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Epithelial-microbial diplomacy: escalating border tensions drive inflammation in inflammatory bowel disease

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Inflammatory bowel diseases (IBD) are chronic conditions of the gastrointestinal tract-the main site of host-microbial interaction in the body. Development of IBD is not due to a single event but rather is a multifactorial process where a patient's genetic background, behavioral habits, and environmental exposures contribute to disease pathogenesis. IBD patients exhibit alterations to gut bacterial populations "dysbiosis" due to the inflammatory microenvironment, however whether this alteration of the gut microbiota precedes inflammation has not been confirmed. Emerging evidence has highlighted the important role of gut microbes in developing measured immune responses and modulating other host responses such as metabolism. Much of the work on the gut microbiota has been correlative and there is an increasing need to understand the intimate relationship between host and microbe. In this review, we highlight how commensal and pathogenic bacteria interact with host intestinal epithelial cells and explore how altered microenvironments impact these connections. **(Intest Res 2019;17:177-191)**

Key Words: Microbiota; Microbiota host interactions; Intestinal epithelium; Inflammatory bowel disease

INTRODUCTION

Inflammatory bowel diseases (IBD), such as CD and UC, are complex, multifactorial disorders characterized by chronic inflammation of the GI tract. In the United States alone, it is estimated that IBD affects up to 1.3% of the population (3 million individuals) and a further 2.2 million individuals in Europe, with incidence rising in newly industrialized countries (www.cdc.gov/ibd/data-statistics.htm).[1-3] Our current understanding suggests IBD manifests largely in genetically susceptible individuals following some perturbation of mucosal homeostasis which permits commensal gut flora to activate inflammatory processes beyond homeostatic levels. In agreement with this hypothesis, studies in gnotobiotic mice have demonstrated that transgenic mouse models of colitis require a microbial community to develop colitis.[4,5] In addition, administration of antibiotic therapy in IBD patients has been shown to induce remission further demonstrating that gut microbes promote disease.[6] Together, these studies point to the importance of gut flora in the pathogenesis of IBD.

The gut microbiota consists of bacteria, viruses, archaea and eukaryotic microbes. Bacteria constitute by far the largest component and both beneficial bacteria "commensals" and opportunistic pathogenic bacteria "pathobionts" are found within the GI tract. Gut bacterial numbers roughly equal the number of intestinal epithelial cells (IECs) and represent a large reservoir of gene expression that can affect host processes essential to GI function.[7] Indeed, the co-evolution of host and microbiota supports the notion that gene expression of the latter can affect the host.[8] The most prominent populations of gut flora

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are present in the large intestine, where bacteria play a critical role in the digestion and absorption of urea, bile acids, sterols, xenobiotics, essential vitamins, plant-based polysaccharides, and certain amino acids. The resulting metabolites are capable of altering IEC signaling and gene expression. In addition to their digestive function, bacteria within the gut are also important for maturation of the immune system and can modulate the immune response by both promoting certain functions and triggering anti-inflammatory responses.[9-12]

The composition of the microbiota is fluid as alterations in diet and the presence of non-infectious disease have been shown to shift certain subpopulations within the gut microbiota.[13,14] These resulting communities can also induce phenotypic traits when administered to gnotobiotic mice.[13] Additionally, various changes in the features of the intestinal epithelium during IBD pathogenesis promote the proliferation and dominance of pathobionts such as adherent-invasive *Escherichia coli* (AIEC). However, whether changes in microbial populations precede disease development onset is still under investigation. Several studies have attempted to understand the role(s) of the microflora in disease pathogenesis by investigating specific bacterial shifts associated with IBD (specifically the prevalence of *Enterobacteriaceae* and *Proteobacteria* in IBD patient intestinal tissues) and the effect of IBD candidate genes on the interaction between IECs and the microflora.

In this review, we will summarize the present understanding of how the interaction of IECs and gut microbiota can either maintain healthy homeostasis or lead to dysbiosis by highlighting specific examples of commensals and pathobionts. We focused attention on the interactions of IECs with luminal bacteria as the interplay of the gut microbiota and mucosal immune cells has been comprehensively described previously.[10,15,16]

MICROBIAL INTERACTIONS WITH THE INTESTINAL BARRIER

In order to understand the impact of the gut microbiota in intestinal diseases we must first consider the major site of interaction: the intestinal barrier. The broader interpretation of the intestinal barrier requires the interplay of multiple components: IECs, the gut microbiota, and mucosal and submucosal immune cells. More specific barrier functions such as permeability to electrolytes and luminal contents are controlled directly by epithelial cells and their key structural components that regulate paracellular permeability, the apical tight junctions. For clarity, we will discriminate between permeability–referring to specific tight junction modifications–and the broader concept of barrier function in the following sections.

