

REVIEW



Role of gut microbiota in intestinal wound healing and barrier function

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ABSTRACT

The mammalian intestine harbors a highly complex and abundant ensemble of bacteria that flourish in a nutrient-rich environment while profoundly influencing many aspects of host biology. The intestine coevolved with its resident microbes in a manner where the mucosa developed a barrier function to segregate the resident microbes from the rest of the body, and yet paradoxically, allowing integration of microbial signals for the host benefit. In this review, we provided a comprehensive overview of why the gut microbiota is key to the efficient development and maintenance of the intestinal barrier. We also highlighted how a destabilized equilibrium between gut microbiota and the host may eventuate in a wide range of intestinal diseases characterized by the disrupted intestinal barrier. Finally, the review delineated how microenvironmental changes in the injured mucosa result in an enrichment of a pro-regenerating consortium of bacteria, which augments mucosal wound repair and restoration of barrier functions.

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Introduction

The mammalian intestine harbors a taxonomically diverse microbial community, which acts as an accessory organ system with distinct functions fundamentally critical in the well-being of their host.^{1–5} This symbiotic and mutualistic host-microbiota relationship has evolved to the extent that the intestine has established a barrier function separating the colonized microbes from the systemic tissues, and yet, also providing a gateway for the cross-talk between the two. The gut barrier function is central to health and breakdown of the barrier is involved in a wide variety of clinical conditions.^{5–8} Although the intestinal barrier depends on the coordinated contributions from a complex network of cellular, immunological, biochemical or microbial factors, ultimately it is the monolayer of tightly junctioned columnar epithelial cells that confer the selective barrier function.^{9–11} Compromised epithelial barrier is a hallmark of IBD, irritable bowel syndrome, obesity and the metabolic syndrome and necrotizing enterocolitis.^{7,12–14} However, mechanistically, it is not well understood how the intestinal resident microbiota influences the efficient maintenance and/or repair of the epithelial barrier. Damage to the barrier -the intestinal wound-

is observed in IBD, enteric infections, as well as following surgical trauma, and environmental insults. Intestinal wound regeneration is a process that restores homeostasis through a complex process of cellular proliferation and migration.^{15–17} This repair process is orchestrated by a coordinated network of different cellular, immunological, biochemical, and also microbial influences.¹⁸ The mechanisms of commensal gut resident microbiota in the restoration of epithelial barrier function and epithelial wound healing is only recently being delineated. Interestingly, several recent studies described the dynamic changes in the physiology and inflammatory responses of the wound microenvironment^{19–22} and the consequent alterations in the bacterial population. Furthermore, it was determined that these events drive a spatiotemporal alteration of the microbial community structure in the mucosal wound microenvironments resulting in the enrichment of a mucosa-associated microbial consortium, which in turn, augments gut epithelial wound healing.¹⁹ This review will particularly focus on the importance of gut microbiota and their products on (1) epithelial barrier functions, (2) resolution of mucosal inflammation, and (3) repair of intestinal wounds.

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The composition of the commensal bacteria of the intestine during homeostasis

Mammals are not autonomous entities, rather can be considered holobionts²³ that harbor microbial communities of astounding density and taxonomic diversity.^{2,24} Holobionts represent the host macro-organism which exists a close symbiosis with the associated microorganisms. The microbiota refers to all of the microorganisms associated with an animal or plant host. The microbiome, on the other hand, is defined as the sum of the genetic information of the microbiota. The components of the gut microbiota – bacteria, viruses, and eukaryotes – interact with one another and with the host immune system that ensure the intestinal homeostasis, and also, at times provoke the development of the disease. This review will focus on the fundamental role of the intestinal bacterial population during repair of the injured mucosa, and also in the restoration of homeostasis. Therefore, the term microbiota will refer to the commensal enteric bacteria in this review.

The past decade has observed a dramatic rise in meta'omics studies of IBD and related inflammatory diseases. As a result, we are beginning to develop an ecological or community-wide understanding of the critical role of the microbiome in intestinal diseases.^{25,26} Recently, these findings have begun to be translated into a functional mechanistic interpretation of the microbiome in the development as well as exacerbation of the inflammatory diseases.^{7,13,25,26} The gut microbiota is currently being surveyed using 16S rRNA gene amplicon sequencing or whole genome sequencing (WGS) techniques. 16S rRNA gene sequencing is popularly used for phylogenetic reconstruction and quantification of microbial diversity. In contrast, WGS explores the functions of the encoded genetic material as well as assesses bacterial diversity.^{7,24,27,28}

