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Microbial interactions with the intestinal epithelium and beyond: Focusing on immune cell maturation and homeostasis

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Abstract

Microbial metabolites influence the function of epithelial, endothelial and immune cells in the intestinal mucosa. Microbial metabolites like SCFAs and B complex vitamins direct macrophage polarization whereas microbial derived biogenic amines modulate intestinal epithelium and immune response. Aberrant bacterial lipopolysaccharide-mediated signaling may be involved in the pathogenesis of chronic intestinal inflammation and colorectal carcinogenesis. Our perception of human microbes has changed from that of opportunistic pathogens to active participants maintaining intestinal and whole body homeostasis. This review attempts to explain the dynamic and enriched interactions between the intestinal epithelial mucosa and commensal bacteria in homeostasis maintenance.

Keywords

Microbes; homeostasis; intestinal epithelium; macrophage; endothelium; metabolites

Compliance with Ethics Guidelines

Conflict of Interest

Dr. Ganesh reports In addition, Dr. Ganesh has a patent P1344SE00 pending to Dr. James Versalovic and Dr. Bhanu Priya Ganesh as Inventors. Filed by BioGaiaAB.

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Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors

Introduction

Mammalian mucosal surfaces serve as substrates to and diverse microbial ecosystems with differences in composition, dependent on gut biogeography. Many commensal microbes are known to colonize the gastrointestinal tract [1]. Intriguing mechanisms have evolved in mammals to handle such an enormous microbial burden in the intestinal mucosa. In the aggregate, the many relationships between a host and its microbiota are, at least in part, symbiotic and mutualistic [2]. The gastrointestinal tract provides a “fertile” ecosystem with space, human cell-generated metabolites and dietary nutrients, while the microbiota contributes to host nutrition, physiology, and immunity by modulating various biochemical functions. The gene set obtained from a recent metagenomic analysis of the human gut microbiome by the MetaHIT consortium revealed that the human microbiome is more than 150 fold larger in terms of gene content, than that of the human genome [3]. This large gene pool likely provides many benefits to the host health. However, the host-microbe interactions are complex, and beneficial coexistence comes via many checks and balances.

This review will discuss recent studies of host-microbe interactions that contribute to our understanding of intestinal mucosal homeostasis, and will review the evidences that suggest microbiota-host interactions under various conditions in the gut drive both systemic beneficial and local pathogenic immune responses (Figure 1). Thus, factors such as host genetics, nutrition and the environment modulate the microbiome and may have a profound impact on host immune responses, with wide-range of implications for human health and disease.

Intestinal microbiota, Intestinal epithelium and their maturation

The gastrointestinal tract contains a wide variety of beneficial microbes and commensals, in addition to potentially pathogenic microbes or canonical pathogens [4]. These interactions place a premium on immune homeostasis with respect to tolerance and inflammation. This microbial:mammalian interface poses a distinct bioregulatory challenge to the immune system of the GI mucosa.

The majority of most microbes in the human gut are bacteria. Four dominant bacterial phyla (Actinobacteria, Bacteroidetes, Firmicutes, Proteobacteria) have been described in the mammalian gastrointestinal tract. [5]. *Bacteroidetes* is a phylum consisting of three classes, of which the class *Bacteroidia*, containing the well-known genera *Bacteroides* and *Prevotella*, is probably the best studied. The largest bacterial phylum by species count, Firmicutes, contains more than 200 genera. Two prominent groups within Firmicutes include the *Clostridium coccoides* group (also known as *Clostridium* cluster XIVa) and the *Clostridium leptum* group (also referred to as *Clostridium* cluster IV). [5,6]. Taxonomically polyphyletic *Clostridium* groups contain members of several genera including *Clostridium*, *Eubacterium* and *Ruminococcus*. In addition to these two prominent phyla *Bacteroidetes* and *Firmicutes*, members of other phyla, such as *Fusobacteria*, *Proteobacteria*, *Actinobacteria*, *Lentisphaerae*, *Spirochaetes* and *Verrucomicrobia*, were detected previously [7,8]. The microbial density in per gram of intestinal content increases from 10^1 – 10^4 microbial cells in

the stomach and duodenum, 10^4 – 10^8 cells in the jejunum and ileum, to 10^{10} – 10^{12} cells in the colon and feces [9].

