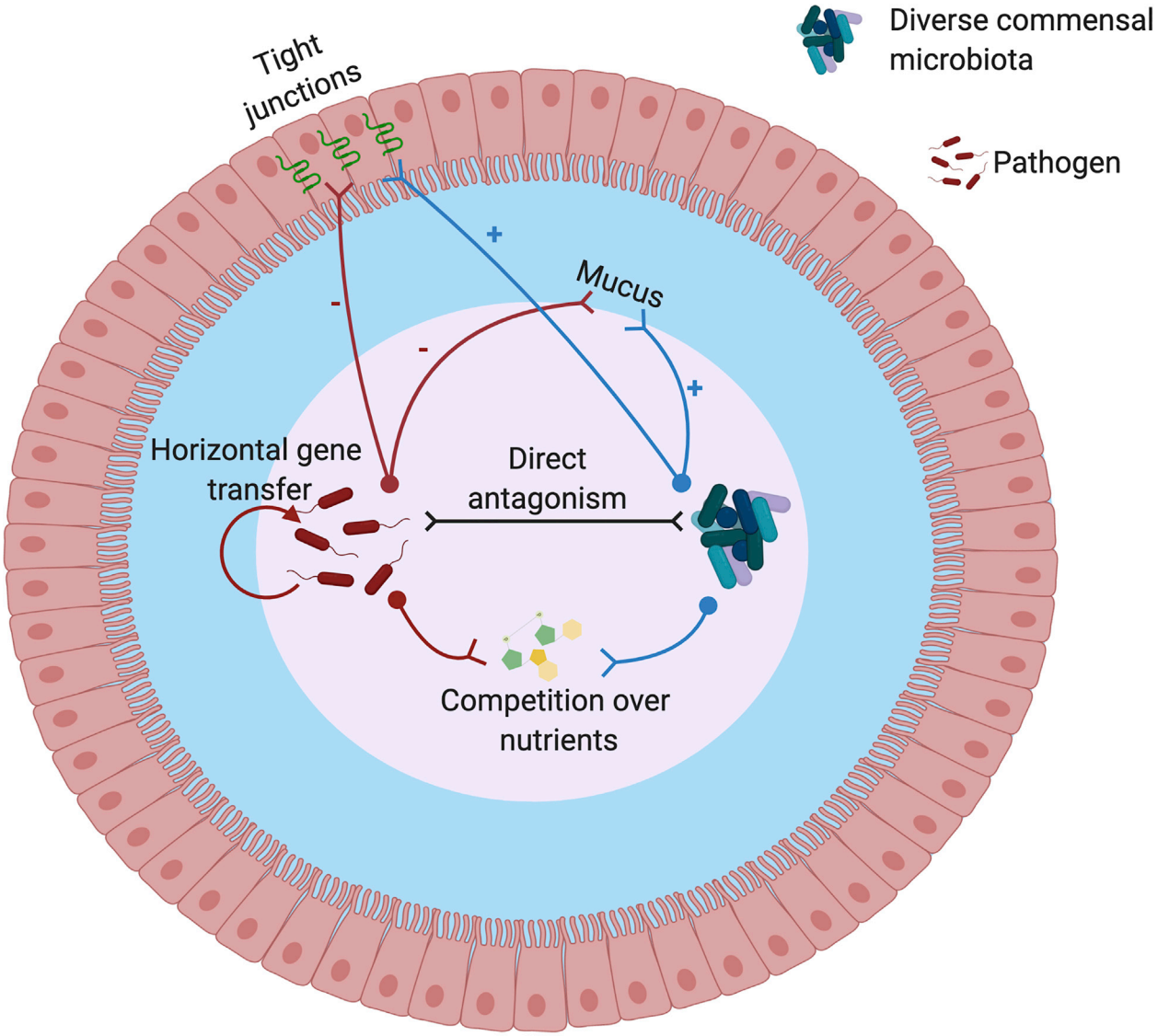

Figure 1. Major Interactions between Host, Microbiota, and Pathogen during Infections

In a homeostatic state, commensal microbiota offer protection to the host against pathogenic agents via a wide array of mechanisms. Commensals such as *Faecalibacterium prausnitzii* enhance mucus production via stimulation of enterocyte genes involved in mucin production and upregulate tight junction expression in an IL-10-dependent manner. Various pathogenic bacteria erode the mucus barrier to promote invasive infection. Moreover, commensals may stimulate intestinal antimicrobial peptide production and stimulate the host's immune response. Commensals compete with pathogens over dietary nutrients and, therefore, occupy the ecological niche required by the pathogen to thrive. Pathogens have developed mechanisms to eliminate commensal competitors, e.g., via bacterial secretion systems. Many commensals directly inhibit pathogens via secreted factors such as bacteriocins, SCFAs, or secondary bile acids. In an evolutionary arms race, pathogens develop mechanisms antagonizing these factors, e.g., by degrading enzymes or efflux pumps. Genes enhancing the pathogen's fitness and virulence may be passed on via horizontal gene transfer, e.g., through nanotubes.

Soluble Factors, Secretion Systems, and Nanotubes

Some commensals directly inhibit other bacteria by secreting soluble factors (Suez et al., 2018), such as bacteriocins (peptides with antimicrobial activity). Such factors restrain the growth of adjacent bacteria by a plethora of mechanisms such as cell-wall synthesis inhibition, pore formation, and nuclease activity (Kommineni et al., 2015). Bacteriocins such as microcin, thuricin, and lantibiotics (a class of polycyclic peptide antibiotics) inhibit pathogens such as *E. coli O157:H7* (Schamberger and Diez-Gonzalez, 2002), *Salmonella enterica* (Sassone-Corsi et al., 2016), *C. difficile* (Rea et al., 2010), and vancomycin-resistant Enterococcus (VRE) (Kim et al., 2019a). Remarkably, small-molecule biosynthetic gene clusters for thiopeptide-class antibiotics are widely distributed in the metagenome of the human microbiota (Donia et al., 2014). Other non-proteinaceous secreted molecules that inhibit the proliferation of neighboring bacteria include short-chain fatty acids (SCFAs) (Cherrington et al., 1991), H_2O_2 (Pircalabioru et al., 2016), and secondary bile acids (Buffie et al., 2015; Wotzka et al., 2019). It was recently discovered that the uptake of commensal *Neisseria*-derived

DNA by pathogenic *Neisseria gonorrhea* induces the pathogen's death as a result of misrecognition of the DNA's methylation pattern (Kim et al., 2019b). Another study discovered that, injecting an adjacent bacteria, a defective enzyme, *P. aeruginosa*, can deplete the neighboring bacteria's ATP and lead to cell death (Ahmad et al., 2019). Whether these findings have any clinical significance in infections remains to be answered and warrant further inquiry. Bacteria develop resistance to such secreted factors through various mechanisms, similar to pharmaceutical antibiotics. Notable examples are enzymatic bacteriocin degradation, modification of bacteriocins' target site (i.e., the cell-wall envelope), and efflux pumps (Bastos et al., 2015).

Secretion systems are protein complexes located at the bacterial cell wall that are used to transport effector molecules (i.e., proteins, toxins, drugs) from the cytoplasm to the exterior of the cell. These structures are of major importance for microbial competition, as they can serve the purpose of transporting toxic substances to their environment or even into nearby eukaryotic or prokaryotic cells. Several different types of secretion systems with various functions have been described (Meuskens et al., 2019; Russell et al., 2014; Sgro et al., 2019). The significant importance of secretion systems (especially type VI secretion systems, or T6SSs) has been implicated for the proliferation of gut commensal species as well as a wide range of invasive pathogens such as *K. pneumoniae*, *P. aeruginosa*, *P. mirabilis*, *E. coli*, *S. enterica*, *Y. entrocolitica*, and *Campylobacter species*. *Vibrio cholera* is a Gram-negative pathogen that induces a severe diarrheal illness and is typically transmitted through consumption of contaminated water in endemic areas of poor sanitation. Following transmission to a new host, *V. cholera* overcomes colonization resistance by antagonizing intestinal commensals using a T6SS (Zhao et al., 2018b). A few papers outlined that T6SSs can serve as interbacterial defense systems and can be upregulated whenever nearby T6SS activity is sensed (Basler and Mekalanos, 2012; Basler et al., 2013; Ross et al., 2019). *B. fragilis* uses a T6SS to secrete a ubiquitin-like protein with potent inhibitory activity against coresident strains of *B. fragilis* (Chatzidaki-Livanis et al., 2016, 2017). Utilization of advanced microscopy technology (cryomicroscopy) uncovered mechanisms by which effector molecules are loaded and transported across T6SSs (Quentin et al., 2018). In addition, secretion systems may also serve functions beyond bacterial antagonism, such as signaling or defense against phages; however, these functions are beyond the scope of this article (Russell et al., 2014). Horizontal transfer of substances can also occur by nanotubes (Bhattacharya et al., 2019). These ubiquitous organelles are composed of membranous projections and are used by bacteria to transport various cytoplasmic entities, such as plasmids that may contain antibiotic resistance genes, or even to extract nutrients from mammalian host cells (Pal et al., 2019).

The Intestinal Barrier Function in Infection

The intestine continuously absorbs nutrients and prevents enteric pathogens from systemic spread while concurrently allowing for immune processing of foodborne antigenic load. The gastrointestinal tract can, thus, be understood as a "barrier tissue," as it selectively separates the body from the external environment. The means by which this intestinal barrier function is achieved include strong inter-cellular epithelial cell adhesion by tight-junction proteins and the secretion of mucus, antibodies (i.e., immunoglobulin A [IgA]), and antimicrobial effector molecules by enterocytes and intestinal immune cells (Martens et al., 2018). If these barrier functions are compromised, the risk of infection significantly increases (Desai et al., 2016b; Thaiss et al., 2018). An extensive body of literature supports the gut microbiota's role in regulating the intestinal barrier function. This section covers some important mechanisms by which microbes either fortify or overcome the intestinal barrier function, a detailed review of microbial-host interactions in the context of the intestinal barrier function is provided elsewhere (Martens et al., 2018).

Mucus is a major component of the intestinal barrier, as microbes are stifled from reaching the epithelium by its diameter. Some commensal bacteria, such as *B. thetaiotaomicron* and *Faecalibacterium prausnitzii*, are capable of enhancing mucus production by inducing the expression of genes involved in mucin glycosylation and mucus-secreting goblet cell differentiation (Wrzosek et al., 2013). Butyrate-producing bacterial species strengthen the barrier function by regulating tight-junction protein expression in an interleukin (IL)-10-dependent manner (Kelly et al., 2015; Zheng et al., 2017). However, various pathogenic bacteria and protozoa such as *C. perfringenes* and *Entamoeba histolytica* produce enzymes that degrade mucus by targeting glycoproteins or their attached glycans (Martens et al., 2018). During dietary fiber deprivation, some commensal microbes undergo a metabolic shift to utilize mucus glycoproteins as an energy source. This process erodes the mucosal barrier and increases the risk of infection (Desai et al., 2016b).

After passing through the mucus layer, bacterial and protozoal pathogens such as *E. coli*, *V. cholerae*, *C. perfringenes*, *C. difficile*, and *Giardia lamblia* can alter tight-junction protein expression to increase gut permeability (Martens et al., 2018; Roxas et al., 2010). Some bacteria overcome the physical barrier imposed by intercellular tight junctions by becoming intracellular and crossing the epithelial layer directly through epithelial cells. *Yersinia pseudotuberculosis* and *Mycobacterium avium* use an outer membrane protein called "invasin" to invade M cells (i.e., epithelial cells that perform endocytosis of antigens and transfer them to antigen-presenting cells) via interaction with their surface integrins (Clark et al., 1998; Secott et al., 2004). *Listeria monocytogenes* invades epithelial cells by interaction with E-cadherin, a trans-membranous protein; it then releases itself from its vacuole using listeriolysin O, travels within the cytoplasm, and spreads to adjacent cells (Mengaud et al., 1996). Other intracellular pathogen species include *Salmonella*, *Neisseria*, *Brucella*, *Fracisella*, and *Legionella* (facultative intracellular), as well as *Rickettsia* and *Chlamydia* (obligate intracellular).

Diet and the Microbiota in Infection

Diet is a major modulator of microbiota composition and function. Therefore, it is also a cornerstone in the evolution of infections. A low-fiber diet increases susceptibility to pathogen invasion by compromising the gut's barrier function (Desai et al., 2016b) and reducing microbial diversity due to increased formation of bile salts (Wotzka et al., 2019). Excess dietary intake of zinc or trehalose (a food additive) promotes *C. difficile* infection by altering the microbiota composition (Collins et al., 2018; Zackular et al., 2016). Reduced food intake is often a

consequence of critical illness and may result in decreased microbial diversity and consecutively increased vulnerability toward infection. To preserve microbial diversity and protect the gut microbiota from nutrient deprivation during infection-associated anorexia, intestinal epithelial cells can produce and secrete fucose, which can be utilized as a dietary carbohydrate source by many bacteria (Pickard et al., 2014). Defective intestinal fucosylation may lead to increased susceptibility to *C. rodentium* (a rodent equivalent of human enteropathogenic *Escherichia coli*) and *S. typhimurium* (Goto et al., 2014; Pickard et al., 2014).

SCFAs are products of bacterial fermentation, i.e., the anaerobic production of energy from carbohydrates. In general, SCFAs are widely perceived as host-beneficial in terms of maintaining colonization resistance and barrier function and regulating host immune response to prevent inflammatory bowel disease and colorectal cancer (Makki et al., 2018). However, the effects of SCFAs are complex and not universally beneficial. The most common SCFAs—acetate, butyrate, and propionate—play a significant role in infections. Butyrate augments the colonic barrier function (Kelly et al., 2015). *Salmonella typhi*'s pathogenicity island SPI1 (which encodes for the type-3 secretion system [T3SS]) is upregulated by acetate (Lawhon et al., 2002) but downregulated by propionate (Lawhon et al., 2002) and butyrate (Gantois et al., 2006). Propionate production by *Bacteroides* species inhibits pathogenic *S. typhimurium* growth by disrupting its intracellular pH levels (Jacobson et al., 2018). SCFAs can induce the expression of Flagella and T3SS in enterohemorrhagic *Escherichia coli* (*EHEC*) (Tobe et al., 2011). A detailed review of dietary fiber and SCFAs can be found elsewhere (Makki et al., 2018).

Microbiota-Host Interactions during Infections

The countless daily encounters of host cells with commensal foodborne microbes and microbial products rarely result in an inflammatory response. However, microbial signaling motifs are continuously surveilled by intestinal innate immune effectors in critical processes that shape the immunological landscape of the gut (Littman and Pamer, 2011). Defects of this system may result in a wide spectrum of immune-mediated aberrations driving disorders ranging from allergy (Feehley et al., 2019) over autoimmunity (Greiling et al., 2018) and infections to altered vaccine responses (Hagan et al., 2019). Immune maturation begins *in utero*, but postpartum environmental cues, especially during the first few years of life, are essential for healthy immune system development (Chung et al., 2012).