The neonatal intestinal tract is rapidly colonized by an array of commensal enteric bacteria after birth eventually forming a complex and dynamic ecosystem. However, the temporal patterns of these changes remain unique to each infant.^{29–32} Furthermore, by around 2.5 years of age, the composition, diversity and functional capabilities of the infant microbiota resemble those of adult.^{30–32} Members of the microbiota may exist in a planktonic state, free-living in the

luminal stream; they may be partially adherent to the intestinal mucosa or mucus layer; or they may be resident in the cecal and proximal-colonic crypt structures. The total number of commensal enteric bacteria vary greatly from $\sim 10^{11,12}$ cells/gram of intestinal luminal content within the ascending colon, to $\sim 10^{7,8}$ in the distal ileum, and $\sim 10^{2,3}$ in proximal ileum and jejunum. Recent human studies identified more than 2100 species classified into 12 different phyla. However, high throughput sequencing analyses 16S rRNA genes have revealed that more than 90% of bacterial species found within the gut belong to 4 phyla including *Proteobacteria*, *Actinobacteria*, *Firmicutes* and *Bacteroidetes*.²⁴ The higher redundancy of the gut bacterial species is reflected in the richness and diversity of the microbial community and their genomes, which ensure diverse functionality and overall stability. Nonetheless, the relatively resilient composition of the adult gut microbiota is still subject to perturbation by different factors including diet and host genetics leading to the alteration in microbial community structure.^{30,31,33}

At homeostasis, the composition and number of the intestinal microbiota community are shaped by physiological (oxygen, reactive oxygen species, acids), biochemical (enzymes, metabolites), nutritional and immunological gradients (antimicrobials, sIgA) along the transverse and longitudinal axis of the GI tract.^{21,22,34} For example, the pH in the mouth is close to neutral and the saliva contains enzymes inhibiting bacterial growth. On the other hand, the stomach is extremely acidic (pH 2). The pH gradually increases in the small intestine and the colon (pH 4–5 and 6.7 respectively).³⁵ Hence, within single individuals, the bacterial community of the small intestine is different from that of the colon. Similarly, the luminal and mucosal compositions of the microbiota are significantly divergent. Moreover, several environmental factors have been implicated in influencing the microbiota including geographical location, surgery, smoking, depression and living arrangements (urban or rural). In addition, antibiotics and other drugs alter the physiology and microbial community of the active human gut microbiome. Therefore, the relatively stable composition of the adult gut microbiota is still subject to perturbation by different factors.^{30,33}

Complex microbial communities are an integral component of our bodies in health and disease. In

fact, the taxonomically diverse human microbiome complements us with over 100 times more genes than those in our genome. Hence, microbes, their metagenome, and their biochemical activities are crucial factors determining the balance between health and disease state of the hosts.⁴ Recently, an extensive catalog of the functional capacity of the human gut microbiome has been determined, where 9,879,896 genes were identified.^{24,36,37} However, the microbiota's nearly 10 million genes and their metabolic functions remained largely unrecognized.^{7,36,38,39} The early studies involving microbial community composition of a given ecosystem has moved well beyond this observational stage, progressing towards functional analysis of the genes and pathways carried by a microbial community, i.e., metagenome. High-throughput analytical approaches, such as metagenomics, metatranscriptomics, metametabolomics and other meta'omics analysis provide comprehensive technologies to explore functional and biomolecular activities of the microbiome and its host.^{39,28} (Figure 1, Mass spectrometry-based and chromatography-based techniques have recently been applied to host-microbiome studies for targeted and untargeted metabolomic and metaproteomic strategies to systematically discover the chemical diversity of the commensal bacteria and the hosts.²⁸ This multidisciplinary approach in bioinformatics and molecular biology to discover genes, products and pathways of the holoorganism are important for dissecting disease mechanisms as well as pivotal in the development of the potential therapeutic, diagnostic, or prognostic applications (Figure 1).

Function of gut microbiota during intestinal homeostasis

Intestinal barrier function is key to the maintenance of gut homeostasis. This is consistent with the fact that many of the IBD susceptibility genes are related to intestinal barrier function. Furthermore, investigations with germ-free mice have demonstrated that the resident bacteria profoundly influence epithelial barrier function, metabolism, proliferation, differentiation and survival.^{1,2,40–42} Although recent studies have implicated the gut microbiota in numerous aspects of the proper development and efficient functions of the intestine, this section will

limit the discussion on the influence of commensal microbiota on (1) perception of gut microbiota (2) epithelial proliferation and turnover, (3) epithelial selective barrier, and (4) mucus barrier.