1. Proliferation and Epithelial Turnover

The intestinal epithelium is a continuous monolayer of specialized cells generated by intestinal stem cells at the base of the crypt. IEC differentiation from the crypt to the villus tip (small intestine), or surface epithelium (large intestine), and their subsequent turnover is critical for the chemical and physical functions of the barrier. IEC apoptosis and shedding into the lumen is tightly regulated to promote continuous turnover of the epithelium and maintain barrier integrity.[17-19] In a healthy gut, only 1% to 2% of the epithelia are undergoing apoptosis at any one time, and neighboring IECs act to seal gaps arising from apoptosis or cell "shedding events" in order to prevent unregulated access across the epithelium by luminal contents or microbes. Indeed, the pro-inflammatory cytokine TNF-α increases the number of shedding events however, this is not associated with a barrier defect due to the "gap-sealing" actions of adjacent epithelial cells.[18,19] The life cycle of an IEC is driven by changes in enterocyte function and epithelial differentiation. Villus or surface enterocytes display an absorptive phenotype while IECs at the crypt base secrete Cl^- ions and water that aid in hydrating the mucus layer and flushing bacteria out of the crypt.[20] These functions are critical for the principal roles of the intestine (digestion, nutrient absorption, and waste excretion) and maintaining balanced interactions with luminal bacteria.

2. Differentiation and Epithelial Functions

In addition to their absorptive and secretory functions, IECs also differentiate into specialized cells that contribute to intestinal barrier homeostasis. Paneth cells that are situated in the crypt base of the small intestine secrete anti-microbial factors such as α-defensins (or cryptidins in mice), RegIIIγ, lysozyme, and phospholipase A2 in response to microbial components.[21] These secreted factors aid in preventing pathogenic bacterial attachment and promote growth of beneficial bacteria. Positioned along the length of crypts, goblet cells secrete mucin proteins to establish a protective mucus layer overlying the intestinal epithelium that also functions as a diffusive medium for metabolites and nutrients. The composition and physical properties of the mucus layer differ by location in the GI tract. The small intestine has a loose, non-adherent mucus layer which facilitates nutrient absorption. In the colon, the mucus

layer is composed of 2 sublayers–an outer layer much like that of the small intestine and a thick inner adherent layer that is impermeable to bacteria in the distal colon and somewhat permeable in the proximal colon.[22] Commensal microbes aid in maintaining this mucus layer through stimulation of mucin secretion. This observation has also been supported by analysis of the mucus layer in germ-free mice where the colonic inner layer was less developed.[23] Finally, the mucus binding capacity of probiotic bacteria is correlated with their colonization capacity, providing a selective advantage over pathogenic bacteria.[24] Studies on cystic fibrosis transmembrane conductance regulator knockout mice, which display accumulations of mucus, have shown an increase in IEC inflammatory gene expression and bacterial overgrowth, suggesting an abnormal mucus layer can promote dysbiosis.[25,26]

3. Mucosal Immune Functions of Intestinal Epithelium

A primary function of IECs is their ability to act as mediators of innate immunity. Similar to other cell types, IECs express cell-surface and intracellular pattern recognition receptors such as Toll-like receptors (TLRs) and NOD-like receptors, respectively. TLRs expressed by IECs function to respond primarily to commensal bacteria and different regions of the intestinal tract exhibit distinct patterns of TLR expression.[27] Accordingly, TLR expression is highest in the colon where their activation promotes expression of host defense genes.[27] In addition, the presence of gut flora or inflammation can also alter the expression profile of IEC TLRs. Activation of TLRs by commensal bacteria promotes tight junction protein expression to fortify the barrier and increases secretion of anti-microbial peptides.[28-30]

Immune cells located in the mucosa and submucosa also function in shaping the microbial environment and hampering colonization by pathogenic bacteria. Lymphoid follicles called Peyer's patches are located at various intervals in the ileum and consist of macrophages, lymphocytes, and M or micro-fold cells. These sites function in maintaining immune tolerance of commensal bacteria. In addition, secretion of IgA and components of the complement system aid in inhibiting pathogenic bacterial growth. Finally, certain bacteria are also necessary for immune maturation, such as segmented filamentous bacteria which promote Th17 cell development and *Bacteroides fragilis* which influences regulatory T cell development, and Th2 and Th1 cell balance.[31-38]