Early-Life Microbiome and Resilience to Infection

Early life, including the fetal stage and first years after birth, represents a state of increased susceptibility to infections with a wide variety of microbes. This is, in part, attributable to an immature immune defense (Zhang et al., 2017). Infectious diseases are the leading cause of childhood death (Bhutta and Black, 2013). However, the manifold facets of early-life immune immaturity are currently incompletely characterized. A vital pillar of the infant's immune development is the maturation of the innate immune system. Compared to adults, neonates are less responsive to Toll-like receptor (TLR) signals and show lower pro-inflammatory cytokine release (Kollmann et al., 2012). Cord blood neonate immune cells, including monocyte and dendritic cells, secrete lower amounts of IL-12p70 (required for Th1-cell polarization), interferon (IFN)-α, and tumor necrosis factor alpha (TNF-α) (Kollmann et al., 2009). They produce equal or even higher levels of IL-1β, IL-6, IL-23, and IL-10 than adult immune cells but are less able to produce multiple cytokines simultaneously (Kollmann et al., 2009). IFN regulatory factor 3 (IRF3)-dependent responses to lipopolysaccharide (LPS) are blunted in cord blood cells, highlighting a decreased ability to elicit Th1 responses upon LPS stimulation (Aksoy et al., 2007).

Growing evidence demonstrates that the commensal microbiota educates the developing immune system during early life (Gensollen et al., 2016). There is controversy about whether initial microbial colonization already occurs *in utero* through mother-to-fetus transmission. Live bacteria were detected in cord blood from healthy human neonates and from the amniotic fluid of healthy pregnant mice (Jiménez et al., 2005). Moreover, a quite complex microbiota was detected by metagenomic sequencing of meconium samples from neonates delivered through C-section (Wang et al., 2018). Adopting a careful meticulous technique, researchers demonstrated that the vast majority of bacterial signals in sequenced placental samples stems from acquisition during fetal delivery or DNA-contaminated laboratory reagents, casting doubt on the existence of placental microbiota (de Goffau et al., 2019).

Although the existence of *in utero* colonization still remains uncertain, it is generally agreed that the largest share of colonization takes place after birth. It predominantly originates from maternal skin, vaginal, and gut microbiotas and is greatly influenced by delivery mode (Dominguez-Bello et al., 2010). To allow for rapid colonization, the infant immune system shifts from a propensity to hyperstimulation toward tolerance, which is, in part, mediated by a specific population of $CD71^+$ erythroid cells that dampen innate immune responses (Elahi et al., 2013). During the first years of life, the immature gut microbiota is relatively variable and converges toward a stable adult-like composition approximately at the age of 3 years (Yatsunenko et al., 2012). The timing and order of species dispersal at the first stages of microbial communities assembly will determine, by and large, how species will affect one another (i.e., "priority effect") (Sprockett et al., 2018). This ecological succession may create a time-limited window of opportunity for healthy (or dysfunctional) imprinting of microbiome composition and associated immune functions persisting throughout later life (Gensollen et al., 2016). Early studies on GF animals have demonstrated developmental defects in lymphoid tissues associated with a lack of commensal microbes (Bauer et al., 1963). Moreover, GF mice display reduced frequency of $CD4^+$ and $CD8^+$ intestinal T cells and decreased numbers of αβ T cell receptor expressing intraepithelial lymphocytes (Umesaki et al., 1993). Intestinal mucosa IgA production is largely induced by gut commensal and is dramatically reduced in GF animals and newborns (Macpherson et al., 2008). Intestinal colonization induces *de novo* generation and activation of colonic T-regulatory cells, which maintain intestinal immune homeostasis (Figure 2) (Umesaki et al., 1993). In specific pathogen-free (SPF) colonized mice, Th1 and T_H17 lymphocytes are constitutively present but are absent in GF animals (Ivanov et al., 2006, 2008). $T_H17$cells are a potent source of IL-17 and play a critical role in host defense against infections

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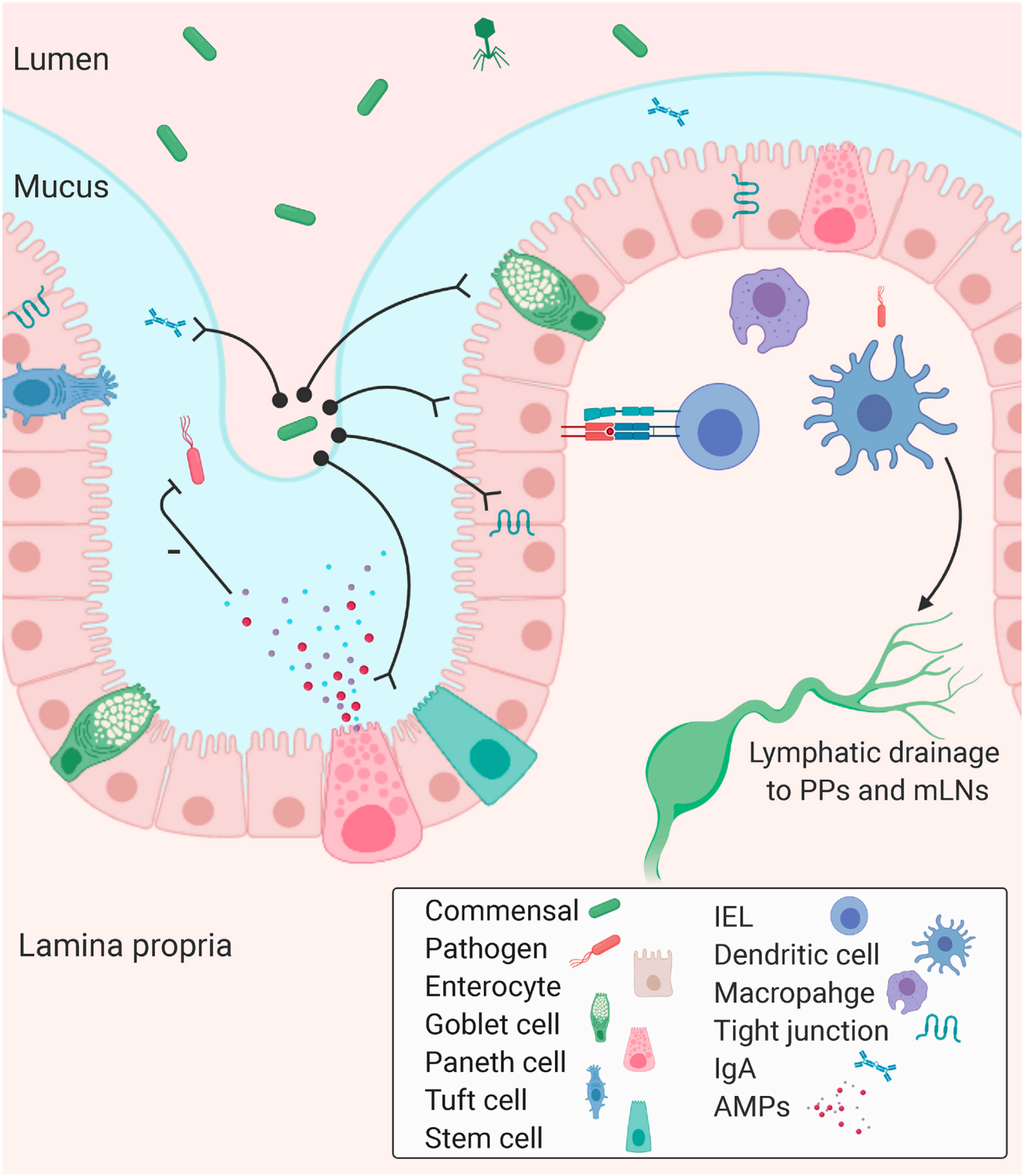

Figure 2. Commensals' Effects in Mucosal Immunity in the Small Intestine

As the intestine is, by far, the largest reservoir of immune cells in the body, many different immune cells drive mucosal immunity. Microbial cells were demonstrated to be involved in almost every aspect of mucosal immunity. The stimulation of Paneth cells' Toll-like receptors (TLRs) by microbial constituents elicits MyD88 signaling, which promotes anti-microbial peptide (AMP) secretion and inhibits bacterial penetration beyond the epithelial barrier. Paneth cells' TLR activation also enhances major histocompatibility complex (MHC) class II expression by intestinal epithelial cells (IELs) (in an IFN-γ-dependent manner), which, in turn, promotes $CD4^+$ intestinal cell activation and differentiation into T_H17 or $Foxp3^+$ T_{reg} cells (among other types of T helper cells). Intestinal microbes can also enhance epithelial cells' tight-junction protein expression, goblet cells' mucus production, and plasma cells' mucosal

(legend continued on next page)

through recruitment of neutrophils and macrophages to infected tissues (Dong, 2008). The emergence of T_H17 cells in mice is linked to specific commensal strains, mainly segmented filamentous bacteria (SFB) in the gut (Gaboriau-Routhiau et al., 2009; Ivanov et al., 2008), or *Malassezia* in the skin (Sparber et al., 2019). Colonization of the murine small intestine with SFB correlates with increased expression of genes associated with inflammation and antimicrobial defenses and increases resistance to the enteric pathogen *Citrobacter rodentium* (Ivanov et al., 2009). Moreover, physical barrier functions of the skin and the intestinal and airway mucosae are enhanced by antimicrobial effector proteins and peptides (APPs), such as β-defensins. Their production is stimulated by postnatal microbial exposure (Kollmann et al., 2017). By using a sophisticated model of gestation-only colonization employing a genetically engineered *Escherichia coli* strain, the authors showed that the maternal microbiota already shapes the offspring immune system *in utero*. Gestational colonization of pregnant dams increased intestinal group 3 innate lymphoid cells and $F4^+/CD11^+$ mononuclear cells in pups and increased expression of genes encoding APPs (Gomez de Agüero et al., 2016).

Recent evidence suggests additional host-immune-system-independent protection of the offspring by microbiota colonization. In a murine model, neonatal microbiota contributed to the acquisition of a protective *Clostridia* consortium before weaning, which prevented the growth of *Citrobacter rodentium* and *S. typhimurium* independent from MyD88/TRIF-domain-containing adapter-inducing interferon-β (TRIF) signaling or adaptive immunity (Kim et al., 2017). The primary gut colonizers are facultative anaerobes belonging to the Enterobacteriaceae family, such as *Escherichia*, and *Enterococcus*. This is perhaps due to their ubiquitous nature in an oxygenated environment. However, in the healthy infant gut, the community composition soon shifts in favor of anaerobes shaped by multiple factors, including the induction of a γ-Proteobacteria-specific IgA response (Mirpuri et al., 2014). Members of the *Bifidobacterium* genus predominate in the intestinal microbiomes of breastfed children. This is facilitated by their competitive nutrient-utilization strategy targeting human milk-oligosaccharides (HMOs) which cannot be utilized by the infant's enzymes (Sela et al., 2008; Zivkovic et al., 2011). The protective effect of breastfeeding against infections, especially compared to formula diet, has long been recognized (Hanson and Winberg, 1972; Victora et al., 2016). Human milk contains a wide variety of bioactive proteins, growth factors, cells, and other ingredients, finely attuning to the infant's requirements as a result of millions of years of evolution (Andreas et al., 2015). The anti-infectious properties of human milk were attributed to various constituents, including maternal antibodies (Caballero-Flores et al., 2019), direct action of HMOs (Hakkarainen et al., 2005), and lactoferrin (Telang, 2018). Live bacteria were cultured from healthy human milk for decades (Heikkilä and Saris, 2003; Martín et al., 2003, 2009). However, only recently, the diversity of the endogenous human milk microbiome has been fully appreciated through next-generation sequencing studies (Moossavi et al., 2019; Togo et al., 2019). Importantly, in breastfed children, the maternal milk microbiota may be the quantitatively most important source to seed the infant gut (Pannaraj et al., 2017). Emerging evidence highlights that gut colonization initiated and shaped by breastfeeding may confer direct protection against pathogens. In mice, colonization with surface-associated exopolysaccharide producing *Bifidobacterium breve* reduced levels of the gut pathogen *C. rodentium* (Fanning et al., 2012). However, the origins of *Bifidobacterium* colonization are still unclear, and factors related to child-rearing and feeding practices may determine its prevalence in the infant's microbiota (Vatanen et al., 2019). Other commensal strains from human milk belonging to the species *Staphylococcus epidermidis* and *Streptococcus salivarius* suppress the growth of pathogenic *Staphylococcus aureus* (Heikkilä and Saris, 2003), and numerous *lactobacilli* strains isolated from human milk showed inhibitory activity against enteric pathogens such as *Escherichia coli*, *Shigella* spp., *Pseudomonas* spp., and *Salmonella* spp. (Jara et al., 2011). However, the mechanisms behind those inhibitory effects remain mostly elusive and require further research.

Antimicrobial Peptides

Paneth cells directly surveil the gut lumen for microbes and secrete antimicrobial peptides (AMPs) through a mechanism that is regulated by MyD88-dependent TLR activation (Natividad et al., 2013; Vaishnava et al., 2008). These AMPs have the capacity to kill or inhibit bacteria, fungi, and enveloped viruses by targeting a range of cytoplasmic and cell well structures (Nguyen et al., 2011; Okumura et al., 2016). Therefore, AMPs shape the gut microbiota composition (Vaishnava et al., 2008). However, the microbiota and AMPs are intertwined in bi-directional interactions, as certain commensals also modulate AMP production and secretion. This notion is supported by the fact that GF mice have decreased levels of particular AMPs compared to conventional mice. Further evidence is provided by the observation that conventionalizing GF mice with low-diversity microbiota does not restore AMP secretion to the same extent as colonization with a high-diversity microbiota, suggesting that AMP secretion in the intestine is selectively affected by specific microbes (Natividad et al., 2013). The importance of AMPs and the direct role of the gut microbiota in regulating their secretion in infectious disease was demonstrated in several studies. For example, it was shown that the microbiota provides essential protection against enteric infections during starvation, in which most other AMPs are downregulated. This is achieved by induction of mTOR signaling and by promoting the production of α-defensins (Liang et al., 2019). In addition, some bacteria alter the AMP profile of their host in order to facilitate colonization and to decolonize the gut from competing strains through manipulation of the host's inflammasome signaling (Levy et al., 2015). Important work in this field was carried out by Eric Pamer and colleagues and contributed substantially to our understanding of host-microbe interaction in infections and, especially, of vancomycin-resistant enterococci (VRE), a common, difficult-to-treat,

immunoglobulin A (IgA) secretion. The antigenic load in the intestinal lumen is continuously sampled, processed, and delivered by antigen-presenting cells to lymphoid tissues—i.e., Peyer's patches (PPs) or mesenteric lymph nodes (mLNs)—for mucosal immune surveillance and initiation of appropriate tolerogenic or inflammatory response.

hospital-acquired pathogen. In a series of studies, they show that microbiota depletion by antibiotic exposure might diminish intestinal RegIIIγ secretion in a MyD88-dependent manner, resulting in the defective clearance of pathogens such as VRE (Brandl et al., 2008) and Listeria (Brandl et al., 2007). More recently, Pamer's group showed that a bacterial consortium consisting of four strains (*Clostridium bolteae*, *Blautia producta*, *Bacteroides sartorii*, and *Parabacteroides distasonis* [CBBP]) effectively restores resistance to VRE colonization by replenishing lantibiotic production (Caballero et al., 2017; Kim et al., 2019a). Consistent with other published data, they showed that autologous FMT (fecal microbiota transplantation; i.e., reconstitution of one's gut microbiota with a fecal sample taken at an earlier time, prior to initiation of disease/treatment) is safe and effective in replenishing microbiota diversity to baseline following antibiotic exposure (Suez et al., 2018; Taur et al., 2018). However, it remains to be determined whether autologous FMT is clinically beneficial in terms of reestablishing colonization resistance, and further investigation is warranted. Gut microbes become resistant to AMPs through subtle modifications in the structure or electrical charge of their outer membranes. Interestingly, these mechanisms of resistance are deployed at times of inflammation by certain pathogens (Martens et al., 2018) and commensals alike (Cullen et al., 2015).