Intestinal perception of the microbiota

Intestinal homeostasis, and also, the development of many inflammatory diseases, are regulated in part by tolerance towards the commensal microbiota that influences the development and training of innate and adaptive immune systems,⁴³ processes modulated by the broad class of pattern recognition receptors (PRRs).⁴⁴ PRRs include Toll-like receptors (TLRs), NOD-like receptors (NLRs), C-type lectin receptors (CLRs) and also some G-protein coupled receptors (GPCRs).⁴⁵ TLRs are characterized structurally by the presence of leucine-rich repeat (LRR) motif in their extracellular ligand-binding domain, a transmembrane domain and a conserved Toll/IL-1R homology domain in the cytosolic region for signal transduction. PRRs recognize microbe-associated molecular patterns (MAMPs) which are conserved molecular motifs derived from commensals and pathogens. TLRs initiate immune responses via signaling adaptors, such as myeloid-differentiation primary response gene 88 (MyD88), which is required for several TLRs.⁴⁵ Additionally, G-protein coupled receptors (GPCRs) are seven transmembrane (7 TM) receptors, and upon ligand binding, signal inside the cell through a complex network of signaling, adaptor and regulatory proteins, including heterotrimeric G proteins. Several GPCRs, including N-formyl peptide receptors (FPRs), have been demonstrated to mediate host-microbiota interactions by sensing microbial peptides, small molecules, and metabolites.

Microbial effects on epithelial proliferation and protection via epithelial prrs

During homeostasis, the epithelial surface is continually renewed by the proliferation and differentiation of pluripotent intestinal epithelial stem cells of the crypts. The differentiation results in the development of six specialized cell types with defined functions: enterocytes, enteroendocrine cells, Paneth cells, tuft cells, goblet cells and microfold (M) cells. The intestinal epithelium is equipped with different PRRs that recognizes microbial components, and

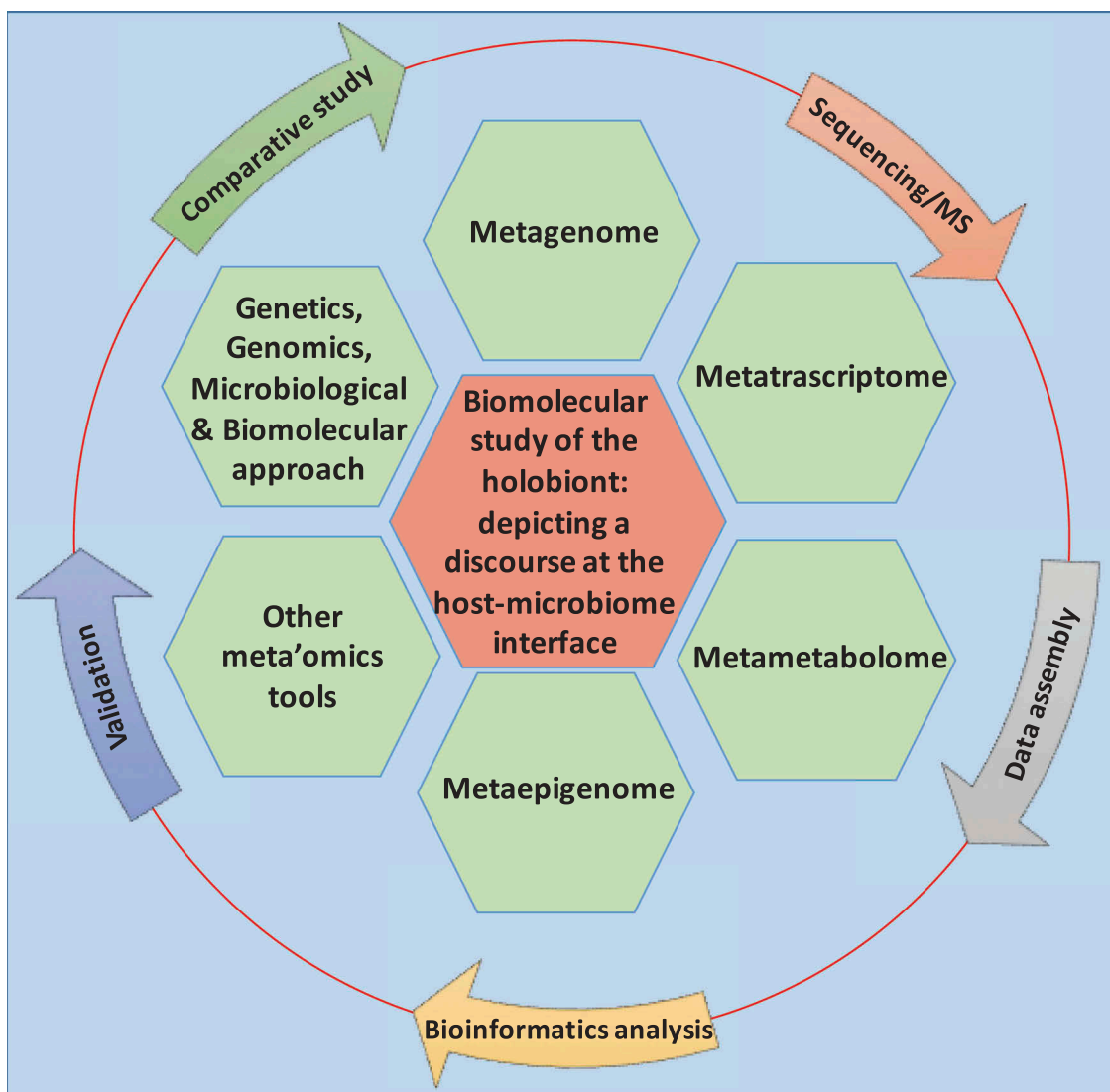


Figure 1. A multidisciplinary approach in bioinformatics and experimental biology to discover genes, pathways, and molecules important for dissecting the biomolecular cross-talk at the host-microbiome interface. Outer circle depicts the workflow of meta'omics study.