Secretory IgA is a mucosal Ig produced by intestinal lamina propria B cells that captures microbial pathogens and their toxins to prevent adherence and invasion. Induction of IgA secretion occurs at Peyer's patches and isolated lymphoid follicles within the GI wall where signals from T cells, dendritic cells, and IECs cause class switching of B cells to IgA-producing plasma cells.[39] Importantly, IgA is able to protect the barrier from bacteria without increasing inflammation. Commensal bacteria can promote IgA production directly through bacterial proteins binding host receptors on IECs, and indirectly through microbial metabolites such as short-chain fatty acids (SCFAs) binding the GPR43 receptor.[40,41] In turn, IgA binding to commensals can promote their colonization and stability within their colonization niche.[42-44] A continuing question in the field is how host responses differentiate between commensal and pathogenic microorganisms. It is thought that IgA binding to commensals is low-affinity whereas increased coating of bacteria by IgA indicates the increased pathogenic potential of the bacterium. This has been supported by transfer of highly IgA-coated bacteria isolated from IBD patients to germ-free mice which led to increased susceptibility to dextran sulfate sodium (DSS) colitis.[45] Thus, IgA is an important discriminator of commensal and pathogenic bacteria and promotes mucosal homeostasis through increased colonization of healthy commensals.

4. IBD Candidate Genes and Epithelial Function

Normal antigen-sampling of the GI luminal contents by IECs and submucosal immune cells elicits a tolerogenic immune response essential for shaping GI microbial composition. The role of IECs as mediators of innate immunity is underscored by studies of single-nucleotide polymorphisms (SNPs) associated with IBD, in which genes critical for antigen recognition and bacterial destruction have been identified, such as nucleotide-binding oligomerization domain 2 (NOD2) and autophagy-related protein 16-like 1 (ATG16L1).[46-50] Deficiency of these genes impairs normal maintenance of commensal populations and restriction of pathobionts, leading to dysbiosis.[51-55] The influence of IBD candidate genes on epithelial barrier function and bacterial sensing are discussed in greater detail in several comprehensive review articles.[56,57]

COMMENSAL BACTERIA THAT REGULATE EPITHELIAL FUNCTION

Commensal microbes are those normally present in a healthy gut and who contribute to host mucosal homeostasis. In addition to their critical roles in digestion for the host, they also

serve to support the intestinal barrier by promoting tolerogenic immune responses, as alluded to earlier. In particular there are a number of commensals that have been well-studied for their ability to improve barrier function and promote intestinal health. In this section, we review a number of these commensal organisms in an effort to understand how microbes contribute to maintenance of the intestinal epithelial barrier.

1. Muciniphilic Commensals

The mucus layer is composed of mucins with oligosaccharide chains whose terminal ends cannot be metabolized by the majority of gut bacteria.[58] However, certain bacteria termed mucin specialists have evolved specific enzymes that allow degradation of mucin oligosaccharide chains for sugar and protein, thereby facilitating their own colonization as well as growth of other commensals by increasing nutrient availability.[59-62] In fact, commensals such as *Faecalibacterium prausnitzii rely* on these mucin specialists to facilitate their colonization to the mucus layer.[63-68] *Akkermansia muciniphila* is a widely studied mucin specialist capable of tolerating the hypoxic environment near the epithelial surface and that utilizes host-derived proteins for survival.[69-71] *A. muciniphila* colonization increases mucin production and mucus thickness, and improves barrier function.[70,72-74] Butyrate production by *A. muciniphila* is also beneficial to the host by upregulating energetic pathways, such as increased β-oxidation and intestinal gluconeogenesis in colonocytes that maintain a commensal-dominated microenvironment and protect from metabolic impairment.[75-78] Finally, increased abundance of *A. muciniphila* at sites of intestinal injury where oxygen is depleted, allows expanded colonization by anaerobic bacteria. In this setting, *A. muciniphila* also promotes intestinal epithelial formyl peptide receptor 1 (FPR1) signaling and NADPH oxidase (NOX1) activity to increase enterocyte migration and proliferation to facilitate mucosal healing.[79]

In addition, *A. muciniphila* has been inversely associated with intestinal inflammation.[62,80-84] Recent evidence has also shown that *A. muciniphila*-secreted extracellular vesicles protect the epithelium from colitis and diet-induced barrier dysfunction.[85] Another muciniphile *Peptostreptococcus russellii*, is also protective against DSS-induced chemical injury colitis model in mice. In addition to its mucin-degrading activity, *P. russellii* expresses aromatic amino acid metabolic enzymes that utilize tryptophan to produce indoleacrylic acids, which have anti-inflammatory effects and promote goblet cell function.[86] The phenyllactate dehydratase gene cluster responsible for expression of these enzymes was decreased in IBD patients compared to healthy controls.[86] Collectively, these findings indicate both host and commensal act synergistically to encourage mucosal homeostasis and repress inflammation.