The glycans present in the outer mucus layer are accessible as energy sources for the intestinal microbiota and glycan utilization accelerates microbial survival and proliferation in the rodent intestine, [10–12]. In contrast to the outer mucus layer, the inner stratified and firmly attached mucus layer inhibits intestinal bacteria from entering indirect contact with the colonic epithelial cells (IEC) [11]. The mucus layers vary in organization and composition in different parts of the GI tract. The mucus layers in the stomach and the colon are continuous and defined in human, mouse and rat [13] in contrast to the less evenly distributed mucus layer in the small intestine [11,14]. Differences in mucus layer organization and composition will most likely be associated with variation in the mucosa-associated microbiota along the GI tract.

The intestinal epithelium exhibits various physical adaptations to protect the host from the external environment. Numerous biochemical adaptations such as a glycocalyx formed by the secretion and apical attachment of a heavily glycosylated mucin-rich layer by goblet cells have previously been demonstrated to reinforce this physical barrier [10]. Together, these mucin-rich aggregations in the lumen form a viscous layer on the apical surface of the epithelium [10,11]. A recent study demonstrates that *B. thetaiotaomicron* colonization increased the ratio of sialylated (NeuAc and NeuGc) to sulfated mucins in mono-associated rats compared to germ-free (GF) rats followed by decreased mucins in rats di-associated with *B. thetaiotaomicron* and *F. prausnitzii* [15]. As observed for goblet cell differentiation, *F. prausnitzii* seemed to attenuate the effects of *B. thetaiotaomicron*. The two bacteria were found to be metabolically complementary both *in vitro* and *in vivo*. *F. prausnitzii* consumes acetate produced by *B. thetaiotaomicron*, and *F. prausnitzii* produces butyrate which modulates the intestinal mucus barrier by affecting goblet cell biology and mucin glycosylation [15]. The study demonstrated how the balance between two microbes, *B. thetaiotaomicron* and *F. prausnitzii*, could be instrumental for maintaining colonic epithelial homeostasis and health *via* their respective effects on mucus [15].

IECs secrete a broad range of antimicrobial peptides (AMPs), including defensins, cathelicidins [16] lysozyme and calprotectins. Recent study demonstrate that nucleotide-binding oligomerization domain protein 2 (NOD2), upon activation through its ligand muramyl dipeptide (MDP), mediates the expression of the inducible AMP human beta defensin 2 (hBD)-2 [17]. Lysozyme is a glycosidase that hydrolyzes the 1, 4- β -glycosidic linkages between the N-acetylglucosamine and N-acetylmuramic acid moieties of peptidoglycan. Lysozyme is found to be effective against gram-positive microbes, where the peptidoglycan is more accessible, in contrast to gram-negative organisms which contain an outer membrane [18]. Gelatinase, an extracellular metalloproteinase produced by some strains of the opportunistic pathogen *E. faecalis*, cleaves the human cathelicidin, LL-37, resulting in the loss of antimicrobial activity *in vitro* [19]. AMPs contain hydrophobic and basic residues that confer broad-spectrum antimicrobial properties through pore formation in the bacterial cell wall [18]. These modifications are consistent with the perspective that IECs impede the entry of commensal and pathogenic bacteria into the lamina propria, in addition to accelerating digestion and absorption of nutrients.

An intact IEC lining is of utmost importance for preventing the uncontrolled intrusion of pathogenic bacteria. However, pathogenic bacterial translocation can reduce barrier integrity by disrupting the tight junctions present between epithelial cells [10,20]. Probiotic *Lactobacillus* GG has been identified to improve epithelial integrity. This *Lactobacillus* strain produces two proteins that protect epithelial cells from apoptosis and thereby increases mucosal integrity. The secreted proteins activate anti-apoptotic protein kinase B (PKB/Akt) in a phosphatidylinositol-3'-kinase (PI3K)-dependent pathway and inhibit the pro-apoptotic p38/mitogen-activated protein kinase (MAPK) [20].