ultimately, elicits cell signaling pathways involved in cellular proliferation. Rakoff-Nahoum et al. have established that commensal enteric bacteria are recognized by TLRs (TLR2 and TLR4) and that this interaction plays a crucial role in the maintenance of intestinal epithelial homeostasis as well as protection from mucosal injuries in a mouse model of chemically-induced colitis.⁴⁶ TLR/MyD88 and Nucleotide oligomerization domain (NOD)-like receptors (NLRs) are essential for the regulation of inflammation and production of antimicrobial proteins, such as RegIII γ , cathelicidins, and defensins, which prevent encroachment of epithelial cells by gut microbiota and thereby maintain homeostasis.⁴⁷ For example, it has been previously shown that the

development of gut inflammation in flagellin receptor TLR5-deficient mice was associated with failure to restrict pro-inflammatory proteobacteria, particularly in the post-weaning period.^{48,49} Direct activation of epithelial TLR signaling has been shown to alter intestinal homeostasis by affecting the proliferation and apoptosis of epithelial cells.^{45,50} How gut microbiota drives the intestinal epithelial cell self-renewal and turnover via the PRRs, and hence maintain, epithelial homeostasis, are matters of immense current interest.

G-protein-coupled receptors (GPCRs) mediate myriad cellular functions including proliferation, differentiation, adhesion, and migration and play pivotal roles in development, homeostasis,

inflammation, immunity, and oncogenesis. GPCRs are also important pharmaceutical targets, as about half of the drugs currently used are either agonists or antagonists for various GPCRs. Recent studies have shown that GPCRs can mediate host-microbiota interactions by sensing microbial products and small molecules. N-formyl peptide receptors (FPRs) can sense and bind an array of formylated and nonformylated peptides as well as nonpeptide ligands synthesized either endogenously by the host or exogenously by the microbiota.⁵¹ Although FPRs are not considered as typical leucine rich repeat bearing PRRs such as TLRs and Nods, the FPRs are clearly, by definition, postulated to function as the pattern recognition receptors that recognize and respond to bacterial products. FPRs are expressed on the surface of neutrophils and macrophages, where they perceive bacterial products and stimulate phagocyte functions. While aberrant PRR activation, including TLRs and NOD2 is associated with the onset of inflammation,^{52,53} we have shown FPR can induce non-proinflammatory signaling in epithelial cells *in vitro* and *in vivo*.⁵⁴ Humans have three FPR paralogs.⁵⁵ FPR1 is the high-affinity receptor for the bacterially synthesized fMLF. FPR2 has been shown to respond to endogenous ligands including annexin A1, lipoxin A4, and serum amyloid A.^{55,56} Importantly, FPRs are also expressed in and localized to the apical and basolateral surface of the intestinal epithelial cells.⁵⁷ Although FPRs are well characterized in phagocytes, their roles in intestinal epithelial cells are not well understood. Recent studies demonstrated that commensal bacteria can activate FPR1 to stimulate the proliferation of intestinal epithelial cells in a redox-dependent manner.^{58,59} In a recent review article (please see refs.⁵³ and ⁵⁹), Jones et al. comprehensively discussed how physiological level of reactive oxygen species (ROS) induced by the gut microbiota promotes epithelial proliferation and intestinal barrier functions.⁵⁹ The study demonstrated that human commensal lactobacilli induce ROS generation in intestinal epithelial cells via the catalytic action of Nox enzymes ultimately resulting in the efficient cellular proliferation.⁶⁰ Moreover, using a *Drosophila* model, mono-colonized *Lactobacillus plantarum* induced the dNox-dependent generation of cellular ROS, and ROS-stimulated epithelial