2. *Faecalibacterium prausnitzii*

Another commensal microbe that is consistently decreased in UC patients is *F. prausnitzii*.[87-89] *F. prausnitzii* is a highly abundant butyrate-producer in the intestinal tract, encompassing 5% of bacteria in feces.[90,91] Since increased *F. prausnitzii* is correlated with improved outcomes following surgical resection, it was thought that this microbe could directly affect host immunity although its presence is not required for patient recovery.[92,93] In accordance with this, Quévrain et al.[94] identified a 15 kDa anti-inflammatory protein was secreted by *F. prausnitzii*. Subsequently, a study by Breyner et al.[95] demonstrated *F. prausnitzii* secreted peptides termed "microbial anti-inflammatory molecules" can inhibit nuclear factor (NF)-κB promoter activity and interleukin (IL)-6 production, leading to protection from experimental colitis in mice through alteration of T cell immune responses. Interestingly, co-culture studies have demonstrated *F. prausnitzii* does not enhance barrier function but can induce activation of NF-κB signaling downstream of IL-1β in Caco-2 cells.[92,96] In contrast, Caco-2 cells treated with *F. prausnitzii* supernatant decreases basal and IL-1β-induced NF-κB activity.[92]

3. *Lactobacillus rhamnosus* Gorbach-Goldin

L. rhamnosus Gorbach-Goldin (LGG) has been well-studied as a probiotic used in the production of dairy yogurt. This commensal microbe can indirectly and directly regulate epithelial function to repress inflammation and resist enteric infections. Administration of LGG culture supernatant to neonatal rats was associated with increased IEC proliferation, decreased paracellular barrier permeability, and increased expression of mucin-2, zonula occludens-1 (ZO-1), and IgA, together promoting resistance to neonatal *E. coli* K1 infection.[97] In addition, LGG enhances wound healing through enterocyte activation of FPR1 and NOX1, similar to *A. muciniphila*.[98] Direct actions of LGG are performed p40, were found to inhibit pro-inflammatory cytokine-induced IEC apoptosis.[99] In addition, p40 can also modulate IgA production by increased expression of a proliferation-inducing ligand in IECs.[41] More recently, p40 was found to exert its responses through the epidermal growth factor receptor in neonatal mice, ultimately promoting IgA production and T regulatory cell (Treg) differentiation in adult mice.[100]

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4. *Bacteroides fragilis*

B. fragilis is a commensal found within the glycocalyx and in colonic crypts of mice that exhibits single-strain stability or the ability of a single strain of bacteria to persist in the microbial community for years.[44] Its colonization of the mucosa is facilitated by IgA binding, which allows the bacteria to aggregate and contribute to colonization resistance against pathogenic bacteria.[44] In addition, its expression of cytochrome bd oxidase permits its growth in low concentrations of oxygen, such as those present in the colon.[101] *B. fragilis* secretes an immunomodulatory molecule, polysaccharide A (PSA) that regulates immune cells such as induction of $Foxp3^+$ Treg cell differentiation to reduce intestinal inflammation and repression of IL-17 secretion.[36-38] Furthermore, *B. fragilis* strains expressing PSA are able to prevent colonization by the pathobiont *Helicobacter hepaticus* and subsequent inflammation, indicating commensals can prevent pathobiont expansion.[37] Intriguingly, *B. fragilis* also accounts for a majority of *Bacteroides* infections in the body and the enterotoxigenic *B. fragilis* (ETBF) strain is a causative pathogen of diarrhea.[102] Recently, Chan et al.[103] demonstrated stable colonization of non-enterotoxigenic *B. fragilis* (NTBF) ameliorated disease severity following ETBF infection, in a PSA-independent manner. This study confirmed that *B. fragilis* predominance alone can be protective against mucosal inflammation. Whether increased virulence results from changes in the GI microenvironment and microbial exchange of genetic information is not well understood, although this is an emerging topic among research groups.[102]