While the immune response is normally directed against infectious agents, some pathogens can exploit it to overcome colonization resistance. By invoking a mucosal anti-bacterial response to which it is resistant (i.e., RegIIIβ, an AMP), *S. typhimurium* promotes its colonization by utilizing the immune system for the clearance of competing strains (Miki et al., 2017; Rivera-Chávez et al., 2016; Stecher et al., 2007; Stelter et al., 2011). During infection, innate immune effectors limit iron availability at the infection site by local production of iron chelators, i.e., lactoferrin, hepcidin, and lipocalin-2 (Cassat and Skaar, 2013). *S. typhimurium* has developed resistance to lipocalin-2, giving it a fitness advantage in the competition for host iron (Raffatellu et al., 2009). The inflammatory environment generated as a result of *S. typhimurium* presence not only inhibits other strains but also increases the availability of alternative nutrients such as lactate (Gillis et al., 2018), ethanolamine (Thiennimitr et al., 2011), and tetrathionate (Winter et al., 2010), which *S. typhimurium* has adapted to metabolize in contrast to most other bacteria. *B. fragilis* is able to modify the outer surface of its capsule to facilitate IgA binding (Donaldson et al., 2018). IgA-binding enables *B. fragilis* and other commensals to adhere to and occupy mucosal niches under exclusion of competitors.

Non-bacterial Enteric Microbiota in Infection

Non-bacterial members of the microbiota are often neglected. However, emerging evidence points toward significant interactions between the gut virome, fungome, and parasitome with pathogens and host immunity in infections. A common fungal member of the gut microbiota, *Candida albicans*, elicits a strong T_H17 response that may cross-react with the pathogenic fungus *Aspergillus fumigatus* during lung infection and accelerate lung inflammation (Bacher et al., 2019). Mono-colonization with either *C. albicans* or *Saccharomyces cerevisiae* emulated the anti-infectious protective effects that the bacterial microbiota is well known to exert (Jiang et al., 2017). This effect was mediated by the induction of the $CD8^+$ T cell immune response by TLR4 recognition of Mannan, a component of the fungal cell wall. In another study, infection with the parasitic helminth *Trichuris muris* triggered a type 2 immune response which led to the expansion of *Clostridium* species and prevented *Bacteriodes vulgatus* colonization (Ramanan et al., 2016). In mice, colonization by the protozoon *Tritrichomonas musculis* induces IL-18 release in an inflammasome-dependent manner, which protects against *S. typhimurium* infection (Chudnovskiy et al., 2016).

The enteric microbiota contains a considerable number of viruses, referred to as "the enteric virome." This viral community is very often neglected in metagenomic studies, due to technical issues such as difficulties in annotation and the exclusion of RNA viruses, which account for a significant proportion of enteric viruses. Akin to bacteria, enteric viruses interact with and modulate the host immune system. Infection with murine norovirus can correct the immune immaturity characterizing GF mice, to some extent, with regard to intestinal morphology, lymphocyte function, and transcriptional profile (Kernbauer et al., 2014). This reconstitution was achieved in the absence of any overt inflammatory or intestinal bacteria and proved protective against *C. rodentium* infection. Human data suggest that viral infections may shape the gut microbiota in a stage-dependent manner. In AIDS patients, low peripheral $CD4^+$ T cell counts are associated with altered enteric microbiota, characterized by a decreased microbial diversity and expansion of *Adenoviridae* (Monaco et al., 2016). In some instances, viruses enter a dormant state ("latency"), which is often perceived to be parasitic. However, latent infection with murine herpesviruses, which serve as models for the common human viruses cytomegalovirus (CMV) and Epstein-Barr virus (EBV), protected mice against infection with *L. monocytogenes* and *Yersinia pestis*. This was achieved through basal activation of innate immunity by the induction of IFN-γ production (Barton et al., 2007). Induction of IFN-γ secretion by enteric viruses is common in enteric viral infections and was shown to be involved in epithelial turnover and tissue repair (Barton et al., 2007; Kernbauer et al., 2014; Sun et al., 2015). Many viruses exploit the native microbiota to colonize and infect the host, for example, by attaching to bacterial surface components such as LPS or peptidoglycan (Jones et al., 2014; Kane et al., 2011; Kuss et al., 2011; Robinson et al., 2014). Interestingly, an opportunistic parasitic infection can impair host antiviral immunity and promote viral infection (Osborne et al., 2014) or reactivation (Goodwin et al., 2010).

Bacteriophages (phages) are viruses that exclusively infect bacteria. Their host tropism to prokaryotic cells and high specificity to a relatively narrow host range (sometimes a single species or strain) gave rise to renewed interest within the scientific community. Certain phages can produce mRNA within human cells that suppress phagocytosis of *Pseudomonas aeruginosa* in wound infections (Sweere et al., 2019). By overcoming the eukaryotic host immune system, phage-bacteria mutualism may offer a selective advantage to both organisms in certain scenarios. Together, these studies suggest the existence of complex trans-kingdom host interactions in clinically relevant infections, which merit further research.

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Questions and Challenges in Uncovering Commensal-Pathogen Interactions

Despite considerable progress in the mechanistic understanding of commensal-pathogen interactions in infections, this scientific field is extremely far from being deciphered and currently faces several limitations and challenges. Although next-generation sequencing aids with overcoming some complexities of the microbiota and provides mechanistic insights in commensal-pathogen interactions, culture-independent methods are not without limitations. First, methodological differences in sequencing technique and analysis limit conclusion generalization (Knight et al., 2018). Second, low biomass samples are highly prone to bias, due to non-proportional target amplification and contamination (Eisenhofer et al., 2019), leaving non-gut mucosal microbiotas (i.e., oral, lung, skin) often overlooked. Third, as outlined in the previous section, the commensal virome, fungome, and parasitome presumably plays a significant role in infections but is commonly left unsequenced or discarded in the process of data analysis. Furthermore, microbes can rapidly adapt to environmental changes in a process that is faster than that of generational random mutation and involves alteration of the gene-expression profile. In an infection-driven inflammatory environment, this process is of primary importance for both commensals and the infectious agent virulence; however, such alterations in gene expression aren't captured by genomic sequencing, and bacterial RNA sequencing is technically challenging and is thus rarely carried out (Anjuwon-Foster and Tamayo, 2018). Advancements in this field, such as dual RNA sequencing of both pathogen and host cells, may potentially provide important revelations in commensal-pathogen interaction in infection (Westermann et al., 2012). Another major challenge in the field of commensal-pathogen interaction is that the majority of microbial species detected by metagenomics are unculturable (Lagier et al., 2012). *In vitro* bacterial culturing is of primary importance, for example, when studying new antimicrobial agents (see the following section) or when viability should be assessed. Modern culturomics facilitates the identification of previously unknown commensal strains and allows their cultivation for further experimentation (Lagier et al., 2018). Recently, isolation and culturing of previously uncultured species were achieved by "reverse genomics," i.e., the design of labeled antibodies to target the cell-surface proteins of a previously uncultured species, by predicting its structure based on its genomic-sequencing data and later using that antibody to isolate it by flow-cytometric sorting (Cross et al., 2019).

An additional limitation of commensal-pathogen interaction studies stems from colonization modeling methods. Most studies modulate the colonization of pathogens/probiotics/microbiota transplantations in antibiotic-treated or GF animals; however, it was shown that both antibiotic-treated (Lundberg et al., 2016) and GF (Al-Asmakh and Zadjali, 2015) animals differ from naive animals in some immunologic properties among other physiologic traits. Such differences mandate that the interpretation of results from such studies is done with caution and warrant studies that modulate colonization in SPF, "humanized" (colonized by human microbiota) (Al-Asmakh and Zadjali, 2015), and "wilding" (colonized by wild-mouse microbiota) (Rosshart et al., 2017) animals. Lastly, the effects of antibiotics and other microbiota-perturbing medical interventions such as surgery or hematopoietic stem-cell transplantation (HSCT) on barrier function, colonization resistance, and their restoration thereafter require scientific attention. In this regard, preliminary studies have shown that autologous FMT, i.e., administrating a patient with its own microbiota taken prior to medical intervention, has great potential to rapidly and safely reconstitute the baseline microbiota of that patient following antibiotic exposure in HSCT (Suez et al., 2018; Taur et al., 2018).

Future of Microbiome-Based Interventions against Infection

The rapid scientific progress in the field of microbiome research opened potential new therapeutic avenues and revitalized "new old tools" targeting the microbiota, which may apply in the combat of infectious diseases.

Microbiota Transplantation

Interest in the therapeutic potential of FMT has increased in recent years, as witnessed in a series of randomized controlled trials (RCTs), which mainly focused on recurrent *C. difficile* infection (CDI) (Hui et al., 2019). CDI is a common enteric nosocomial infection, particularly in the aftermath of antibiotic treatment, and its pathology is mediated by toxins secreted by the bacterium (Abt et al., 2016). The evolving epidemiology of CDI, including the emergence of hypervirulent strains, high risk of recurrence after standard antibiotic treatment, and increasing incidence in the community setting (Desai et al., 2016a), warrants exploration of novel therapeutic avenues. Despite the growing popularity, FMT currently should only be considered in patients with recurrent CDI who have previously failed appropriate antibiotic therapy, and it is not recommended for patients with primary CDI (Mullish et al., 2018). Nevertheless, published preliminary results from a small, exploratory, industry-funded trial suggest that FMT may be useful in primary CDI as well (Juul et al., 2018).

Currently, all available RCTs have significant limitations; therefore, the efficacy and safety profile of FMT cannot been fully evaluated yet. A great heterogeneity in the FMT process exists across studies regarding donor selection, sample processing, and administration regimen (pretreatment, dose, route, frequency, etc.). There is no clear evidence to prefer one protocol over another, which highlights the need for further standardization (Mullish et al., 2018). Of note, despite growing evidence for its efficacy from RCTs, the U.S. Food and Drug Administration (FDA) did not approve FMT for recurrent CDI. Despite recommended donor feces screening for pathogens, the long-term safety profile of FMT is currently uncertain. Recently, a MedWatch alert was declared after two immunocompromised patients acquired systemic infections with a donor-derived strain of antibiotic-resistant *E. coli* (DeFilipp et al., 2019). Of note, both cases were linked to the same stool donor. Moreover, it has been criticized that patient cohorts in existing RCTs are not sufficiently reflective of real-life CDI patients eligible for FMT (Kelly et al., 2019; Tariq et al., 2017). The mechanisms mediating FMT efficacy are elusive, as recent studies suggest that, rather than engraftment of complete live microbiota, transfer of specific bacterial consortia or even bacteria-free filtrates containing phages or secondary bile acids may suffice to achieve a treatment effect

(Buffie et al., 2015; Lee et al., 2016; Ott et al., 2017; Staley et al., 2016; Zuo et al., 2018).

An emerging application of FMT is restoration of colonization resistance to antibiotic-resistant pathogens. The introduction of antibiotics in the mid-20th century facilitated unprecedented medical and societal progress; today, they are indispensable in all health care systems. However, the emergence of antibiotic-resistant bacteria (ARB) has proven ineluctable, prompting the urgent need for strategies to combat them (Laxminarayan et al., 2013). To date, several case reports and uncontrolled trials reported successful decolonization of ARB, such as VRE and extended-spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae*, by FMT (Davido et al., 2019). However, the only RCT published to date failed to demonstrate a clear benefit from FMT in eradicating multidrug-resistant *Enterobacteriaceae* (Huttner et al., 2019). Nevertheless, evidence from basic science supports the concept that specified commensal clades can be harnessed to achieve ARB decolonization, e.g., through their natural capability to produce bacteriocins or lantibiotics (Caballero et al., 2017; Kim et al., 2019a; Kommineni et al., 2015).

Bacterial vaginosis (BV) is a common cause of vaginal discharge and is associated with vaginal microbiota alterations. Recent evidence suggests that BV is a sexually transmitted infection (Muzny et al., 2019), and the mainstay of treatment is antibiotics. However, a subpopulation of women experiences persistent or recurrent infection despite multiple antibiotics courses. A recent first in-human trial demonstrated that vaginal microbiota transfer (VMT) from healthy donors can shift the microbiota toward a normal *Lactobacillus*-dominated configuration, which was accompanied with BV amelioration in a small group of women (Lev-Sagie et al., 2019). Further clinical trials are warranted to evaluate the safety and efficacy of VMT.

Together, these advances indicate a limited utility of microbiota transplantation against infectious diseases. A better understanding of microbiota transplantation may facilitate reductionist identification of particular microbes or small microbial molecules, such as secondary bile acids, mediating its efficacy. This could, ultimately, lead to the replacement of microbiota transplantation by more efficient and safer next-generation tools.