cell proliferation in axenic larval flies.⁶⁰ Furthermore, *L. rhamnosus* GG was shown to induce ROS generation and cell proliferation in the murine intestine.^{58,61,62} Importantly, it has been shown that specific probiotic bacteria activate FPR1 and epithelial NOX1 to stimulate physiological ROS that increase phosphorylation of ERK^{58,63} via oxidative inactivation of the redox-sensitive and ERK-specific phosphatase called DUSP3 (dual specific phosphatase 3), and consequently induce cell proliferation.⁵⁸ The molecular mechanism by which ROS control cell signaling pathways is by the oxidation of reactive cysteine residues within regulatory proteins.⁶⁴ A number of redox-sensitive regulatory proteins have been characterized and shown to be sensitive to microbiota-induced ROS generation, which includes the lipid phosphatase (PTEN), low-molecular weight (LMW)-PTP, and enzymes involved in ubiquitination processes.^{61,62,65,66} Together, these data from the cultured human enterocytes, *Drosophila* and murine models demonstrate that commensal bacteria and their products activate ROS signaling in an FPR-dependent manner and define a conserved mechanism by which cellular ROS enhance epithelial development and homeostasis. In addition, these discoveries establish the function for PRRs, including FPRs, in perceiving the commensal enteric microbial products and mediating an efficient epithelial turnover and barrier integrity.

Microbial effects on intestinal epithelial barrier

Intestinal epithelial cells establish and maintain a continuous barrier to avert potential encroachment of harmful luminal components; nonetheless, it remains selectively and dynamically permeable. The tasks of regulating paracellular transport and intercellular connections between the polarized columnar epithelial cells are achieved by a series of intercellular junctions, which are conferred by complex junctional structures, the tight junctions (TJs), adherens junctions, the desmosomes and the gap junctions.^{10,11,14} A network of actin and myosin supports these types of junctions, which are composed of transmembrane proteins, scaffolding proteins, and junctional molecules.⁶⁷ The physiological structures, properties, and functions of the epithelial apical junctional complex have been reviewed extensively elsewhere

(please see refs. ^{9,11,14}, and ⁶⁷). In response to different stimuli, these junctional complexes also dynamically change epithelial permeability by rapid assembly and disassembly.^{68,69} Increased epithelial permeability and dysfunctional epithelial integrity are widely acknowledged to play a critical role in the pathophysiology of various intestinal disorders, including the enteric infectious disease, inflammatory bowel disease (IBD), irritable bowel syndrome obesity Graft-versus-host disease (GVHD), celiac disease, the metabolic syndrome, necrotising enterocolitis and even in human immunodeficiency virus HIV/AIDS syndrome.^{14,70,71} Transcriptional, structural and functional changes have been reported in the TJs of intestinal epithelial cells in patients with IBD, including claudin proteins.^{68,70}

It is widely reported that commensal enteric bacteria can have profound effects on epithelial permeability and integrity, particularly, on TJ repair and maintenance (Table 1).⁷²⁻⁷⁶ For example, in 1996, Mao et al. demonstrated that the human *L. plantarum* and the rat-originating strain *L. reuteri* could reduce permeability dysfunction in a methotrexate-induced colitis model in the rat. Intriguingly, this study determined that the rats administered *L. plantarum* show less *Enterobacteriaceae* and Gram-negative anaerobes in the intestine. Ewaschuk et al. demonstrated that soluble factors produced by *B. infantis* elevated expression of occludin, which resulted in decreased paracellular permeability.⁷⁷ It has been shown that bifidobacteria isolated from infants and cultured on human milk oligosaccharides (HMO) increased expression of ZO-1, occludin in HT-29 cells. Furthermore, both *B. infantis* and *B. bifidum* grown on HMO prevented the significant intracellular redistribution of occludin.⁷⁸ In addition, a recent study demonstrated that specific *B. animalis* strain protected barrier functions by

decreasing intestinal permeability in a mouse model characterized by low grade inflammation.⁷⁹ Importantly, this strain specifically normalized the level of several TJ proteins, in particular for claudin-4, and thereby, potently ameliorated compromised epithelial barrier in the mouse. A separate study demonstrated that *L. rhamnosus* and *Faecalibacterium prausnitzii* exhibit a protective effect in a murine model of induced-barrier hyperpermeability. An oral administration of these bacteria restored epithelial permeability defect by increasing the levels of tight junction proteins occludin and E-cadherin in murine colonic epithelial cells. The authors also postulated that these events were mediated by the microbiota-induced reduction of NF- κ B activation leading to the amelioration of epithelial permeability dysfunction.⁸⁰ Using a neonatal mouse NEC model, Bergmann et al. demonstrated that bifidobacteria stabilize claudins at tight junctions and prevent epithelial barrier dysfunction. In mice, administration of *B. infantis* attenuated increases in intestinal permeability, preserved claudin 4 and occludin localization at TJs, and decreased NEC incidence in mice.⁸¹ Taken together, this body of data collectively demonstrates that endogenous bacteria can have beneficial effects on the physiology of the intestinal barrier (Table 1).

Microbial effects on mucus barrier

The intestinal epithelium surface is covered by a mucus layer which differs in composition, organization, and thickness at different locations along the intestinal tract, and represents the first site of mucosal contact with the gut microbiota. The mucus layer is a dynamic and complex structure that is mainly composed of the glycoprotein mucin-2 (MUC2). MUC2 is a large 5,197-amino

Table 1. Microbial effects on intestinal epithelial barrier.