DYSBIOSIS IN THE INFLAMMATORY GUT MICROENVIRONMENT

Inflammation in the GI tract is able to both alter the microbial community composition and promote expansion of pathogenic bacteria, as described in extensive detail elsewhere.[14-16] Metagenomic studies of fecal matter, intestinal wash, and intestinal tissues have observed reduced diversity and temporal instability of the microflora in IBD patients compared to healthy controls despite an increased number of microflora at involved tissue sites in IBD patients.[104] Bacterial communities can be influenced by inflammation-induced alterations of nutrient sources, luminal pH, and growth of other bacterial species such as those producing SCFAs which can also affect luminal pH due to their acidic profile. In some cases, inflammation can support the virulent activity of commensals known as pathobionts.[105-107] For example, Chassaing and Gewirtz[108] demonstrated that expansion of AIEC in mice lacking TLR5 required a pro-inflammatory niche. Here we summarize how inflammation-induced changes in mucosal homeostasis, particularly the mucosal microenvironment, promotes dysbiosis (Table 1).[78,79,109-118]

Table 1. Factors Influencing Microbial Colonization

Factor	Regulated by	Effect
O_2 concentration	Butyrate producers decrease O_2 (antibiotic therapy)	Decreased numbers of commensal microbes and increased pathogenic bacteria[109]
	Inflammation (ROS secretion and increased blood flow) increase O_2	Increased number of aerotolerant bacteria[110]
	Decreased at sites of injury	*Akkermansia muciniphila* increases mucosal healing wound sites[79]
Reactive oxygen/ nitrogen species	Inflammation	Increase O_2 concentration[110] Promote alternative electron acceptors for microbial respiration, increasing pathogen number[78,111]
Inflammatory mediators	Inflammation–ethanolamine	Promotes expansion of pathogenic bacteria[112]
	Inflammation–IL22RA1	Increases intestinal fucosylation promoting diversity of anaerobic commensals and represses *Enterococcus faecalis*[113]
Dietary transition metals	Inflammation–molybdenum	Utilized for microbial respiration to promote *Enterobacteriaceae* expansion[114]
	Inflammation–iron	Utilization by *Escherichia coli* Nissle 1917 restricts expansion of pathogenic bacteria[115]
	Zinc	Deficiency increases enteroaggregative *E. coli* virulence[116]
Dietary metabolites	Host behavior and environment	Increased sulfur promotes *Bilophila wadsworthia* expansion[117]
	Milk fats	
pH gradients	Substrate fermentation	Shifts in microbial communities[118]

O_2, molecular oxygen; ROS, reactive oxygen species; IL22RA1, interleukin 22 receptor subunit α-1.

1. Hypoxia and the Oxygen Gradient

Under healthy conditions, an oxygen gradient in the colonic mucosa exists as a result of the high oxygen consumption required to maintain the inward Na^+ gradient driving fluid absorption. However, bacteria can also contribute to the colonic oxygen gradient. Butyrate-producing bacteria increase levels of CO_2 and together these mechanisms promote a hypoxic environment in the colonic lumen. Decreased levels of O_2 promote anaerobic growth of commensal bacteria such as *B. fragilis* and *F. prausnitzii* closer to the surface of IECs.[101,110] However, in a chronic inflammatory state, oxygen levels near IECs increase due to secretion of reactive oxygen species and increased blood flow. This allows facultative anaerobic, or "aerotolerant," bacteria to predominate near the intestinal epithelium such as *Actinobacteria* and *Proteobacteria*, phyla that are also associated with IBD.[119] For example, mice exposed to DSS had increased numbers of aerobic bacteria compared to healthy controls, a phenomenon that has also been observed in newly-diagnosed CD patients.[109,120] Depletion of butyrate-producers such as *Clostridia* by antibiotic therapy induces a subsequent increase in luminal oxygen, allowing increased growth of *Enterobacteriaceae* such as *Salmonella* serovar *typhimurium*.[121] The concept of increased epithelial oxygenation acting as a driver of facultative anaerobe expansion at the expense of butyrate-producing obligate anaerobes is elegantly described in a recent review by Litvak et al.[78]