Germ-free mice can serve as useful model systems to understand the importance of the intestinal microbiota in orchestrating the intestinal epithelial and immune homeostasis. The host responds to microbial colonization by preventing microbial invasion, responding to health-promoting microbial signals, and maintaining intestinal homeostasis. Recently it has been demonstrated that colonization by one bacterial species e.g., *Lactobacillus reuteri* PTA 6475 and its secretory factors facilitated epithelial and immune cell maturation [21,22]. The biogenic amine, histamine, synthesized by *L. reuteri* via conversion of dietary L-histidine, suppressed inflammation by suppressing MAP kinase signaling via histamine receptor 2. The gut microbe, *L. reuteri*, suppressed inflammation via histamine receptor 1 signaling by secreting the bacterial enzyme diacylglycerol kinase (Dgk) [23]. Dgk synthesized by *L. reuteri* blocks protein kinase C (PKC) phosphorylation by converting the lipid signal, diacylglycerol (DAG), to phosphatidic acid of H1R downstream signaling, thereby attenuating NF κ B activation induced inflammation. *L. reuteri* 6475 may be considered as a H1R blocker and could suppress intestinal inflammation via inhibition of H1R-mediated cell signaling [23]. In addition, *L. reuteri* also increases mucus fucosylation and increased mucus synthesizing goblet cells compared to GF mice [23]. Suppression of H1R led to reduced systemic IL-1 levels followed by reduced immature myeloid cells (IMC) in the spleen [23]. Increased IMCs were reported to be found in inflammation-associated colorectal cancer of mammals lacking histidine decarboxylase (HDC). HDC is a key enzyme converting L-histidine to histamine. Supplementing *L. reuteri* derived histamine reduced colorectal cancer and significantly suppressed IMCs in spleen of HDC^{-/-} inflammation associate-colorectal cancer mice [24]. These findings clearly demonstrate that gut microbes can generate metabolites capable of suppressing inflammation and cancer.

Pathogens and their effects on immune cell maturation

Current understanding of the interactions between indigenous intestinal microbes and the host, is fragmentary at best. Recent findings indicate that commensals and the host immune system exist in a dynamic state of equilibrium. Disruption of the dynamics between microbes and mammal may be a key trigger regarding pathogenesis of intestinal inflammation, in conditions such as inflammatory bowel disease (IBD) and necrotizing enterocolitis (NEC) [25]. Intestinal bacteria contribute to healthy maturation of the intestinal immune system in the postnatal period. Alterations in intestinal bacterial composition in infancy have been strongly associated with the subsequent development of immune-mediated disorders [2,25].

Intestinal microbiota promote resistance to colonization by pathogens [26–28]. Recent studies show that enteric bacterial pathogens induce disruption of the microbial ecosystem by promoting inflammation to overcome resistance to colonization [29]. Enteric pathogens can selectively utilize nutrients and cofactors produced during inflammation. For example, a fermentation end-product generated by commensal microbes is hydrogen sulphide (H_2S), a cytotoxic compound that is converted to thiosulphate ($\text{S}_2\text{O}_3^{2-}$) by the colonic epithelium. During inflammation, neutrophils that transmigrate into the intestinal lumen release reactive oxygen species (ROS) in an attempt to kill enteric bacteria. A by-product of releasing ROS leads to the oxidation of thiosulphate ($\text{S}_2\text{O}_3^{2-}$) to tetrathionate ($\text{S}_4\text{O}_6^{2-}$). In contrast to the commensal fermenting microbiota, *S. Typhimurium* (a murine pathogen) can use tetrathionate as a terminal electron acceptor to support its growth by anaerobic respiration, which is more efficient for energy production than fermentation [30–32]. A similar mechanism was observed in *Campylobacter jejuni* [33]. These findings emphasize the concept that pathogens evolved virulence mechanisms to allow access to host nutrients. Invasion, colonization and induction of inflammation by *S. Typhimurium* require the function of two type III secretion systems, T3SS-1 and T3SS-2. T3SS-1 and T3SS-2, encoded within *Salmonella* pathogenicity islands 1 and 2 (SPI-1 and SPI-2), respectively [34,35]. These microbial secretion systems deliver effector proteins into host cells to trigger specific responses in favor of the pathogen. Inflammatory responses in the intestine against pathogens, including the epithelial transmigration of neutrophils, cause changes in the availability of cofactors and nutrients that allow pathogenic bacteria remaining in the intestinal lumen to out-compete the resident microbiota. Increases in cell numbers or “blooms” of *S. Typhimurium* in the intestine facilitate transmission and are thus critical for its success as a pathogen [36,37]. Apart from dedicated pathogenic bacteria, some commensals can also act as pathogens under certain circumstances. *Akkermansia muciniphila*, a mucin degrading bacterium, is normally a non-pathogenic organism but under specific conditions can behave like pathobionts, in this case infection-associated inflammation by *Salmonella Typhimurium* can be exacerbated in the presence of *A. muciniphila* [38].