Prebiotics, Probiotics, and Postbiotics

Probiotics are nowadays defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (Hill et al., 2014, p. 506). Over the past century, numerous studies have examined the effects of probiotics. In more recent years, many RCTs were conducted on the utility of probiotics in the prevention of different infectious diseases and were exhaustively summarized in systematic reviews and meta-analyses (Hao et al., 2011; Shen et al., 2017). Generally, results were mixed, as was the quality of the underlying studies, and differed by underlying indication and the probiotic strain applied. To cite notable recent studies: in a rural Thai population, the consumption of probiotic *Bacillus* strains abolished colonization by pathogenic *Staphylococcus aureus*. This effect was achieved by the interference of a class of *Bacillus* lipopeptides (fengycins) with *S. aureus*' quorum sensing (Piewngam et al., 2018). However, in a multicenter phase-3 RCT, no efficacy of the probiotic strain *Bifidobacterium breve* BBG-001 could be demonstrated in preventing necrotizing enterocolitis or sepsis in preterm neonates (Costeloe et al., 2016). The mechanisms underlying probiotic activity, and the potential undesired effects, are yet to be understood for many commonly used strains. The current next-generation sequencing era provides new tools for investigating probiotics (Suez et al., 2020). In a study using a widely available over-the-counter preparation of several probiotic *bifidobacterial* and *lactobacilli* strains in mice and humans, probiotic treatment resulted in the delay, rather than aid, of post-antibiotic microbiota recovery (Suez et al., 2018). This inhibitory effect on post-antibiotic microbiota reconstitution also occurred upon exposure of probiotic-derived, cell-free filtrate, suggesting that inhibition was mediated by probiotics-derived molecules. Moreover, intestinal engraftment of probiotic strains proved challenging and depended on individualized predictors such as the host's baseline microbiota structure (Zmora et al., 2018). This highlights the need for innovative personalized probiotic approaches to complement traditional "empiric" antibiotic treatments. Innovative probiotic strategies using rationally selected consortia and recombinant strains are on the horizon but still await testing *in vivo* (Sanders et al., 2019). It was recently discovered that some gut commensal bacteria express immunogenic glycans that elicit a specific anti-glycan humeral response with a protective effect against *Plasmodium* spp. (malaria) infection (Soares and Yilmaz, 2016). This discovery of cross-reactivity between commensal antigens and a vector-born infection introduces the possibility that colonization by a bacterial consortium or by a probiotic strain genetically engineered to express a specific glycan may help to prevent malaria transmission. A group of researchers identified segmented filamentous bacteria as protective against rotavirus infection by both reducing rotavirus's infectivity and increasing intestinal epithelial turnover (Shi et al., 2019); however, human data are still lacking. It would be interesting to test whether the four-bacterial-strain consortia proposed by Eric Pamer and colleagues to prevent VRE colonization (see the CPPB consortium discussed earlier in Antimicrobial Peptides) is beneficial in hospitalized patients. It is also worth mentioning that Kenya Honda's patient-derived bacterial consortium was shown to enhance $CD8^+$ T cell response against infection and against cancer and is currently undergoing clinical trials (Tanoue et al., 2019) (ClinicalTrials.gov: NCT03788434).

A "prebiotic" is defined as "a substrate that is selectively utilized by host microorganisms conferring a health benefit" (Gibson et al., 2017, p. 491). Numerous were reported to convey such prebiotic effects, including a wide range of oligosaccharides, several types of dietary fiber, phenolics, linoleic acid, and polyunsaturated fatty acids (Gibson et al., 2017). Combinations of probiotic strains with complementary prebiotic substrates are termed "synbiotics." Clinical trials evaluating the effects of pre- or synbiotics are rare. A synbiotic combination of the probiotic strain *Lactobacillus plantarum ATCC-202195* and fructooligosaccharide showed promising results in preventing sepsis in a cohort of >4,500 Indian children (Panigrahi et al., 2017). In another RCT in preterm infants, oral administration of a prebiotic mixture (galacto-oligosaccharide and polydextrose) effectively reduced the incidence of rhinovirus infections (Luoto et al., 2014). Many effects of the commensal microbiota on the host are mediated by a vast array of microbe-derived bioactive

small molecules spanning the entire spectrum of chemical classes (Donia and Fischbach, 2015). "Postbiotics" is an evolving term coined to describe probiotic strain-derived non-viable products that suffice to achieve the desired health-promoting effect (Tsilingiri and Rescigno, 2013). Postbiotics may have several advantages over probiotics, in that the direct application of the bioactive compound may increase the probiotic potency and circumvent the challenge of keeping the probiotic microorganism viable and achieve sufficient colonization. Moreover, they may ease pharmaceutic packaging, storage, and delivery (Wegh et al., 2019). Given the increasing appreciation of the adverse effects of probiotics that may outweigh their benefits, such as—particularly alarming—direct causation of bacteremia in hospitalized patients (Yelin et al., 2019), postbiotics may also represent a safer alternative. Emerging data highlight that certain microbiota-derived metabolites may protect against infections. For example, desaminotyrosine (which is, e.g., produced by the human commensal *Clostridium orbiscindens*) may protect against influenza infection through augmentation of type 1 IFN signaling (Steed et al., 2017). It is a formidable challenge to identify such beneficial compounds among a myriad of microbiota-derived metabolites. Recent progress in high-throughput screening techniques, such as genomic inquiry for small-molecule biosynthetic gene clusters (Donia et al., 2014) or utilizing the host receptor-ome as a lens for mining microbe-derived ligands (Chen et al., 2019), may greatly advance the identification of postbiotic agents.

Dietary Interventions

Diet has a profound influence on human health, partly by modulating the gut microbiome (Kolodziejczyk et al., 2019; Muegge et al., 2011). As mentioned earlier, growing evidence shows that dietary choices may affect susceptibility to some common infections. A Western-style, high-fat diet and excess zinc and trehalose consumption were shown to predispose to infection by impairing colonization resistance or barrier function in a microbiome-dependent manner (Agus et al., 2016; Collins et al., 2018; Desai et al., 2016b; Zackular et al., 2016). On the contrary, retinoic acid (vitamin A) and high fiber intake were demonstrated to benefit microbiota homeostasis and protect the host from pathogens (Grizotte-Lake et al., 2018; Ng et al., 2019; Park et al., 2011). Clinical trials utilizing microbiota-targeted diets are emerging, and, so far, are focused on metabolic disorders, malnourishment, and inflammatory bowel disease (Gehrig et al., 2019; Levine et al., 2019; Zeevi et al., 2015; Zhao et al., 2018a). Given the evidence from basic research, microbiota-targeted diets may hold great potential for the management of infectious diseases; however, currently, no microbiota-directed diet is used in clinical microbiology, and further studies are needed. We believe that, in addition to traditional "one-size-fits-all" interventions, personalized dietary approaches may greatly advance the harnessing of diet for microbiota-targeted prevention and therapy of infectious diseases.

Phage Therapy

The capability of phages to replicate within their bacterial prey is one of their potential advantages over antibiotics, in that they operate as "self-amplifying drugs" (Kortright et al., 2019). In the light of emergence of antibiotics resistance and the detrimental effects of antibiotic treatment on the gut microbiome composition with potential long-term consequences, the broad-spectrum activity of most clinically applied antibiotics is nowadays perceived as a major disadvantage. In contrast, phages usually target bacteria with species, or even strain, specificity. For example, a phage was successfully used to specifically eradicate *E. faecalis* and its hepatotoxic effect in mice colonized with human microbiota from alcoholic hepatitis patients, but clinical trials are warranted (Duan et al., 2019). A method to increase the very narrow activity spectrum, and to decrease the risk of phage-resistance development, is to use combinations ("cocktails") of several defined phages (Duyvejonck et al., 2019). In a recently published murine model, an *E. coli/Salmonella* spp./*Listeria monocytogenes*-targeting bacteriophage cocktail achieved the depletion of pathogenic *E. coli* without altering the gut microbiota (Dissanayake et al., 2019). Since the turn of the millennium, several pre-clinical studies demonstrated the efficacy of phage treatment against various local and systemic infections (Yen et al., 2017). However, at the same time, evidence for safety and efficacy in humans was mostly supported by case reports (Dedrick et al., 2019; Jennes et al., 2017). Recently, large-scale funding initiatives for clinical research on phage therapy were launched by government and private institutions (Moelling et al., 2018). A first high-quality RCT was recently published, demonstrating the tolerability, but inferior efficacy, of a cocktail of 12 natural lytic anti-*P. aeruginosa* bacteriophages (PP1131) in burn-wound infections (Jault et al., 2019).

Phage therapy will probably not be able to replace antibiotics entirely in the future. However, adjuvant strategies—i.e., mixed application of antibiotics and phages—could be worthwhile to explore (Bedi et al., 2009). Currently, phage therapy faces many challenges, including regulatory approval obstacles, the narrow host range, uncertain evolutionary dynamics and (long-term) impact on host and microbiota, unclear phage selection criteria, and lack of well-curated publicly accessible phage libraries. More mechanistic and pre-clinical insights on phage-pathogen interactions and host-microbiota-phage dynamics are required to pave the way for further RCTs and to bring forth regulatory approvals.

Summary and Future Prospects

Recent advances in microbiota research unveiled the complex dynamics on the microbe-microbe and host-microbe levels in infectious diseases. Exciting insights were gained in studies on developmental trajectories in early-life commensal colonization and protection against infection, mainly relying on GF animal models. Nevertheless, many gaps in our knowledge of these highly dynamic developmental processes still need to be filled. Moreover, more mechanistic research on commensal-pathogen-immune interactions is required for non-bacterial members of the endogenous microbiota.

With progress in DNA sequencing, microbial culturing, and transgenic and gnotobiotic animal technologies, engineering the microbiota for therapeutic purposes becomes feasible. Despite the recent demonstration of the efficacy of FMT in treating CDIs and emerging novel applications such as ARB decolonization, the technique is still relatively crude and "noisy," in that the mechanisms mediating its efficacy and the potential

undesired effects are insufficiently understood. A challenging but important next step will be to rationally determine specific microbial consortia or metabolites that can be administered in a minimally invasive and efficient way. The growing global threat of antibiotics resistance warrants novel antibacterial strategies, whereby a narrower host range than that covered by classical antibiotics is desirable. Recombinant bacteriophages may offer an attractive alternative. However, more efforts in basic biology and RCTs are urgently required. To summarize, a more holistic view on the pathophysiology of infectious diseases and deeper mechanistic understanding of how the microbiota interacts with pathogens and confers antimicrobial resistance hold great potential to tackle the multiple challenges posed by infectious diseases in the 21st century.

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All authors researched data for the article; made substantial contributions to discussion of content; and wrote, reviewed, and edited the manuscript before submission.

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E.E. is a paid scientific consultant for DayTwo and BiomX. The other authors declare no competing interests.

REFERENCES

Abt, M.C., McKenney, P.T., and Pamer, E.G. (2016). Clostridium difficile colitis: pathogenesis and host defence. Nat. Rev. Microbiol. *14*, 609–620.

Agus, A., Denizot, J., Thévenot, J., Martinez-Medina, M., Massier, S., Sauvanet, P., Bernalier-Donadille, A., Denis, S., Hofman, P., Bonnet, R., et al. (2016). Western diet induces a shift in microbiota composition enhancing susceptibility to adherent-invasive *E. coli* infection and intestinal inflammation. Sci. Rep. *6*, 19032.

Ahmad, S., Wang, B., Walker, M.D., Tran, H.R., Stogios, P.J., Savchenko, A., Grant, R.A., McArthur, A.G., Laub, M.T., and Whitney, J.C. (2019). An interbacterial toxin inhibits target cell growth by synthesizing (p)ppApp. Nature *575*, 674–678.

Aksoy, E., Albarani, V., Nguyen, M., Laes, J.F., Ruelle, J.L., De Wit, D., Willems, F., Goldman, M., and Goriely, S. (2007). Interferon regulatory factor 3-dependent responses to lipopolysaccharide are selectively blunted in cord blood cells. Blood *109*, 2887–2893.

Al-Asmakh, M., and Zadjali, F. (2015). Use of germ-free animal models in microbiota-related research. J. Microbiol. Biotechnol. *25*, 1583–1588.

Andreas, N.J., Kampmann, B., and Mehring Le-Doare, K. (2015). Human breast milk: a review on its composition and bioactivity. Early Hum. Dev. *91*, 629–635.

Anjuwon-Foster, B.R., and Tamayo, R. (2018). Phase variation of *Clostridium difficile* virulence factors. Gut Microbes *9*, 76–83.

Bacher, P., Hohnstein, T., Beerbaum, E., Röcker, M., Blango, M.G., Kaufmann, S., Röhmel, J., Eschenhagen, P., Grehn, C., Seidel, K., et al. (2019). Human anti-fungal Th17 immunity and pathology rely on cross-reactivity against *Candida albicans*. Cell *176*, 1340–1355.e15.

Barton, E.S., White, D.W., Cathelyn, J.S., Brett-McClellan, K.A., Engle, M., Diamond, M.S., Miller, V.L., and Virgin, H.W., 4th (2007). Herpesvirus latency confers symbiotic protection from bacterial infection. Nature *447*, 326–329.

Basler, M., and Mekalanos, J.J. (2012). Type 6 secretion dynamics within and between bacterial cells. Science *337*, 815.

Basler, M., Ho, B.T., and Mekalanos, J.J. (2013). Tit-for-tat: type VI secretion system counterattack during bacterial cell-cell interactions. Cell *152*, 884–894.

Bastos, M.do.C., Coelho, M.L., and Santos, O.C. (2015). Resistance to bacteriocins produced by Gram-positive bacteria. Microbiology *161*, 683–700.

Bauer, H., Horowitz, R.E., Levenson, S.M., and Popper, H. (1963). The response of the lymphatic tissue to the microbial flora. Studies on germfree mice. Am. J. Pathol. *42*, 471–483.

Bedi, M.S., Verma, V., and Chhibber, S. (2009). Amoxicillin and specific bacteriophage can be used together for eradication of biofilm of *Klebsiella pneumoniae* B5055. World J. Microbiol. Biotechnol. *25*, 1145–1151.

Bhattacharya, S., Baidya, A.K., Pal, R.R., Mamou, G., Gatt, Y.E., Margalit, H., Rosenshine, I., and Ben-Yehuda, S. (2019). A ubiquitous platform for bacterial nanotube biogenesis. Cell Rep. *27*, 334–342.e10.

Bhutta, Z.A., and Black, R.E. (2013). Global maternal, newborn, and child health–so near and yet so far. N. Engl. J. Med. *369*, 2226–2235.

Bohnhoff, M., Drake, B.L., and Miller, C.P. (1955-1956). The effect of an antibiotic on the susceptibility of the mouse's intestinal tract to Salmonella infection. Antibiot. Annu. *3*, 453–455.

Brandl, K., Plitas, G., Schnabl, B., DeMatteo, R.P., and Pamer, E.G. (2007). MyD88-mediated signals induce the bactericidal lectin RegIII γ and protect mice against intestinal *Listeria monocytogenes* infection. J. Exp. Med. *204*, 1891–1900.

Brandl, K., Plitas, G., Mihu, C.N., Ubeda, C., Jia, T., Fleisher, M., Schnabl, B., DeMatteo, R.P., and Pamer, E.G. (2008). Vancomycin-resistant enterococci exploit antibiotic-induced innate immune deficits. Nature *455*, 804–807.