2. Nutrient Sources and Colonization Resistance

A primary method of preventing pathobiont expansion is colonization resistance by homeostatic microbes. Efficient nutrient assimilation provides a selective advantage to certain bacteria that can hinder growth of pathogens. In addition, metabolites in the GI lumen can also activate signaling processes within IECs that promote selective microbial growth in both the healthy and inflamed gut. During inflammation, secreted reactive nitrogen species such as nitric oxide degrade to nitrate in the lumen where they are utilized by microbial nitrogen respiration pathways. Similarly, production of reactive oxygen species promotes alternative sources of electron acceptors that are then utilized in microbial respiration. For example, hydrogen sulfide (H_2S) produced by commensals is oxidized to thionate during inflammation which is then utilized by *S. typhimurium*, although there has been some evidence indicating H_2S is also anti-inflammatory and promotes mucosal healing.[111,122] Another metabolite increased by intestinal inflammation includes ethanolamine (EA), which is produced from phosphatidylethanolamine. Enzymes that utilize EA as a nutrient source are found in the genomes of many enteric pathogens so it is unsurprising that EA promotes predominance of *S. typhimurium* and enterohemorrhagic *E. coli* in mice.[112,123] Similarly, the bacterial fermentation product 1,2-propanediol is exploited by *S. typhimurium* during inflammation with increased expression of virulence factors.[124] Finally, GPR43, a G-protein coupled receptor bound by bacterial-produced SCFAs, is important for resolution of intestinal inflammation in mice, and thus indicates a link between gut microbiota and inflammation resolution.[125]

Availability of dietary transition metals can also restrict pathogenic bacteria growth. In a recent study Zhu et al.[114] confirmed that microbial molybdenum cofactor-dependent respiration is a metabolic signature of DSS-induced dysbiosis and promotes *Enterobacteriaceae* expansion during colitis.[126] Consistently, pathobiont expansion was reversed by tungstate treatment.[114] Competition for iron by the *E. coli* strain Nissle 1917 restricts *S. typhimurium* during colitis and infection.[115] Interestingly, the host protein lipocalin-2 is required for this effect, indicating an intricate interaction between host and commensal bacteria that promotes a healthy gut environment. In addition to colonization resistance, dietary metals can also alter bacterial gene expression. Zinc deficiency elevates the risk of pediatric diarrhea and is associated with pathogenic *E. coli*.[127] Indeed, low zinc causes increased virulence of enteroaggregative *E. coli*, the pathogen responsible for traveler's diarrhea.[116]

3. Intestinal pH Gradients

Another factor affecting the gut microbial community is luminal pH, which can range from 5.7 to 6.8 in human cecum and pH 6.1 to 7.5 in the colon.[128] There are major shifts in the gut microbiota between luminal pH values of 5.5 and 6.5. In a healthy colon, a gradient from a mildly acidic to a neutral environment exists from the proximal to distal colon. The acidic pH in the proximal colon is maintained by substrate fermentation where acetate and other SCFAs are produced from the fermentation of indigestible polysaccharides facilitating expansion of butyrate-producing species such as *Roseburia*.[129,130] During spontaneous colitis, accumulation of lactate drives a decrease in luminal pH, potentially due to repressed growth of lactate-utilizing bacteria.[131,132]

From these examples, it is clear that environmental changes of the microbial niche can alter composition of the gut microbiota, occurring as both small perturbations and large displacement of commensal bacteria. Both diet and inflammatory sta-

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tus contribute to these effects. Apart from these shifts in population, changes in pathogenicity of seemingly innocuous bacteria are also of great interest since chronic GI conditions are associated with increased numbers of more virulent bacteria including "pathobionts."

PATHOBIONT BACTERIA AND INTESTINAL FUNCTION IN IBD

Pathobionts are bacteria normally found in the intestines that exhibit pathogenic activity during a chronic disease state or in a genetically-susceptible host.[133] Examples of pathobionts include *Helicobacter* species, virulent strains of *E. coli*, *Campylobacter concisus*, *Klebsiella* species, and Vancomycin-resistant *Enterococcus faecalis*, among others. These bacteria promote inflammation by disruption of the intestinal barrier, invasion of the epithelium, and/or modulation of inflammatory responses. Notably, in mouse models monoassociated with pathobiont bacteria increased disease severity is dependent upon the presence of resident microbiota.[134] In addition, activated immune cell responses against both resident and pathogenic bacteria suggest that background inflammatory responses to resident commensals drives development of pathobiont pathogenicity.[135] These data suggest that expansion of pathobionts in the intestinal mucosa result from the complex interaction of commensals, IECs, and mucosal immune responses all of which contribute to the intestinal microenvironment. The extent of pathobiont-induced dysbiosis can vary as well. For example, *Helicobacter bilis* does not alter microbial composition while adherent-invasive *E. coli* induces dysbiosis despite commensal-directed immune responses.[135,136] In this section we review the current understanding of various pathobionts in the GI tract.