Microbial metabolites and host immunity

The intestinal microbiome affects mucosal immune homeostasis in myriad ways (Figure 1). Metabolites secreted by the microbiota affect host immunity by acting through the epithelium as well as by engaging directly with leukocytes. Macrophages and dendritic cells migrate through the connective tissue layer beneath the epithelium, embed and extend dendrites through the epithelial layer, into the lumen, to engage intimately in environmental sensing [39]. The unique characteristics of these cells allow them to be exceptionally adept, sensing, and responsive to microbial metabolites as environmental queues.

Substantial progress has been made in previous years toward understanding the influence of microbial metabolites on macrophage polarization in the intestinal mucosa. Populations of proinflammatory macrophages and anti-inflammatory macrophages, designated M1 and M2 respectively, in the gut, have major implications for host health. M1/M2 polarization of macrophages affects inflammatory functions of the cell, such as cytokine production, and thus has a direct influence on tissue homeostasis [40]. Recent work, focused on polarization

of macrophages by short chain fatty acids (SCFAs), demonstrated that butyrate induced M2 polarization of bone marrow-derived macrophages by upregulation of STAT-6 phosphorylation, resulting in expression of the M2-associated markers Ym1 and Arg1. Going further, the authors of this study demonstrated that butyrate, given to mice, reduced severity of DSS colitis and were associated with decreased serum IL-6, IL-1 β , and TNF. STAT-6 mediated M2 polarization of macrophages is mediated by butyrate. Butyrate acts as a histone deacetylase inhibitor, and acetylated histone H3K9 was shown to be increased in M2 macrophages exposed to butyrate. Taken together the data suggest that butyrate contributes to epigenetic modifications within macrophages which result in M2 polarization. [41]. SCFAs are widely recognized as key immunomodulatory metabolites, produced by microbial fermentation of dietary fiber. Acetate, propionate, and butyrate, are extensively well studied in their capacity as immunomodulatory compounds and exert their effects via G-protein coupled receptors, including GPR43 and GPR109a, which are expressed by macrophages and dendritic cells [42]. In addition to its role as a key receptor for the SCFA butyrate, GPR109a is also a receptor for niacin, a vitamin produced by gut microbiota. Stimulation of GPR109a in macrophages and dendritic cells by butyrate and niacin promotes alternative activation of macrophages and enables dendritic cells to induce differentiation of Tregs and IL-10 producing T cells. These effects reduce the severity colitis and colorectal cancer in mouse models [43].

Other vitamins synthesized by the microbiome also affect mucosal immunity. Lactobacilli are key generators of B complex vitamins in the microbiome, capable of synthesizing folate, thiamine, and riboflavin [44]. Many links have been made between these vitamins and important immunological process that help maintain homeostasis including strong connections to modulation of macrophage physiology. For instance, *in vitro*, riboflavin is associated with macrophage proliferation and protection against apoptosis [45]. Additionally, folate has been shown to be important in macrophages as well. Activated macrophages express the folate receptor which mediates the internalization of folate and folylpolyglutamates, including potentially therapeutic agents [46].

Vascular endothelial cells and their importance in maintaining gut homeostasis

Systemic dissemination of microbes and toxins were limited by intestinal mucosa while allowing nutrients to pass through the endothelium, access the blood circulation and thereby maintain the homeostasis [47]. The portal venous system ensures that substances absorbed in the intestine pass through the liver first, where they can be further metabolized and detoxified [48]. Spadoni *et al.* [49] characterize the gut-vascular barrier (GVB) and propose how bacteria can disrupt it, allowing their passage into the bloodstream and spread to other internal organs. In the GVB, the endothelial cells are surrounded by enterogial cells and pericytes which gives a hint that blood-brain barrier (BBB) and GVB may share key features. GVB allows diffusion of molecules as large as 4 kD, eight times the maximal size observed for the BBB and whether this plays any significant physiological role needs to be studied [50].

Bacteria cannot easily translocate to the bloodstream, since the GVB has a size exclusion range below Mw 70 Kd. *Salmonella* Typhimurium, Gram negative bacterial pathogen disrupts the GVB, allowing the dissemination of microbes to the bloodstream, livers, and spleens in infected mice [50]. β -catenin signaling in intestinal epithelial cells was increased by *S. Typhimurium*, effectively promoting dissemination of this enteric pathogen [50,51]. The authors reported a surprising decrease in the β -catenin-inducible gene *Axin 2*, suggesting that the pathogen may decrease β -catenin signaling in vascular endothelial cells. Wnt and β -catenin signaling in intestinal vascular endothelial cells, and the interactions between glial and endothelial cells may regulate GVB and prevent translocation of bacteria.