Brandtzaeg, P. (2010). The mucosal immune system and its integration with the mammary glands. J. Pediatr. *156*, S8–S15.

Buffie, C.G., Bucci, V., Stein, R.R., McKenney, P.T., Ling, L., Gobourne, A., No, D., Liu, H., Kinnebrew, M., Viale, A., et al. (2015). Precision microbiome reconstitution restores bile acid mediated resistance to *Clostridium difficile*. Nature *517*, 205–208.

Caballero, S., Kim, S., Carter, R.A., Leiner, I.M., Sušac, B., Miller, L., Kim, G.J., Ling, L., and Pamer, E.G. (2017). Cooperating commensals restore colonization resistance to vancomycin-resistant *Enterococcus faecium*. Cell Host Microbe *21*, 592–602.e4.

Caballero-Flores, G., Sakamoto, K., Zeng, M.Y., Wang, Y., Hakim, J., Matus-Acuña, V., Inohara, N., and Núñez, G. (2019). Maternal immunization confers protection to the offspring against an attaching and effacing pathogen through delivery of IgG in breast milk. Cell Host Microbe *25*, 313–323.e4.

Cassat, J.E., and Skaar, E.P. (2013). Iron in infection and immunity. Cell Host Microbe *13*, 509–519.

Cerasi, M., Ammendola, S., and Battistoni, A. (2013). Competition for zinc binding in the host-pathogen interaction. Front. Cell. Infect. Microbiol. *3*, 108.

Chatzidaki-Livanis, M., Geva-Zatorsky, N., and Comstock, L.E. (2016). *Bacteroides fragilis* type VI secretion systems use novel effector and immunity proteins to antagonize human gut Bacteroidales species. Proc. Natl. Acad. Sci. USA *113*, 3627–3632.

Chatzidaki-Livanis, M., Coyne, M.J., Roelofs, K.G., Gentyala, R.R., Caldwell, J.M., and Comstock, L.E. (2017). Gut symbiont *Bacteroides fragilis* secretes a eukaryotic-like ubiquitin protein that mediates intraspecies antagonism. MBio *8*, e01902-17.

Chen, H., Nwe, P.K., Yang, Y., Rosen, C.E., Bielecka, A.A., Kuchroo, M., Cline, G.W., Kruse, A.C., Ring, A.M., Crawford, J.M., and Palm, N.W. (2019). A forward chemical genetic screen reveals gut microbiota metabolites that modulate host physiology. Cell *177*, 1217–1231.e18.

Cherrington, C.A., Hinton, M., Pearson, G.R., and Chopra, I. (1991). Short-chain organic acids at ph 5.0 kill *Escherichia coli* and *Salmonella* spp. without causing membrane perturbation. J. Appl. Bacteriol. *70*, 161–165.

Chudnovskiy, A., Mortha, A., Kana, V., Kennard, A., Ramirez, J.D., Rahman, A., Remark, R., Mogno, I., Ng, R., Gnjatic, S., et al. (2016). Host-protozoan interactions protect from mucosal infections through activation of the inflammasome. Cell *167*, 444–456.e14.

Chung, H., Pamp, S.J., Hill, J.A., Surana, N.K., Edelman, S.M., Troy, E.B., Reading, N.C., Villablanca, E.J., Wang, S., Mora, J.R., et al. (2012). Gut immune maturation depends on colonization with a host-specific microbiota. Cell *149*, 1578–1593.

Clark, M.A., Hirst, B.H., and Jepson, M.A. (1998). M-cell surface β1 integrin expression and invasin-mediated targeting of *Yersinia pseudotuberculosis* to mouse Peyer's patch M cells. Infect. Immun. *66*, 1237–1243.

Collins, J., Robinson, C., Danhof, H., Knetsch, C.W., van Leeuwen, H.C., Lawley, T.D., Auchtung, J.M., and Britton, R.A. (2018). Dietary trehalose enhances virulence of epidemic *Clostridium difficile*. Nature *553*, 291–294.

Costeloe, K., Hardy, P., Juszczak, E., Wilks, M., and Millar, M.R.; Probiotics in Preterm Infants Study Collaborative Group (2016). *Bifidobacterium breve* BBG-001 in very preterm infants: a randomised controlled phase 3 trial. Lancet *387*, 649–660.

Cross, K.L., Campbell, J.H., Balachandran, M., Campbell, A.G., Cooper, S.J., Griffen, A., Heaton, M., Joshi, S., Klingeman, D., Leys, E., et al. (2019). Targeted isolation and cultivation of uncultivated bacteria by reverse genomics. Nat. Biotechnol. *37*, 1314–1321.

Cullen, T.W., Schofield, W.B., Barry, N.A., Putnam, E.E., Rundell, E.A., Trent, M.S., Degnan, P.H., Booth, C.J., Yu, H., and Goodman, A.L. (2015). Antimicrobial peptide resistance mediates resilience of prominent gut commensals during inflammation. Science *347*, 170–175.

Curtis, M.M., Hu, Z., Klimko, C., Narayanan, S., Deberardinis, R., and Sperandio, V. (2014). The gut commensal *Bacteroides thetaiotaomicron* exacerbates enteric infection through modification of the metabolic landscape. Cell Host Microbe *16*, 759–769.

Davido, B., Batista, R., Dinh, A., de Truchis, P., Terveer, E.M., Roberts, B., Kuijper, E.J., and Caballero, S. (2019). Fifty shades of graft: how to improve the efficacy of faecal microbiota transplantation for decolonization of antibiotic-resistant bacteria. Int. J. Antimicrob. Agents *53*, 553–556.

de Goffau, M.C., Lager, S., Sovio, U., Gaccioli, F., Cook, E., Peacock, S.J., Parkhill, J., Charnock-Jones, D.S., and Smith, G.C.S. (2019). Human placenta has no microbiome but can contain potential pathogens. Nature *572*, 329–334.

Dedrick, R.M., Guerrero-Bustamante, C.A., Garlena, R.A., Russell, D.A., Ford, K., Harris, K., Gilmour, K.C., Soothill, J., Jacobs-Sera, D., Schooley, R.T., et al. (2019). Engineered bacteriophages for treatment of a patient with a disseminated drug-resistant *Mycobacterium abscessus*. Nat. Med. *25*, 730–733.

DeFilipp, Z., Bloom, P.P., Torres Soto, M., Mansour, M.K., Sater, M.R.A., Huntley, M.H., Turbett, S., Chung, R.T., Chen, Y.-B., and Hohmann, E.L. (2019). Drug-resistant *E. coli* bacteremia transmitted by fecal microbiota transplant. N. Engl. J. Med. *381*, 2043–2050.

Deriu, E., Liu, J.Z., Pezeshki, M., Edwards, R.A., Ochoa, R.J., Contreras, H., Libby, S.J., Fang, F.C., and Raffatellu, M. (2013). Probiotic bacteria reduce *Salmonella* Typhimurium intestinal colonization by competing for iron. Cell Host Microbe *14*, 26–37.

Desai, K., Gupta, S.B., Dubberke, E.R., Prabhu, V.S., Browne, C., and Mast, T.C. (2016a). Epidemiological and economic burden of *Clostridium difficile* in the United States: estimates from a modeling approach. BMC Infect. Dis. *16*, 303.

Desai, M.S., Seekatz, A.M., Koropatkin, N.M., Kamada, N., Hickey, C.A., Wolter, M., Pudlo, N.A., Kitamoto, S., Terrapon, N., Muller, A., et al. (2016b). A dietary fiber-deprived gut microbiota degrades the colonic mucus barrier and enhances pathogen susceptibility. Cell *167*, 1339–1353.e21.

Dissanayake, U., Ukhanova, M., Moye, Z.D., Sulakvelidze, A., and Mai, V. (2019). Bacteriophages reduce pathogenic *Escherichia coli* counts in mice without distorting gut microbiota. Front. Microbiol. *10*, 1984.

Dominguez-Bello, M.G., Costello, E.K., Contreras, M., Magris, M., Hidalgo, G., Fierer, N., and Knight, R. (2010). Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. Proc. Natl. Acad. Sci. USA *107*, 11971–11975.

Donaldson, G.P., Ladinsky, M.S., Yu, K.B., Sanders, J.G., Yoo, B.B., Chou, W.-C., Conner, M.E., Earl, A.M., Knight, R., Bjorkman, P.J., et al. (2018). Gut microbiota utilize immunoglobulin A for mucosal colonization. Science *360*, 795–800.

Dong, C. (2008). TH17 cells in development: an updated view of their molecular identity and genetic programming. Nat. Rev. Immunol. *8*, 337–348.

Donia, M.S., and Fischbach, M.A. (2015). Small molecules from the human microbiota. Science *349*, 1254766.

Donia, M.S., Cimermancic, P., Schulze, C.J., Wieland Brown, L.C., Martin, J., Mitreva, M., Clardy, J., Linington, R.G., and Fischbach, M.A. (2014). A systematic analysis of biosynthetic gene clusters in the human microbiome reveals a common family of antibiotics. Cell *158*, 1402–1414.

Duan, Y., Llorente, C., Lang, S., Brandl, K., Chu, H., Jiang, L., White, R.C., Clarke, T.H., Nguyen, K., Torralba, M., et al. (2019). Bacteriophage targeting of gut bacterium attenuates alcoholic liver disease. Nature *575*, 505–511.

Duyvejonck, H., Merabishvili, M., Pirnay, J.-P., De Vos, D., Verbeken, G., Van Belleghem, J., Gryp, T., De Leenheer, J., Van der Borght, K., Van Simaey, L., et al. (2019). Development of a qPCR platform for quantification of the five bacteriophages within bacteriophage cocktail 2 (BFC2). Sci. Rep. *9*, 13893.

Eisenhofer, R., Minich, J.J., Marotz, C., Cooper, A., Knight, R., and Weyrich, L.S. (2019). Contamination in low microbial biomass microbiome studies: issues and recommendations. Trends Microbiol. *27*, 105–117.

Elahi, S., Ertelt, J.M., Kinder, J.M., Jiang, T.T., Zhang, X., Xin, L., Chaturvedi, V., Strong, B.S., Qualls, J.E., Steinbrecher, K.A., et al. (2013). Immunosuppressive CD71+ erythroid cells compromise neonatal host defence against infection. Nature *504*, 158–162.

Fanning, S., Hall, L.J., Cronin, M., Zomer, A., MacSharry, J., Goulding, D., Motherway, M.O., Shanahan, F., Nally, K., Dougan, G., and van Sinderen, D. (2012). Bifidobacterial surface-exopolysaccharide facilitates commensal-host interaction through immune modulation and pathogen protection. Proc. Natl. Acad. Sci. USA *109*, 2108–2113.

Feehley, T., Plunkett, C.H., Bao, R., Choi Hong, S.M., Culleen, E., Belda-Ferre, P., Campbell, E., Aitoro, R., Nocerino, R., Paparo, L., et al. (2019). Healthy infants harbor intestinal bacteria that protect against food allergy. Nat. Med. *25*, 448–453.

Fonseca, D.M., Hand, T.W., Han, S.J., Gerner, M.Y., Glatman Zaretsky, A., Byrd, A.L., Harrison, O.J., Ortiz, A.M., Quinones, M., Trinchieri, G., et al. (2015). Microbiota-dependent sequelae of acute infection compromise tissue-specific immunity. Cell *163*, 354–366.

Gaboriau-Routhiau, V., Rakotobe, S., Lécuyer, E., Mulder, I., Lan, A., Bridonneau, C., Rochet, V., Pisi, A., De Paepe, M., Brandi, G., et al. (2009). The key role of segmented filamentous bacteria in the coordinated maturation of gut helper T cell responses. Immunity *31*, 677–689.

Gantois, I., Ducatelle, R., Pasmans, F., Haesebrouck, F., Hautefort, I., Thompson, A., Hinton, J.C., and Van Immerseel, F. (2006). Butyrate specifically down-regulates salmonella pathogenicity island 1 gene expression. Appl. Environ. Microbiol. *72*, 946–949.

Gehrig, J.L., Venkatesh, S., Chang, H.-W., Hibberd, M.C., Kung, V.L., Cheng, J., Chen, R.Y., Subramanian, S., Cowardin, C.A., Meier, M.F., et al. (2019). Effects of microbiota-directed foods in gnotobiotic animals and undernourished children. Science *365*, eaau4732.

Gensollen, T., Iyer, S.S., Kasper, D.L., and Blumberg, R.S. (2016). How colonization by microbiota in early life shapes the immune system. Science *352*, 539–544.

Gibson, G.R., Hutkins, R., Sanders, M.E., Prescott, S.L., Reimer, R.A., Salminen, S.J., Scott, K., Stanton, C., Swanson, K.S., Cani, P.D., et al. (2017). Expert consensus document: the International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. Nat. Rev. Gastroenterol. Hepatol. *14*, 491–502.

Gielda, L.M., and DiRita, V.J. (2012). Zinc competition among the intestinal microbiota. MBio *3*, e00171-12.

Gillis, C.C., Hughes, E.R., Spiga, L., Winter, M.G., Zhu, W., Furtado de Carvalho, T., Chanin, R.B., Behrendt, C.L., Hooper, L.V., Santos, R.L., and Winter, S.E. (2018). Dysbiosis-associated change in host metabolism generates lactate to support *Salmonella* growth. Cell Host Microbe *23*, 54–64.e6.

Gomez de Agüero, M., Ganal-Vonarburg, S.C., Fuhrer, T., Rupp, S., Uchimura, Y., Li, H., Steinert, A., Heikenwalder, M., Hapfelmeier, S., Sauer, U., et al. (2016). The maternal microbiota drives early postnatal innate immune development. Science *351*, 1296–1302.

Goodwin, M.M., Canny, S., Steed, A., and Virgin, H.W. (2010). Murine gammaherpesvirus 68 has evolved gamma interferon and stat1-repressible promoters for the lytic switch gene 50. J. Virol. *84*, 3711–3717.

Goto, Y., Obata, T., Kunisawa, J., Sato, S., Ivanov, I.I., Lamichhane, A., Takeyama, N., Kamioka, M., Sakamoto, M., Matsuki, T., et al. (2014). Innate lymphoid cells regulate intestinal epithelial cell glycosylation. Science *345*, 1254009.

Greiling, T.M., Dehner, C., Chen, X., Hughes, K., Iñiguez, A.J., Boccitto, M., Ruiz, D.Z., Renfroe, S.C., Vieira, S.M., Ruff, W.E., et al. (2018). Commensal orthologs of the human autoantigen Ro60 as triggers of autoimmunity in lupus. Sci. Transl. Med. *10*, eaan2306.

Grizotte-Lake, M., Zhong, G., Duncan, K., Kirkwood, J., Iyer, N., Smolenski, I., Isoherranen, N., and Vaishnava, S. (2018). Commensals suppress intestinal epithelial cell retinoic acid synthesis to regulate interleukin-22 activity and prevent microbial dysbiosis. Immunity *49*, 1103–1115.e6.