Bacteria	TJ proteins	Experimental system
<i>L. plantarum</i> and <i>L. reuteri</i>		<i>In vivo</i>
<i>B. infantis</i>	Occludin	<i>In vivo</i> and <i>in vitro</i>
<i>B. infantis</i> and <i>B. bifidum</i> cultured on human milk oligosaccharides (HMO)	ZO-1, occludin	<i>In vitro</i>
<i>L. rhamnosus</i> GG	Claudin 3	<i>In vivo</i>
<i>B. animalis</i>	Claudin 4	<i>In vivo</i>
<i>L. rhamnosus</i> and <i>Faecalibacterium prausnitzii</i>	Occludin and E-cadherin	<i>In vivo</i>
<i>B. infantis</i>	Claudin 4 and occludin	<i>In vivo</i>
<i>L. plantarum</i>	ZO-1 and occludin	human volunteers
<i>B. longum</i> and VSL#3	ZO-1, occludin, and claudin-1	<i>In vivo</i>

acid protein core containing two mucin domains rich in proline, threonine, and serine repeats that become heavily O-glycosylated.⁸² Due to the cross-links, MUC2 forms a large, net-like polymer that is secreted by the goblet cells. Mice deficient in Muc2 demonstrate a defective mucus layer and development of spontaneous colitis. Importantly, biopsy samples of ulcerative colitis patients also show alterations in MUC2 expression and depletion of the mucus layer.⁸³ The colonic mucus consists of two layers, a loose outer layer, and a dense epithelium-attached inner layer.⁸⁴ In the distal colon, the inner layer acts as a physical barrier restricting bacteria from accessing the epithelium, whereas the outer layer is the habitat and food source of a distinct population of commensal microbes. In contrast, the small intestine is coated with the single thin layer of mucus, which is loose and penetrable by a much less numerous and diverse microbiota^{85,86}, likely due to the secreted antimicrobial peptides from small intestinal Paneth cells.⁴⁷ In the colon, some commensal bacterial contact with the epithelial cells occurs⁸⁷ For example, the close association of bacteria with the epithelial surface and within crypts have been demonstrated in the mouse proximal colon and cecum.^{88,89} The inner mucus layer of this region is less dense and more penetrable by bacteria.⁸⁶ Interestingly, the crypt-dwelling bacteria are distinct from the luminal community, and predominantly include *Proteobacteria*, *Firmicutes*, some *Bacteroidetes*. It is postulated that the deep channel of the crypts and transverse folds of the proximal colon provide a protected microhabitat for these bacteria.⁸⁹ Similarly, it has been recently shown that during regeneration of epithelial wounds, a consortium of anaerobic, mucinophilic bacteria transiently bloom in the close proximity of the murine intestinal epithelial cells.¹⁹ In addition, the segmented filamentous bacteria (SFB) adhere tightly to epithelial cells of villi and Peyer's patches in the ileum of mice, which confers pronounced immunomodulatory effects to the host.⁹⁰

Certain commensal microbes are enzymatically equipped to utilize mucin glycans as their energy source. However, an aberrant abundance of these bacteria has been reported to be associated with different inflammatory diseases. For example, *Ruminococcus gnavus* and *Ruminococcus torques*,

two prominent mucin degraders, are increased in IBD.^{91,92} Interestingly, a number of studies also provided evidence that gut microbiota is essential for the proper function and integrity of the intestinal mucus layers. For example, the mucus layer of the germ-free mice is thinner in comparison with the conventionally raised animals, and monocolonization of the germ-free mice with a commensal bacteria resulted in the expansion of the volume of mucus layer.^{93,94} Furthermore, germ-free mice monocolonized with short chain fatty acids (SCFA)-producing *B. thetaiotaomicron* or *Faecalibacterium prausnitzii* increased goblet cell differentiation and elevated mucus production.⁹³ Thus, during intestinal homeostasis, the mucin-utilizing bacteria are required for the proper turnover of the mucin protein to ensure an efficient barrier. Paradoxically, the aberrant abundance of the mucin-degrading bacteria may result in the dysfunction in the mucus barrier.

Together, these studies emphasized the importance of intestinal commensal microbiota, which modulates intestinal barrier function by regulating epithelial cell self-renewal and turnover, production of mucus layer or importantly, by influencing tight junctions of the intestinal epithelial cells.