1. Alterations of Intestinal Epithelial Integrity

Pathobionts can interact with IECs to exert their effects both directly through adherence and translocation and indirectly through modulation of IEC functions (Fig. 1). Pathobionts such as *Bilophila wadsworthia* and *Enterobacteriaceae faecalis* are able to adhere to and translocate through IEC monolayers *in vitro* and *in vivo*.[137-139] Adherence and invasion of pathobionts usually requires expression of specific genes such as proteases in *E. faecalis* and *GipA* (growth in Peyer's patches) in AIEC.[140-143] In addition to attachment and invasion, pathobiont species also impair normal IEC functions for intestinal homeostasis. *C. concisus*, a gram-negative microbe that is increased in pediatric and adult IBD patients, inhibits expression of the tight junction-associated proteins ZO-1 and occludin, as well as their association with tight junction complexes leading to a less regulated intestinal barrier.[144-147] Pathobionts can also alter the life cycle of IECs. *C. concisus* increased apoptosis which left large gaps (apoptotic lesions) in the monolayer that per-

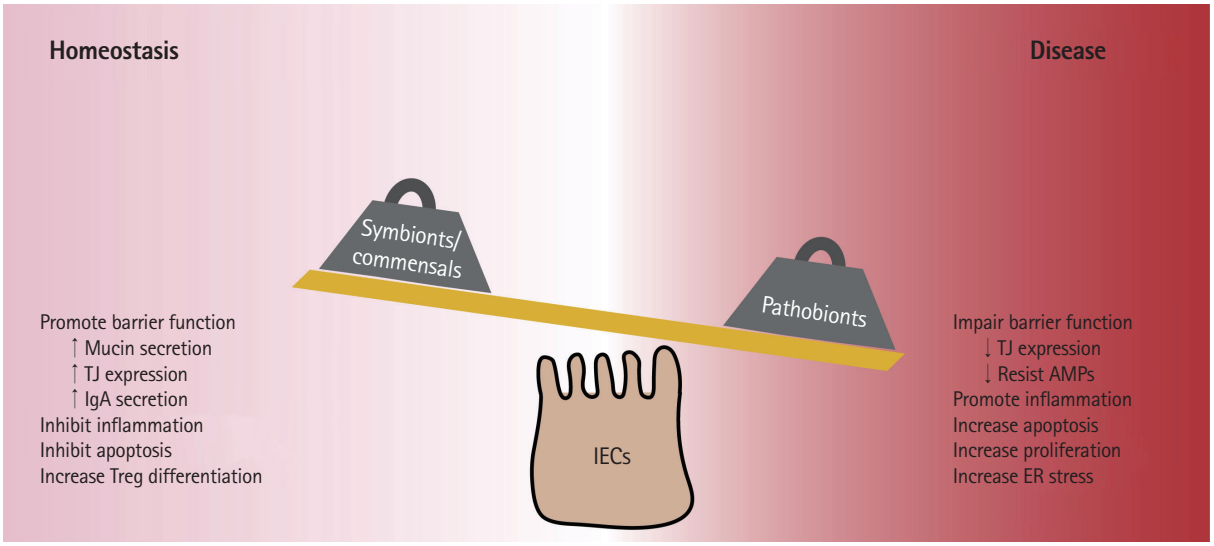

Fig. 1. Effect of bacteria on intestinal epithelial cells (IECs) leading to mucosal health or disease. Symbionts/commensal bacteria facilitate intestinal health by promoting tolerogenic immunity and barrier function. Expansion of pathobionts can lead to alteration of epithelial turnover, increased endoplasmic reticulum (ER) stress, pro-inflammatory signaling, and impaired barrier function. TJ, tight junction; Treg, T regulatory cell; AMP, antimicrobial peptide.

sisted longer than those in control HT-29/B6 IECs.[148] In addition, colibactin-producing *E. coli* strains (which are also associated with inflammation-associated colorectal cancer) activate senescent secretory intestinal cells to increase growth factor production, leading to increased local cell proliferation and tumor growth.[149-151] Finally, expansion of *Bacteroides vulgatus* in *Nod2*-deficient mice was associated with goblet cell dysfunction and subsequently increased numbers of interferon-γ-expressing lymphocytes.[152] Together these studies demonstrated the ability of pathobionts to hijack the proliferation-differentiation process in the intestine in order to reduce important mediators of host defense and promote bacterial invasion.