Hagan, T., Cortese, M., Rouphael, N., Boudreau, C., Linde, C., Maddur, M.S., Das, J., Wang, H., Guthmiller, J., Zheng, N.-Y., et al. (2019). Antibiotics-driven gut microbiome perturbation alters immunity to vaccines in humans. Cell *178*, 1313–1328.e13.

Hakkarainen, J., Toivanen, M., Leinonen, A., Frängsmyr, L., Strömberg, N., Lapinjoki, S., Nassif, X., and Tikkanen-Kaukanen, C. (2005). Human and bovine milk oligosaccharides inhibit *Neisseria meningitidis* pili attachment in vitro. J. Nutr. *135*, 2445–2448.

Hanson, L.Å., and Winberg, J. (1972). Breast milk and defence against infection in the newborn. Arch. Dis. Child. *47*, 845–848.

Hao, Q., Lu, Z., Dong, B.R., Huang, C.Q., and Wu, T. (2011). Probiotics for preventing acute upper respiratory tract infections. Cochrane Database Syst. Rev. *9*, CD006895, https://doi.org/10.1002/14651858.CD006895.pub2.

Heikkilä, M.P., and Saris, P.E.J. (2003). Inhibition of *Staphylococcus aureus* by the commensal bacteria of human milk. J. Appl. Microbiol. *95*, 471–478.

Herp, S., Brugiroux, S., Garzetti, D., Ring, D., Jochum, L.M., Beutler, M., Eberl, C., Hussain, S., Walter, S., Gerlach, R.G., et al. (2019). *Mucispirillum schaedleri* antagonizes *Salmonella* virulence to protect mice against colitis. Cell Host Microbe *25*, 681–694.e8.

Hill, C., Guarner, F., Reid, G., Gibson, G.R., Merenstein, D.J., Pot, B., Morelli, L., Canani, R.B., Flint, H.J., Salminen, S., et al. (2014). Expert consensus document. the International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat. Rev. Gastroenterol. Hepatol. *11*, 506–514.

Hui, W., Li, T., Liu, W., Zhou, C., and Gao, F. (2019). Fecal microbiota transplantation for treatment of recurrent *C. difficile* infection: an updated randomized controlled trial meta-analysis. PLoS ONE *14*, e0210016.

Huttner, B.D., de Lastours, V., Wassenberg, M., Maharshak, N., Mauris, A., Galperine, T., Zanichelli, V., Kapel, N., Bellanger, A., Olearo, F., et al. (2019). A 5-day course of oral antibiotics followed by faecal transplantation to eradicate carriage of multidrug-resistant Enterobacteriaceae: a randomized clinical trial. Clin. Microbiol. Infect. *25*, 830–838.

Ivanov, I.I., McKenzie, B.S., Zhou, L., Tadokoro, C.E., Lepelley, A., Lafaille, J.J., Cua, D.J., and Littman, D.R. (2006). The orphan nuclear receptor RORgammat directs the differentiation program of proinflammatory IL-17+ T helper cells. Cell *126*, 1121–1133.

Ivanov, I.I., Frutos, R. de L., Manel, N., Yoshinaga, K., Rifkin, D.B., Sartor, R.B., Finlay, B.B., and Littman, D.R. (2008). Specific microbiota direct the differentiation of IL-17-producing T-helper cells in the mucosa of the small intestine. Cell Host Microbe *4*, 337–349.

Ivanov, I.I., Atarashi, K., Manel, N., Brodie, E.L., Shima, T., Karaoz, U., Wei, D., Goldfarb, K.C., Santee, C.A., Lynch, S.V., et al. (2009). Induction of intestinal Th17 cells by segmented filamentous bacteria. Cell *139*, 485–498.

Jacobson, A., Lam, L., Rajendram, M., Tamburini, F., Honeycutt, J., Pham, T., Van Treuren, W., Pruss, K., Stabler, S.R., Lugo, K., et al. (2018). A gut commensal-produced metabolite mediates colonization resistance to *Salmonella* infection. Cell Host Microbe *24*, 296–307.e7.

Jara, S., Sánchez, M., Vera, R., Cofré, J., and Castro, E. (2011). The inhibitory activity of Lactobacillus spp. isolated from breast milk on gastrointestinal pathogenic bacteria of nosocomial origin. Anaerobe *17*, 474–477.

Jault, P., Leclerc, T., Jennes, S., Pirnay, J.P., Que, Y.A., Resch, G., Rousseau, A.F., Ravat, F., Carsin, H., Le Floch, R., et al. (2019). Efficacy and tolerability of a cocktail of bacteriophages to treat burn wounds infected by *Pseudomonas aeruginosa* (PhagoBurn): a randomised, controlled, double-blind phase 1/2 trial. Lancet Infect. Dis. *19*, 35–45.

Jennes, S., Merabishvili, M., Soentjens, P., Pang, K.W., Rose, T., Keersebilck, E., Soete, O., François, P.M., Teodorescu, S., Verween, G., et al. (2017). Use of bacteriophages in the treatment of colistin-only-sensitive *Pseudomonas aeruginosa* septicaemia in a patient with acute kidney injury—a case report. Crit. Care *21*, 129.

Jiang, T.T., Shao, T.Y., Ang, W.X.G., Kinder, J.M., Turner, L.H., Pham, G., Whitt, J., Alenghat, T., and Way, S.S. (2017). Commensal fungi recapitulate the protective benefits of intestinal bacteria. Cell Host Microbe *22*, 809–816.e4.

Jiménez, E., Fernández, L., Marín, M.L., Martín, R., Odriozola, J.M., Nueno-Palop, C., Narbad, A., Olivares, M., Xaus, J., and Rodríguez, J.M. (2005). Isolation of commensal bacteria from umbilical cord blood of healthy neonates born by cesarean section. Curr. Microbiol. *51*, 270–274.

Jones, M.K., Watanabe, M., Zhu, S., Graves, C.L., Keyes, L.R., Grau, K.R., Gonzalez-Hernandez, M.B., Iovine, N.M., Wobus, C.E., Vinjé, J., et al. (2014). Enteric bacteria promote human and mouse norovirus infection of B cells. Science *346*, 755–759.

Juul, F.E., Garborg, K., Bretthauer, M., Skudal, H., Øines, M.N., Wiig, H., Rose, Ø., Seip, B., Lamont, J.T., Midtvedt, T., et al. (2018). Fecal microbiota transplantation for primary *Clostridium difficile* infection. N. Engl. J. Med. *378*, 2535–2536.

Kane, M., Case, L.K., Kopaskie, K., Kozlova, A., MacDearmid, C., Chervonsky, A.V., and Golovkina, T.V. (2011). Successful transmission of a retrovirus depends on the commensal microbiota. Science *334*, 245–249.

Kelly, C.R., Fischer, M., Grinspan, A., and Allegretti, J.R. (2019). Patients eligible for trials of microbiota-based therapeutics do not represent the population with recurrent *Clostridioides difficile* infection. Clin. Gastroenterol. Hepatol. *114*, S1558–S1560.

Kelly, C.J., Zheng, L., Campbell, E.L., Saeedi, B., Scholz, C.C., Bayless, A.J., Wilson, K.E., Glover, L.E., Kominsky, D.J., Magnuson, A., et al. (2015). Crosstalk between microbiota-derived short-chain fatty acids and intestinal epithelial HIF augments tissue barrier function. Cell Host Microbe *17*, 662–671.

Kernbauer, E., Ding, Y., and Cadwell, K. (2014). An enteric virus can replace the beneficial function of commensal bacteria. Nature *516*, 94–98.

Khosravi, A., Yáñez, A., Price, J.G., Chow, A., Merad, M., Goodridge, H.S., and Mazmanian, S.K. (2014). Gut microbiota promote hematopoiesis to control bacterial infection. Cell Host Microbe *15*, 374–381.

Kim, Y.G., Sakamoto, K., Seo, S.U., Pickard, J.M., Gillilland, M.G., Pudlo, N.A., Hoostal, M., Li, X., Wang, T.D., Feehley, T., et al. (2017). Neonatal acquisition of Clostridia species protects against colonization by bacterial pathogens. Science *356*, 315–319.

Kim, S.G., Becattini, S., Moody, T.U., Shliaha, P.V., Littmann, E.R., Seok, R., Gjonbalaj, M., Eaton, V., Fontana, E., Amoretti, L., et al. (2019a). Microbiota-derived lantibiotic restores resistance against vancomycin-resistant *Enterococcus*. Nature *572*, 665–669.

Kim, W.J., Higashi, D., Goytia, M., Rendón, M.A., Pilligua-Lucas, M., Bronnimann, M., McLean, J.A., Duncan, J., Trees, D., Jerse, A.E., and So, M.

(2019b). Commensal *Neisseria* kill *Neisseria gonorrhoeae* through a DNA-dependent mechanism. Cell Host Microbe *26*, 228–239.e8.

Knight, R., Vrbanac, A., Taylor, B.C., Aksenov, A., Callewaert, C., Debelius, J., Gonzalez, A., Kosciolek, T., McCall, L.I., McDonald, D., et al. (2018). Best practices for analysing microbiomes. Nat. Rev. Microbiol. *16*, 410–422.

Kollmann, T.R., Crabtree, J., Rein-Weston, A., Blimkie, D., Thommai, F., Wang, X.Y., Lavoie, P.M., Furlong, J., Fortuno, E.S., 3rd, Hajjar, A.M., et al. (2009). Neonatal innate TLR-mediated responses are distinct from those of adults. J. Immunol. *183*, 7150–7160.

Kollmann, T.R., Levy, O., Montgomery, R.R., and Goriely, S. (2012). Innate immune function by Toll-like receptors: distinct responses in newborns and the elderly. Immunity *37*, 771–783.

Kollmann, T.R., Kampmann, B., Mazmanian, S.K., Marchant, A., and Levy, O. (2017). Protecting the newborn and young infant from infectious diseases: lessons from immune ontogeny. Immunity *46*, 350–363.

Kolodziejczyk, A.A., Zheng, D., and Elinav, E. (2019). Diet-microbiota interactions and personalized nutrition. Nat. Rev. Microbiol. *17*, 742–753.

Kommineni, S., Bretl, D.J., Lam, V., Chakraborty, R., Hayward, M., Simpson, P., Cao, Y., Bousounis, P., Kristich, C.J., and Salzman, N.H. (2015). Bacteriocin production augments niche competition by enterococci in the mammalian gastrointestinal tract. Nature *526*, 719–722.

Kortright, K.E., Chan, B.K., Koff, J.L., and Turner, P.E. (2019). Phage therapy: a renewed approach to combat antibiotic-resistant bacteria. Cell Host Microbe *25*, 219–232.

Kuss, S.K., Best, G.T., Etheredge, C.A., Pruijssers, A.J., Frierson, J.M., Hooper, L.V., Dermody, T.S., and Pfeiffer, J.K. (2011). Intestinal microbiota promote enteric virus replication and systemic pathogenesis. Science *334*, 249–252.

Lagier, J.C., Armougom, F., Million, M., Hugon, P., Pagnier, I., Robert, C., Bittar, F., Fournous, G., Gimenez, G., Maraninchi, M., et al. (2012). Microbial culturomics: paradigm shift in the human gut microbiome study. Clin. Microbiol. Infect. *18*, 1185–1193.

Lagier, J.-C., Dubourg, G., Million, M., Cadoret, F., Bilen, M., Fenollar, F., Levasseur, A., Rolain, J.-M., Fournier, P.-E., and Raoult, D. (2018). Culturing the human microbiota and culturomics. Nat. Rev. Microbiol. *16*, 540–550.

Lawhon, S.D., Maurer, R., Suyemoto, M., and Altier, C. (2002). Intestinal short-chain fatty acids alter *Salmonella typhimurium* invasion gene expression and virulence through BarA/SirA. Mol. Microbiol. *46*, 1451–1464.

Lawley, T.D., and Walker, A.W. (2013). Intestinal colonization resistance. Immunology *138*, 1–11.

Laxminarayan, R., Duse, A., Wattal, C., Zaidi, A.K.M., Wertheim, H.F.L., Sumpradit, N., Vlieghe, E., Hara, G.L., Gould, I.M., Goossens, H., et al. (2013). Antibiotic resistance-the need for global solutions. Lancet Infect. Dis. *13*, 1057–1098.

Leatham, M.P., Banerjee, S., Autieri, S.M., Mercado-Lubo, R., Conway, T., and Cohen, P.S. (2009). Precolonized human commensal *Escherichia coli* strains serve as a barrier to *E. coli* O157:H7 growth in the streptomycin-treated mouse intestine. Infect. Immun. *77*, 2876–2886.

Lee, C.H., Steiner, T., Petrof, E.O., Smieja, M., Roscoe, D., Nematallah, A., Weese, J.S., Collins, S., Moayyedi, P., Crowther, M., et al. (2016). Frozen vs fresh fecal microbiota transplantation and clinical resolution of diarrhea in patients with recurrent clostridium difficile infection a randomized clinical trial. JAMA *315*, 142–149.

Leffler, D.A., and Lamont, J.T. (2015). *Clostridium difficile* infection. N. Engl. J. Med. *372*, 1539–1548.

Lev-Sagie, A., Goldman-Wohl, D., Cohen, Y., Dori-Bachash, M., Leshem, A., Mor, U., Strahilevitz, J., Moses, A.E., Shapiro, H., Yagel, S., and Elinav, E. (2019). Vaginal microbiome transplantation in women with intractable bacterial vaginosis. Nat. Med. *25*, 1500–1504.

Levine, A., Wine, E., Assa, A., Sigall Boneh, R., Shaoul, R., Kori, M., Cohen, S., Peleg, S., Shamaly, H., On, A., et al. (2019). Crohn's disease exclusion diet plus partial enteral nutrition induces sustained remission in a randomized controlled trial. Gastroenterology *157*, 440–450.e8.

Levy, M., Thaiss, C.A., Zeevi, D., Dohnalová, L., Zilberman-Schapira, G., Mahdi, J.A., David, E., Savidor, A., Korem, T., Herzig, Y., et al. (2015). Microbiota-modulated metabolites shape the intestinal microenvironment by regulating NLRP6 inflammasome signaling. Cell *163*, 1428–1443.

Ley, R.E., Hamady, M., Lozupone, C., Turnbaugh, P.J., Ramey, R.R., Bircher, J.S., Schlegel, M.L., Tucker, T.A., Schrenzel, M.D., Knight, R., and Gordon, J.I. (2008). Evolution of mammals and their gut microbes. Science *320*, 1647–1651.