Microbial effects on intestinal inflammation

Intestinal homeostasis can be disturbed by the absence or over-representation of certain bacterial groups or altered abundance of microbial genes and products in the intestine. An unfavorable imbalance of microbial composition, altered diversity and defective functions of the intestinal microbiota is termed dysbiosis.^{6-8,12,13} Microbial dysbiosis is intricately associated with the host immune system and barrier functions. As a result, dysbiosis of the gut microbiota is associated with different diseases including IBD, liver diseases, gastrointestinal cancers, metabolic diseases, respiratory diseases, mental or psychological diseases, autoimmune diseases, and also infectious diseases.^{7,13}

Dysbiosis of gut microbiota in IBD

This microbial dysbiosis in IBD is often attributed by the decrease of bacteria with anti-inflammatory capacities and the elevated

abundance of bacteria with inflammatory potentials. Furthermore, dysbiosis can also be associated with major impairments in many crucial microbial metabolic functions with critical impact on the host.^{6,8,13,25,95} For example, a lower abundance of *Firmicutes* and the increases in abundance of *Proteobacteria* and *Bacteroidetes* have been reported to be associated with IBD.⁹⁶ It has been reported that *F. prausnitzii*, *Blautia faecis*, *Roseburia inulinivorans*, and *C. lavalense*, all of which belong to *Firmicutes*, are depleted in patients with CD.^{8,97} Moreover, the number of *F. prausnitzii* is correlated with the risk of relapse of ileal CD after surgery. Furthermore, a significant decrease of *Roseburia* spp. was shown in the gut microbiota of healthy individuals with a high genetic risk for IBD. Interestingly, *F. prausnitzii* has been reported to have a pronounced anti-inflammatory effect by fermenting carbohydrate into butyrate, an abundant short chain fatty acid. Moreover, human peripheral blood mononuclear cells stimulated with *F. prausnitzii* induce the production of IL-10 and inhibit the generation of inflammatory cytokines, such as IL-12 and IFN- γ .⁹⁸ Similarly, a decreased abundance of *Akkermansia* has been reported in both UC and CD patients.⁹⁹ *Akkermansia* has been demonstrated to induce anti-inflammatory immune response and restore intestinal barrier defects.^{100,101} In contrast, a relative increase in pro-inflammatory *Proteobacteria*, mainly *E. coli*, was observed in CD patients, in particular, on mucosa-associated microbiota compared to fecal samples.¹⁰² CD-associated *E. coli* with pro-inflammatory properties includes adhesion-invasive *E. coli* (AIEC), which was originally isolated from adult CD patients. In addition, increased abundance of mucosa-associated bacteria in IBD have been reported. Different mucolytic bacteria, including *Ruminococcus gnavas* and *R. torques*, may degrade the mucin layers in IBD patients, and thereby, may facilitate the colonization of the epithelial surface by an elevated abundance of proinflammatory bacteria.^{92,99,103} Thus the microbial dysbiosis is often contributed by the aberrant balance between the bacteria with anti-inflammatory capacities and the bacteria with pro-inflammatory potentials.

Microbial effects on the pathogenesis of intestinal inflammation and colitis

Although the precise cause of IBD remains unknown, the most accepted hypothesis of IBD pathogenesis is that an aberrant immune response against the gut commensal is elicited by environmental factors in a genetically susceptible host.^{12,13,104} The disruption of the intestinal homeostasis, and thereby the development of the inflammation is initiated in part by the failure of tolerance towards the commensal microbiota that influences the development and training of innate and adaptive immune systems.^{43,44,105} Therefore, many of the known IBD susceptibility genes are associated with the recognition and processing of microbes, which is correlated with a role of the gut microbiota in the pathogenesis of IBD.

Recent studies using different animal models of inflammatory bowel diseases have provided evidence to establish a causal link between gut microbiota, aberrant immune response and disrupted barrier functions in a genetically susceptible host. For example, Kim et al. performed a mono-colonization study, in which a single strain of bacteria was inoculated into germ-free IL-10 knockout mice. The authors demonstrated that different commensal bacterial species selectively initiate CD4 + T cell-mediated intestinal inflammation with distinctly different kinetics and anatomic distribution in the same host. Specifically, *E. coli* induced cecal inflammation, *Enterococcus faecalis* induced distal colitis, and *Pseudomonas fluorescens* failed to cause colitis.¹⁰⁶ A separate study also reported that the mouse enteric commensal bacteria *Helicobacter hepaticus* exacerbated colitis in IL-10-deficient mice. In addition, a disproportionate outgrowth of a non-culturable *Clostridium*-related species of segmented filamentous bacteria promotes T cell-mediated pro-inflammatory immune responses.¹⁰⁷ These studies provided the evidence that the aberrant microbial composition can elicit intestinal immune responses even in an animal with the same genetic background. Interestingly, Garrett et al. demonstrated that mice deficient in both Tbx21/T-bet and Rag developed spontaneous colitis, which was ameliorated by the administration of antibiotics. Tbx21/T-bet

is an essential transcription factor for Th1 differentiation, and Rag is central to the development of the acquired immune system. Importantly, wild-type mice co-housed with colitis-prone T-bet/Rag deficient mice also demonstrated similar colonic inflammation and colitis. These findings suggested that a dysbiotic gut microbiota is communicable and can cause intestinal inflammation without genetic manipulation.¹⁰⁸ In addition, these results highlight that gut microbial dysbiosis may contribute to the intestinal inflammation.