2. Activation of Immune and Stress Responses

Pathobiont expansion can also increase pro-inflammatory signaling in IECs leading to dysbiosis and colitis. As mentioned above, *B. vulgatus* also increased pro-inflammatory cytokine production in *Nod2*-deficient mice.[153] The capacity of pathobionts to drive a pro-inflammatory response does not appear to be uniformly dependent on the presence of a host defect in innate immunity as *E. faecalis* activates NF-κB, p38 MAPK, and ERK1/2 pro-inflammatory signaling via TLR2 resulting in induction of IL-6 and IP-10 secretion in wild-type murine IECs.[154] AIEC secretion of outer membrane vesicles leads to activation of the host ER stress response protein Gp96.[155,156] In addition, AIEC infection in mice also increased *Il6* and *Lcn2* mRNA expression, fecal lipocalin-2 content, and spleen weight.[108] Bretin and colleagues recently suggested that transient AIEC infection in mice can promote inflammatory signaling after clearance of the pathobiont.[136] These data demonstrate that pathobionts are able to induce stress responses and inflammatory signaling in IECs and may continue to promote inflammation long after infection.

3. Expansion of Pathobionts

While much work has focused on how pathobionts induce inflammation, we have only begun to understand how these initially innocuous microbes become virulent and expand. As chronic GI disease is prevalent in developed countries, it has been hypothesized that environmental and behavioral factors such as diet contribute to dysbiosis. In support of this, studies have demonstrated that individuals relocating to developed countries begin to exhibit a more Western-like microbiome composition, similar to that of non-human primates who are captured in the wild and transferred to captivity.[157] *B. wadsworthia* is a gram-negative bacterium usually found at low abundance and has sulfite-reducing activity. Mice that were fed a high milk fat diet with saturated fats developed expanded *B. wadsworthia* leading to colitis, however this did not occur in mice fed other types of saturated fats or polyunsaturated fats.[117] Milk fats have elevated levels of hydrophobic stearate that requires higher concentrations of bile salts for emulsification. Milk fat feeding led to a higher ratio of the bile salt taurocholate, which is a more efficient emulsifier and a source of organic sulfur.[117] The subsequent increase in luminal organic sulfur facilitated a bacterial bloom of *B. wadsworthia* in these mice.[117] In addition to dietary components, inflammatory mediators can also promote pathobiont growth. For example, when IL-22RA1 KO mice were infected with *Citrobacter rodentium* they developed sepsis and had increased mortality due to expansion and increased translocation of *E. faecalis*. IL-22RA increases *Fut2* expression and subsequently increases intestinal fucosylation. This promotes increased diversity of anaerobic commensal symbionts, restricting expansion of *E. faecalis*.[113] Pathobionts can also promote their own survival by escaping or resisting host defense mechanisms. For example, *E. faecalis* can resist antimicrobials targeting its cell envelope through expression of IreK, which is a kinase important for long-term colonization, potentially through inhibition of antagonistic enterococci proteins.[158] Thus, it is clear that a variety of host and environmental factors can precipitate selective expansion of pathobionts and that inflammatory responses can not only alter populations of pathobionts but also modify their level of pathogenicity, in conjunction with increasing host epithelial susceptibility to pathobionts.

CONCLUSIONS

It is increasingly clear that the gut microbiota has a significant impact on human health. Recent literature has described how changes in the intestinal microbiota can affect neurological networks and behavior, host metabolism, cardiovascular health, and many other physiological systems. Commensal bacteria promote mucosal health by fortifying barrier integrity, increasing mucin and IgA secretion, inhibiting pro-inflammatory responses and apoptosis, and promoting commensal colonization. In contrast, pathobiont bacteria repress expression of tight junction proteins and promote mislocalization, cause dysregulation of apoptosis and proliferation, and increase ER stress and pro-inflammatory signaling (Fig. 1). These effects contribute to the development of dysbiosis and prolong inflammatory signaling following infection. While much work

has begun to unravel this intricate network of interactions between the gut microbiota, IECs, immune cells, and environmental factors, many questions still remain due to limitations in technology and methods to adequately study the microbiome. Future studies focusing on the causal roles of bacteria and inflammation in driving changes to the microbiome and the influence of these changes on the intestinal mucosa will not only increase our understanding of how these relationships operate in different setting (i.e., health vs. disease) but has the potential to identify strategies through which we can harness the therapeutic potential of host-gut microbe interactions.

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CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

AUTHOR CONTRIBUTION

King SJ and McCole DF conceived, wrote, and edited the manuscript.

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