Liang, S., Guo, X.K., Ou, J., Huang, R., Xue, Q., Zhang, B., Chung, Y., Wu, W., Dong, C., Yang, X., and Hu, X. (2019). Nutrient sensing by the intestinal epithelium orchestrates mucosal antimicrobial defense via translational control of Hes1. Cell Host Microbe *25*, 706–718.e7.

Littman, D.R., and Pamer, E.G. (2011). Role of the commensal microbiota in normal and pathogenic host immune responses. Cell Host Microbe *10*, 311–323.

Litvak, Y., Mon, K.K.Z., Nguyen, H., Chanthavixay, G., Liou, M., Velazquez, E.M., Kutter, L., Alcantara, M.A., Byndloss, M.X., Tiffany, C.R., et al. (2019). Commensal *Enterobacteriaceae* protect against *Salmonella* colonization through oxygen competition. Cell Host Microbe *25*, 128–139.e5.

Lundberg, R., Toft, M.F., August, B., Hansen, A.K., and Hansen, C.H.F. (2016). Antibiotic-treated versus germ-free rodents for microbiota transplantation studies. Gut Microbes *7*, 68–74.

Luoto, R., Ruuskanen, O., Waris, M., Kalliomäki, M., Salminen, S., and Isolauri, E. (2014). Prebiotic and probiotic supplementation prevents rhinovirus infections in preterm infants: a randomized, placebo-controlled trial. J. Allergy Clin. Immunol. *133*, 405–413.

Macpherson, A.J., McCoy, K.D., Johansen, F.E., and Brandtzaeg, P. (2008). The immune geography of IgA induction and function. Mucosal Immunol. *1*, 11–22.

Makki, K., Deehan, E.C., Walter, J., and Bäckhed, F. (2018). The impact of dietary fiber on gut microbiota in host health and disease. Cell Host Microbe *23*, 705–715.

Martens, E.C., Neumann, M., and Desai, M.S. (2018). Interactions of commensal and pathogenic microorganisms with the intestinal mucosal barrier. Nat. Rev. Microbiol. *16*, 457–470.

Marteyn, B., West, N.P., Browning, D.F., Cole, J.A., Shaw, J.G., Palm, F., Mounier, J., Prévost, M.-C., Sansonetti, P., and Tang, C.M. (2010). Modulation of *Shigella* virulence in response to available oxygen *in vivo*. Nature *465*, 355–358.

Martín, R., Langa, S., Reviriego, C., Jimínez, E., Marín, M.L., Xaus, J., Fernández, L., and Rodríguez, J.M. (2003). Human milk is a source of lactic acid bacteria for the infant gut. J. Pediatr. *143*, 754–758.

Martín, R., Jiménez, E., Heilig, H., Fernández, L., Marín, M.L., Zoetendal, E.G., and Rodríguez, J.M. (2009). Isolation of bifidobacteria from breast milk and assessment of the bifidobacterial population by PCR-denaturing gradient gel electrophoresis and quantitative real-time PCR. Appl. Environ. Microbiol. *75*, 965–969.

McKenney, P.T., and Pamer, E.G. (2015). From hype to hope: the gut microbiota in enteric infectious disease. Cell *163*, 1326–1332.

Mengaud, J., Ohayon, H., Gounon, P., Mege R-M, and Cossart, P. (1996). E-cadherin is the receptor for internalin, a surface protein required for entry of L. monocytogenes into epithelial cells. Cell *84*, 923–932.

Meuskens, I., Saragliadis, A., Leo, J.C., and Linke, D. (2019). Type V secretion systems: an overview of passenger domain functions. Front. Microbiol. *10*, 1163.

Miki, T., Goto, R., Fujimoto, M., Okada, N., and Hardt, W.D. (2017). The bactericidal lectin RegIIIβ prolongs gut colonization and enteropathy in the streptomycin mouse model for *Salmonella* diarrhea. Cell Host Microbe *21*, 195–207.

Mirpuri, J., Raetz, M., Sturge, C.R., Wilhelm, C.L., Benson, A., Savani, R.C., Hooper, L.V., and Yarovinsky, F. (2014). Proteobacteria-specific IgA regulates maturation of the intestinal microbiota. Gut Microbes *5*, 28–39.

Moelling, K., Broecker, F., and Willy, C. (2018). A wake-up call: we need phage therapy now. Viruses *10*, E688.

Momose, Y., Hirayama, K., and Itoh, K. (2008). Competition for proline between indigenous *Escherichia coli* and *E. coli* O157:H7 in gnotobiotic mice associated with infant intestinal microbiota and its contribution to the colonization resistance against *E. coli* O157:H7. Antonie van Leeuwenhoek *94*, 165–171.

Monaco, C.L., Gootenberg, D.B., Zhao, G., Handley, S.A., Ghebremichael, M.S., Lim, E.S., Lankowski, A., Baldridge, M.T., Wilen, C.B., Flagg, M., et al. (2016). Altered virome and bacterial microbiome in human immunodeficiency virus-associated acquired immunodeficiency syndrome. Cell Host Microbe *19*, 311–322.

Moossavi, S., Sepehri, S., Robertson, B., Bode, L., Goruk, S., Field, C.J., Lix, L.M., de Souza, R.J., Becker, A.B., Mandhane, P.J., et al. (2019). Composition and variation of the human milk microbiota are influenced by maternal and early-life factors. Cell Host Microbe *25*, 324–335.e4.

Muegge, B.D., Kuczynski, J., Knights, D., Clemente, J.C., González, A., Fontana, L., Henrissat, B., Knight, R., and Gordon, J.I. (2011). Diet drives convergence in gut microbiome functions across mammalian phylogeny and within humans. Science *332*, 970–974.

Mullish, B.H., Quraishi, M.N., Segal, J.P., McCune, V.L., Baxter, M., Marsden, G.L., Moore, D.J., Colville, A., Bhala, N., Iqbal, T.H., et al. (2018). The use of faecal microbiota transplant as treatment for recurrent or refractory *Clostridium difficile* infection and other potential indications: joint British Society of Gastroenterology (BSG) and Healthcare Infection Society (HIS) guidelines. Gut *67*, 1920–1941.

Muzny, C.A., Taylor, C.M., Swords, W.E., Tamhane, A., Chattopadhyay, D., Cerca, N., and Schwebke, J.R. (2019). An updated conceptual model on the pathogenesis of bacterial vaginosis. J. Infect. Dis. *220*, 1399–1405.

Natividad, J.M.M., Hayes, C.L., Motta, J.P., Jury, J., Galipeau, H.J., Philip, V., Garcia-Rodenas, C.L., Kiyama, H., Bercik, P., and Verdú, E.F. (2013). Differential induction of antimicrobial REGIII by the intestinal microbiota and *Bifidobacterium breve* NCC2950. Appl. Environ. Microbiol. *79*, 7745–7754.

Ng, K.M., Aranda-Díaz, A., Tropini, C., Frankel, M.R., Van Treuren, W., O'Laughlin, C.T., Merrill, B.D., Yu, F.B., Pruss, K.M., Oliveira, R.A., et al. (2019). Recovery of the gut microbiota after antibiotics depends on host diet, community context, and environmental reservoirs. Cell Host Microbe *26*, 650–665.e4.

Nguyen, L.T., Haney, E.F., and Vogel, H.J. (2011). The expanding scope of antimicrobial peptide structures and their modes of action. Trends Biotechnol. *29*, 464–472.

Okumura, R., Kurakawa, T., Nakano, T., Kayama, H., Kinoshita, M., Motooka, D., Gotoh, K., Kimura, T., Kamiyama, N., Kusu, T., et al. (2016). Lypd8 promotes the segregation of flagellated microbiota and colonic epithelia. Nature *532*, 117–121.

Osborne, L.C., Monticelli, L.A., Nice, T.J., Sutherland, T.E., Siracusa, M.C., Hepworth, M.R., Tomov, V.T., Kobuley, D., Tran, S.V., Bittinger, K., et al. (2014). Virus-helminth co-infection reveals a microbiota-independent mechanism of immuno-modulation. Science *345*, 578–582.

Ott, S.J., Waetzig, G.H., Rehman, A., Moltzau-Anderson, J., Bharti, R., Grasis, J.A., Cassidy, L., Tholey, A., Fickenscher, H., Seegert, D., et al. (2017). Efficacy of sterile fecal filtrate transfer for treating patients with *Clostridium difficile* infection. Gastroenterology *152*, 799–811.e7.

Pal, R.R., Baidya, A.K., Mamou, G., Bhattacharya, S., Socol, Y., Kobi, S., Katsowich, N., Ben-Yehuda, S., and Rosenshine, I. (2019). Pathogenic *E. coli* extracts nutrients from infected host cells utilizing injectisome components. Cell *177*, 683–696.e18.

Panigrahi, P., Parida, S., Nanda, N.C., Satpathy, R., Pradhan, L., Chandel, D.S., Baccaglini, L., Mohapatra, A., Mohapatra, S.S., Misra, P.R., et al. (2017). A randomized synbiotic trial to prevent sepsis among infants in rural India. Nature *548*, 407–412.

Pannaraj, P.S., Li, F., Cerini, C., Bender, J.M., Yang, S., Rollie, A., Adisetiyo, H., Zabih, S., Lincez, P.J., Bittinger, K., et al. (2017). Association between breast milk bacterial communities and establishment and development of the infant gut microbiome. JAMA Pediatr. *171*, 647–654.

Park, Y., Subar, A.F., Hollenbeck, A., and Schatzkin, A. (2011). Dietary fiber intake and mortality in the NIH-AARP diet and health study. Arch. Intern. Med. *171*, 1061–1068.

Pickard, J.M., Maurice, C.F., Kinnebrew, M.A., Abt, M.C., Schenten, D., Golovkina, T.V., Bogatyrev, S.R., Ismagilov, R.F., Pamer, E.G., Turnbaugh, P.J., and Chervonsky, A.V. (2014). Rapid fucosylation of intestinal epithelium sustains host-commensal symbiosis in sickness. Nature *514*, 638–641.

Piewngam, P., Zheng, Y., Nguyen, T.H., Dickey, S.W., Joo, H.S., Villaruz, A.E., Glose, K.A., Fisher, E.L., Hunt, R.L., Li, B., et al. (2018). Pathogen elimination by probiotic *Bacillus* via signalling interference. Nature *562*, 532–537.

Pircalabioru, G., Aviello, G., Kubica, M., Zhdanov, A., Paclet, M.H., Brennan, L., Hertzberger, R., Papkovsky, D., Bourke, B., and Knaus, U.G. (2016). Defensive mutualism rescues NADPH oxidase inactivation in gut infection. Cell Host Microbe *19*, 651–663.

Quentin, D., Ahmad, S., Shanthamoorthy, P., Mougous, J.D., Whitney, J.C., and Raunser, S. (2018). Mechanism of loading and translocation of type VI secretion system effector Tse6. Nat. Microbiol. *3*, 1142–1152.

Raffatellu, M., George, M.D., Akiyama, Y., Hornsby, M.J., Nuccio, S.P., Paixao, T.A., Butler, B.P., Chu, H., Santos, R.L., Berger, T., et al. (2009). Lipocalin-2 resistance confers an advantage to *Salmonella enterica* serotype Typhimurium for growth and survival in the inflamed intestine. Cell Host Microbe *5*, 476–486.

Ramanan, D., Bowcutt, R., Lee, S.C., Tang, M.S., Kurtz, Z.D., Ding, Y., Honda, K., Gause, W.C., Blaser, M.J., Bonneau, R.A., et al. (2016). Helminth infection promotes colonization resistance via type 2 immunity. Science *352*, 608–612.

Rea, M.C., Sit, C.S., Clayton, E., O'Connor, P.M., Whittal, R.M., Zheng, J., Vederas, J.C., Ross, R.P., and Hill, C. (2010). Thuricin CD, a posttranslationally modified bacteriocin with a narrow spectrum of activity against *Clostridium difficile*. Proc. Natl. Acad. Sci. USA *107*, 9352–9357.

Rivera-Chávez, F., Zhang, L.F., Faber, F., Lopez, C.A., Byndloss, M.X., Olsan, E.E., Xu, G., Velazquez, E.M., Lebrilla, C.B., Winter, S.E., and Bäumler, A.J. (2016). Depletion of butyrate-producing *Clostridia* from the gut microbiota drives an aerobic luminal expansion of *Salmonella*. Cell Host Microbe *19*, 443–454.

Robinson, C.M., Jesudhasan, P.R., and Pfeiffer, J.K. (2014). Bacterial lipopolysaccharide binding enhances virion stability and promotes environmental fitness of an enteric virus. Cell Host Microbe *15*, 36–46.

Ross, B.D., Verster, A.J., Radey, M.C., Schmidtke, D.T., Pope, C.E., Hoffman, L.R., Hajjar, A.M., Peterson, S.B., Borenstein, E., and Mougous, J.D. (2019). Human gut bacteria contain acquired interbacterial defence systems. Nature *575*, 224–228.

Rosshart, S.P., Vassallo, B.G., Angeletti, D., Hutchinson, D.S., Morgan, A.P., Takeda, K., Hickman, H.D., McCulloch, J.A., Badger, J.H., Ajami, N.J., et al. (2017). Wild mouse gut microbiota promotes host fitness and improves disease resistance. Cell *171*, 1015–1028.e13.

Roxas, J.L., Koutsouris, A., Bellmeyer, A., Tesfay, S., Royan, S., Falzari, K., Harris, A., Cheng, H., Rhee, K.J., and Hecht, G. (2010). Enterohemorrhagic *E. coli* alters murine intestinal epithelial tight junction protein expression and barrier function in a Shiga toxin independent manner. Lab. Invest. *90*, 1152–1168.

Russell, A.B., Peterson, S.B., and Mougous, J.D. (2014). Type VI secretion system effectors: poisons with a purpose. Nat. Rev. Microbiol. *12*, 137–148.

Sanders, M.E., Merenstein, D.J., Reid, G., Gibson, G.R., and Rastall, R.A. (2019). Probiotics and prebiotics in intestinal health and disease: from biology to the clinic. Nat. Rev. Gastroenterol. Hepatol. *16*, 605–616.

Sassone-Corsi, M., Nuccio, S.P., Liu, H., Hernandez, D., Vu, C.T., Takahashi, A.A., Edwards, R.A., and Raffatellu, M. (2016). Microcins mediate competition among Enterobacteriaceae in the inflamed gut. Nature *540*, 280–283.

Schamberger, G.P., and Diez-Gonzalez, F. (2002). Selection of recently isolated colicinogenic *Escherichia coli* strains inhibitory to *Escherichia coli* O157:H7. J. Food Prot. *65*, 1381–1387.

Secott, T.E., Lin, T.L., and Wu, C.C. (2004). *Mycobacterium avium* subsp. *paratuberculosis* fibronectin attachment protein facilitates M-cell targeting and invasion through a fibronectin bridge with host integrins. Infect. Immun. *72*, 3724–3732.