Bacteria interact with ECs by releasing toxins that can modulate the endothelial recruitment of immune cells. Shiga-toxicogenic *Escherichia coli* can release cytotoxins [52] that disrupt endothelial cells and their ability to sense microbes through TLR receptors or other innate sensing mechanisms [53] thereby impairing defense mechanisms. Outer membrane vesicles (OMVs) from *E. coli* increase ICAM-1, E-selectin, vascular cell adhesion molecule-1 (VCAM-1), and IL-6 expression in ECs *in vitro* and *in vivo* [54,55]. Further, *E. coli* can use OMVs as another mechanism to secrete toxins. Although autophagy helps endothelial cells counterattack by decreasing the levels of α -toxin receptor, ADAM10, in a post-transcriptional manner [56], pathogens have developed mechanisms to overcome their resilience mechanisms and promote infection and survival within the host [57]. Reducing gut bacterial diversity and population richness with broad-spectrum antibiotics decreases miR-204, increases Sirt1 and bioavailable vascular nitric oxide, and improves endothelium-dependent vasorelaxation [58]. In addition to bacteria and toxin specific therapies, statins and angiotensin receptor blockers could exert protective effects on the vasculature during overwhelming infection [59–61].

Paneth cells which reside at the bottom of the intestinal crypts play an important role in intestinal microbial angiogenesis by communicating with endothelial cells. They express members of angiopoietin family and act as key effectors of innate mucosal defense by secreting antimicrobial peptides [62,63].

Endothelial activation and expansion of microvasculature occurs by immune-driven or pathologic angiogenesis [63–65]. Exposure of human intestinal microvascular endothelial cells (HIMECs) to the bacterial ligands Toll-like receptors (TLR) and NOD-like receptors (NLR), resulted in angiogenesis with increased proliferation, migration, vessel sprouting, tube formation and production of proangiogenic factors [66]. This involved mitogen activated protein kinase (MAPK) and nuclear factor-Kappa B (NF- κ B) pathways along with upregulation of vascular endothelial growth factor receptor 2 (VEGFR2) and focal adhesion kinase (FAK). Since angiogenesis is an essential part of IBD, anti-angiogenic therapy seems to be an ideal target for IBD treatment. However a thorough study of the angiogenic players is necessary to prevent any side effects since these molecules may behave diversely in different environments [67].

Taken together, trans-endothelial and trans-epithelial communication plays a pivotal role in maintaining homeostasis by interacting with the gut microbiome. An in-depth study on the interactions between the gut microvasculature, growth factors, cytokines, chemokines,

platelets, and cell adhesion molecules are needed to create new therapeutic strategies for patients with intestinal inflammatory disorders.

Conclusions

Commensal and beneficial microbes have a profound impact on intestinal immunity and physiology by influencing various cell types within the intestine such as IEC, Paneth cells, immune cells and endothelial cells (Figure 1). In addition diet-induced bacterial derived metabolites and factors have well defined effects on shaping the gut epithelium and play a major role in maintaining intestinal homeostasis (Figure 1). Specific signals provided by intestinal microbial colonization facilitates the maturation of the intestinal immune system. Such transient immune activation, results in changes in epithelial glycosylation patterns, functional maturation, and tolerance toward the indigenous microbiota. In addition to this observation, different species of commensal microbes as well as pathogens have developed a number of strategies to modulate cell-signaling pathways such as NF- κ B and MAP kinases, resulting in suppression or stimulation of inflammation in vivo.

Commensal microbial interactions are required for the development and sustained function of the intestinal immune system. The wide range of microbial composition and broad scope of microbial functions must be considered when considering the impact of the microbiome on mammalian physiology and disease pathogenesis. The microbial:mammalian interface extends throughout the gastrointestinal mucosa and beyond the intestine to many body surfaces including the respiratory tract, skin, and oral cavity. In addition to local effects, microbial:mammalian interactions yield remote effects in tissues such as the liver, brain and vasculature. Microbial enzymes and metabolites alter mammalian biochemistry and physiology in many different ways resulting in mammalian holobionts, a menagerie of microbial and mammalian cells working intimately together.

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Figure 1.

Intestinal homeostasis is maintained mainly by several factors including immune cell maturation, nutrients, genetic susceptibility and environmental factors. These factors orchestrate the structure and physiology of different cell types in the intestinal mucosa and help maintain gut homeostasis.