Sela, D.A., Chapman, J., Adeuya, A., Kim, J.H., Chen, F., Whitehead, T.R., Lapidus, A., Rokhsar, D.S., Lebrilla, C.B., German, J.B., et al. (2008). The

genome sequence of *Bifidobacterium longum* subsp. *infantis* reveals adaptations for milk utilization within the infant microbiome. Proc. Natl. Acad. Sci. USA *105*, 18964–18969.

Sender, R., Fuchs, S., and Milo, R. (2016). Revised estimates for the number of human and bacteria cells in the body. PLoS Biol. *14*, e1002533.

Sgro, G.G., Oka, G.U., Souza, D.P., Cenens, W., Bayer-Santos, E., Matsuyama, B.Y., Bueno, N.F., Dos Santos, T.R., Alvarez-Martinez, C.E., Salinas, R.K., and Farah, C.S. (2019). Bacteria-killing type IV secretion systems. Front. Microbiol. *10*, 1078.

Shen, N.T., Maw, A., Tmanova, L.L., Pino, A., Ancy, K., Crawford, C.V., Simon, M.S., and Evans, A.T. (2017). Timely use of probiotics in hospitalized adults prevents *Clostridium difficile* infection: a systematic review with meta-regression analysis. Gastroenterology *152*, 1889–1900.e9.

Shi, Z., Zou, J., Zhang, Z., Zhao, X., Noriega, J., Zhang, B., Zhao, C., Ingle, H., Bittinger, K., Mattei, L.M., et al. (2019). Segmented filamentous bacteria prevent and cure rotavirus infection. Cell *179*, 644–658.e13.

Soares, M.P., and Yilmaz, B. (2016). Microbiota control of malaria transmission. Trends Parasitol. *32*, 120–130.

Sparber, F., De Gregorio, C., Steckholzer, S., Ferreira, F.M., Dolowschiak, T., Ruchti, F., Kirchner, F.R., Mertens, S., Prinz, I., Joller, N., et al. (2019). The skin commensal yeast *Malassezia* triggers a type 17 response that coordinates anti-fungal immunity and exacerbates skin inflammation. Cell Host Microbe *25*, 389–403.e6.

Sprinz, H., Kundel, D.W., Dammin, G.J., Horowitz, R.E., Schneider, H., and Formal, S.B. (1961). The response of the germfree guinea pig to oral bacterial challenge with *Escherichia coli* and *Shigella flexneri*. Am. J. Pathol. *39*, 681–695.

Sprockett, D., Fukami, T., and Relman, D.A. (2018). Role of priority effects in the early-life assembly of the gut microbiota. Nat. Rev. Gastroenterol. Hepatol. *15*, 197–205.

Staley, C., Kelly, C.R., Brandt, L.J., Khoruts, A., and Sadowsky, M.J. (2016). Complete microbiota engraftment is not essential for recovery from recurrent *Clostridium difficile* infection following fecal microbiota transplantation. MBio *7*, e01965-16.

Stecher, B., Robbiani, R., Walker, A.W., Westendorf, A.M., Barthel, M., Kremer, M., Chaffron, S., Macpherson, A.J., Buer, J., Parkhill, J., et al. (2007). *Salmonella enterica* serovar Typhimurium exploits inflammation to compete with the intestinal microbiota. PLoS Biol. *5*, 2177–2189.

Steed, A.L., Christophi, G.P., Kaiko, G.E., Sun, L., Goodwin, V.M., Jain, U., Esaulova, E., Artyomov, M.N., Morales, D.J., Holtzman, M.J., et al. (2017). The microbial metabolite desaminotyrosine protects from influenza through type I interferon. Science *357*, 498–502.

Stelter, C., Käppeli, R., König, C., Krah, A., Hardt, W.D., Stecher, B., and Bumann, D. (2011). Salmonella-induced mucosal lectin RegIIIβ kills competing gut microbiota. PLoS ONE *6*, e20749.

Suez, J., Zmora, N., Zilberman-Schapira, G., Mor, U., Dori-Bachash, M., Bashiardes, S., Zur, M., Regev-Lehavi, D., Ben-Zeev Brik, R., Federici, S., et al. (2018). Post-antibiotic gut mucosal microbiome reconstitution is impaired by probiotics and improved by autologous FMT. Cell *174*, 1406–1423.e16.

Suez, J., Zmora, N., and Elinav, E. (2020). Probiotics in the next-generation sequencing era. Gut Microbes *11*, 77–93.

Sun, L., Miyoshi, H., Origanti, S., Nice, T.J., Barger, A.C., Manieri, N.A., Fogel, L.A., French, A.R., Piwnica-Worms, D., Piwnica-Worms, H., et al. (2015). Type I interferons link viral infection to enhanced epithelial turnover and repair. Cell Host Microbe *17*, 85–97.

Sweere, J.M., Van Belleghem, J.D., Ishak, H., Bach, M.S., Popescu, M., Sunkari, V., Kaber, G., Manasherob, R., Suh, G.A., Cao, X., et al. (2019). Bacteriophage trigger antiviral immunity and prevent clearance of bacterial infection. Science *363*, eaat9691.

Tanoue, T., Morita, S., Plichta, D.R., Skelly, A.N., Suda, W., Sugiura, Y., Narushima, S., Vlamakis, H., Motoo, I., Sugita, K., et al. (2019). A defined commensal consortium elicits CD8 T cells and anti-cancer immunity. Nature *565*, 600–605.

Tariq, R., Pardi, D.S., Bartlett, M., and Khanna, S. (2017). Low resolution rates in open-label and randomized controlled trials of fecal microbiota transplantation for recurrent *Clostridium difficile* infection: a systematic review and meta-analysis. Am. J. Gastroenterol. *112* (*Suppl.*), S50.

Taur, Y., Coyte, K., Schluter, J., Robilotti, E., Figueroa, C., Gjonbalaj, M., Littmann, E.R., Ling, L., Miller, L., Gyaltshen, Y., et al. (2018). Reconstitution of the gut microbiota of antibiotic-treated patients by autologous fecal microbiota transplant. Sci. Transl. Med. *10*, eaap9489.

Telang, S. (2018). Lactoferrin: a critical player in neonatal host defense. Nutrients *10*, 1–16.

Thaiss, C.A., Levy, M., Grosheva, I., Zheng, D., Soffer, E., Blacher, E., Braverman, S., Tengeler, A.C., Barak, O., Elazar, M., et al. (2018). Hyperglycemia drives intestinal barrier dysfunction and risk for enteric infection. Science *359*, 1376–1383.

Thiennimitr, P., Winter, S.E., Winter, M.G., Xavier, M.N., Tolstikov, V., Huseby, D.L., Sterzenbach, T., Tsolis, R.M., Roth, J.R., and Bäumler, A.J. (2011). Intestinal inflammation allows *Salmonella* to use ethanolamine to compete with the microbiota. Proc. Natl. Acad. Sci. USA *108*, 17480–17485.

Tobe, T., Nakanishi, N., and Sugimoto, N. (2011). Activation of motility by sensing short-chain fatty acids via two steps in a flagellar gene regulatory cascade in enterohemorrhagic *Escherichia coli*. Infect. Immun. *79*, 1016–1024.

Togo, A., Dufour, J.C., Lagier, J.C., Dubourg, G., Raoult, D., and Million, M. (2019). Repertoire of human breast and milk microbiota: a systematic review. Future Microbiol. *14*, 623–641.

Tsilingiri, K., and Rescigno, M. (2013). Postbiotics: what else? Benef. Microbes *4*, 101–107.

Umesaki, Y., Setoyama, H., Matsumoto, S., and Okada, Y. (1993). Expansion of alpha beta T-cell receptor-bearing intestinal intraepithelial lymphocytes after microbial colonization in germ-free mice and its independence from thymus. Immunology *79*, 32–37.

Vaishnava, S., Behrendt, C.L., Ismail, A.S., Eckmann, L., and Hooper, L.V. (2008). Paneth cells directly sense gut commensals and maintain homeostasis at the intestinal host-microbial interface. Proc. Natl. Acad. Sci. USA *105*, 20858–20863.

van der Waaij, D., Berghuis-de Vries, J.M., and Lekkerkerk-van der Wees, J.E.C. (1971). Colonization resistance of the digestive tract in conventional and antibiotic-treated mice. J. Hyg. (Lond.) *69*, 405–411.

Vatanen, T., Plichta, D.R., Somani, J., Münch, P.C., Arthur, T.D., Hall, A.B., Rudolf, S., Oakeley, E.J., Ke, X., Young, R.A., et al. (2019). Genomic variation and strain-specific functional adaptation in the human gut microbiome during early life. Nat. Microbiol. *4*, 470–479.

Victora, C.G., Bahl, R., Barros, A.J.D., França, G.V.A., Horton, S., Krasevec, J., Murch, S., Sankar, M.J., Walker, N., Rollins, N.C., et al. (2016). Breastfeeding in the 21st century: epidemiology, mechanisms, and lifelong effect. Lancet *387*, 475–490.

Voravuthikunchai, S.P., and Lee, A. (1987). Cecectomy causes long-term reduction of colonization resistance in the mouse gastrointestinal tract. Infect. Immun. *55*, 995–999.

Wang, J., Zheng, J., Shi, W., Du, N., Xu, X., Zhang, Y., Ji, P., Zhang, F., Jia, Z., Wang, Y., et al. (2018). Dysbiosis of maternal and neonatal microbiota associated with gestational diabetes mellitus. Gut *67*, 1614–1625.

Wegh, C.A.M., Geerlings, S.Y., Knol, J., Roeselers, G., and Belzer, C. (2019). Postbiotics and their potential applications in early life nutrition and beyond. Int. J. Mol. Sci. *20*, E4673.

Westermann, A.J., Gorski, S.A., and Vogel, J. (2012). Dual RNA-seq of pathogen and host. Nat. Rev. Microbiol. *10*, 618–630.

Winter, S.E., Thiennimitr, P., Winter, M.G., Butler, B.P., Huseby, D.L., Crawford, R.W., Russell, J.M., Bevins, C.L., Adams, L.G., Tsolis, R.M., et al. (2010). Gut inflammation provides a respiratory electron acceptor for *Salmonella*. Nature *467*, 426–429.

Wotzka, S.Y., Kreuzer, M., Maier, L., Arnoldini, M., Nguyen, B.D., Brachmann, A.O., Berthold, D.L., Zünd, M., Hausmann, A., Bakkeren, E., et al. (2019).

Escherichia coli limits *Salmonella* Typhimurium infections after diet shifts and fat-mediated microbiota perturbation in mice. Nat. Microbiol. *4*, 2164–2174.

Wrzosek, L., Miquel, S., Noordine, M.-L., Bouet, S., Joncquel Chevalier-Curt, M., Robert, V., Philippe, C., Bridonneau, C., Cherbuy, C., Robbe-Masselot, C., et al. (2013). *Bacteroides thetaiotaomicron* and *Faecalibacterium prausnitzii* influence the production of mucus glycans and the development of goblet cells in the colonic epithelium of a gnotobiotic model rodent. BMC Biol. *11*, 61.

Yatsunenko, T., Rey, F.E., Manary, M.J., Trehan, I., Dominguez-Bello, M.G., Contreras, M., Magris, M., Hidalgo, G., Baldassano, R.N., Anokhin, A.P., et al. (2012). Human gut microbiome viewed across age and geography. Nature *486*, 222–227.

Yelin, I., Flett, K.B., Merakou, C., Mehrotra, P., Stam, J., Snesrud, E., Hinkle, M., Lesho, E., Mcgann, P., McAdam, A.J., et al. (2019). Genomic and epidemiological evidence of bacterial transmission from probiotic capsule to blood in ICU patients. Nat. Med. *25*, 1728–1732.

Yen, M., Cairns, L.S., and Camilli, A. (2017). A cocktail of three virulent bacteriophages prevents *Vibrio cholerae* infection in animal models. Nat. Commun. *8*, 14187.

Zachar, Z., and Savage, D.C. (1979). Microbial interference and colonization of the murine gastrointestinal tract by Listeria monocytogenes. Infect. Immun. *23*, 168–174.

Zackular, J.P., Moore, J.L., Jordan, A.T., Juttukonda, L.J., Noto, M.J., Nicholson, M.R., Crews, J.D., Semler, M.W., Zhang, Y., Ware, L.B., et al. (2016). Dietary zinc alters the microbiota and decreases resistance to *Clostridium difficile* infection. Nat. Med. *22*, 1330–1334.

Zeevi, D., Korem, T., Zmora, N., Israeli, D., Rothschild, D., Weinberger, A., Ben-Yacov, O., Lador, D., Avnit-Sagi, T., Lotan-Pompan, M., et al. (2015). Personalized nutrition by prediction of glycemic responses. Cell *163*, 1079–1094.

Zhang, X., Zhivaki, D., and Lo-Man, R. (2017). Unique aspects of the perinatal immune system. Nat. Rev. Immunol. *17*, 495–507.

Zhao, L., Zhang, F., Ding, X., Wu, G., Lam, Y.Y., Wang, X., Fu, H., Xue, X., Lu, C., Ma, J., et al. (2018a). Gut bacteria selectively promoted by dietary fibers alleviate type 2 diabetes. Science *359*, 1151–1156.

Zhao, W., Caro, F., Robins, W., and Mekalanos, J.J. (2018b). Antagonism toward the intestinal microbiota and its effect on *Vibrio cholerae* virulence. Science *359*, 210–213.

Zheng, L., Kelly, C.J., Battista, K.D., Schaefer, R., Lanis, J.M., Alexeev, E.E., Wang, R.X., Onyiah, J.C., Kominsky, D.J., and Colgan, S.P. (2017). Microbial-derived butyrate promotes epithelial barrier function through IL-10 receptor-dependent repression of claudin-2. J. Immunol. *199*, 2976–2984.

Zivkovic, A.M., German, J.B., Lebrilla, C.B., and Mills, D.A. (2011). Human milk glycobiome and its impact on the infant gastrointestinal microbiota. Proc. Natl. Acad. Sci. USA *108* (*Suppl 1*), 4653–4658.

Zmora, N., Zilberman-Schapira, G., Suez, J., Mor, U., Dori-Bachash, M., Bashiardes, S., Kotler, E., Zur, M., Regev-Lehavi, D., Brik, R.B.-Z., et al. (2018). Personalized gut mucosal colonization resistance to empiric probiotics is associated with unique host and microbiome features. Cell *174*, 1388–1405.e21.

Zuo, T., Wong, S.H., Lam, K., Lui, R., Cheung, K., Tang, W., Ching, J.Y.L., Chan, P.K.S., Chan, M.C.W., Wu, J.C.Y., et al. (2018). Bacteriophage transfer during faecal microbiota transplantation in *Clostridium difficile* infection is associated with treatment outcome. Gut *67*, 634–643.