Disequilibrium in the abundance microbial metabolites and small molecules are also involved in the regulation of inflammatory response. Microbially fermented short-chain fatty acids (SCFAs) can bind and activate GPCRs, which are expressed on the surface of epithelial cells and immune cells and have numerous regulatory functions on host physiology and immunity. These GPCRs include GPR41, GPR43 and GPR109A. One of the important functions of the SCFA-sensing GPCRs is the regulation of the effector and regulatory T cell development and expansion. Specifically, SCFAs influence peripheral T cell numbers and function, particularly regulatory T (Treg) cells, through epigenetic regulator histone deacetylases (HDAC) inhibition. SCFA-dependent inhibition of HDAC9 increased forkhead box P3 (FOXP3) expression, elevated Treg cell numbers, and finally, enhanced the immunosuppressive function of FOXP3+ Treg cells, which inhibits inflammatory response in a murine model of colitis.¹⁰⁹ In addition, activation of IEC-specific GPR43 and GPR109A by microbial SCFAs increases production of the cytokine IL-18, and further, prevents inflammation in a murine model of colitis via the activation of inflammasome assembly.^{110–112} Thus, a dysbiotic gut microbiota characterized by the depletion of SCFA producing bacteria may result in the defective immunosuppressive function of various cell types leading to the development of intestinal inflammation and colitis. Findings from animal models have revealed diverse roles of the gut microbiota in protective or pro-inflammatory functions. As discussed above, recent studies performed in animal models clearly provided data supporting an etiological role of gut microbiota in development of model colitis. However, a direct cause and effect

relationship between dysbiosis and IBD has not been definitively established in humans.¹⁰⁴

Effect of gut microbiota on intestinal epithelial cells during repair of injured mucosa

Mucosal wound repair is coordinated by a spatio-temporal network of different cell types, which include the epithelium itself, inflammatory cells, endothelial cells, and mesenchymal cells. Intermolecular and intercellular communication among these cells creates a microenvironment conducive for epithelial regeneration and mucosal healing, which is characterized by efficient migration and proliferation of intestinal epithelial cells followed by tissue remodeling.¹¹³ Recent studies provided evidence that commensal microbiota profoundly influences the repair of the injured mucosa by regulating the functions of the wound-associated cellular network and ultimately modulating migration and proliferation of epithelial cells adjacent to the wounded area.

Effect of microbiota on epithelial migration during wound healing

In larger wounds, the denuded mucosal surface is initially covered by the migrating epithelial cells adjacent to the wound. The migration of epithelial cells as a sheet is also referred to as “epithelial restitution”, which requires dynamic and coordinated remodeling of cell-cell and cell-matrix adhesions. Within minutes to hours, epithelial restitution begins when epithelial cells undergo de-differentiation, reorganize the actin cytoskeleton and migrate to cover the damaged area.¹¹⁴ The organization and remodeling of the actin cytoskeleton are controlled by the Rho family of small GTPases, which includes Rho, Rac, and Cdc42. These proteins regulate the formation of stress fibers, lamellipodia, and filopodia.¹¹⁵ In addition, formyl peptide receptor-1 (FPR1) signaling activates Rac1 and Rho kinase to regulate intestinal epithelial cell migration and wound repair.^{57,116}

Investigations in germ-free mice revealed an impaired rate of intestinal epithelial cell migration, a crucial cellular process of wound repair.^{15,117} This finding demonstrated that commensal bacteria are

required for efficient migration and wound healing. Cell migration is regulated by a coordinated restructuring of the actin cytoskeleton at the advancing edge of the cell called focal adhesions (FA) of the extracellular matrix. FAs are large macromolecular assemblies that link the extracellular matrix (ECM) to the cytoplasmic cytoskeleton of an interacting cell. Cell migration, and thus wound repair, is critically dependent upon the dynamic assembly and disassembly of focal adhesions.¹¹³ The dynamics of the FA assembly is controlled by an enzyme called focal adhesion kinase (FAK), which is a protein tyrosine kinase. FAK remains in its inactive state under the dephosphorylating influences of the redox-sensitive tyrosine phosphatases LMW-PTPase and SHP-2. Furthermore, interactions between growth factors and integrins at the basement membrane trigger Nox1-dependent cellular ROS production which inactivates PTPase. These events result in the elevated levels of phosphorylated FAK thereby initiating FA turnover and cell motility. We demonstrated that specific commensal bacteria induce the generation of ROS in intestinal epithelia, especially elevated at the leading edge of the migrating epithelial sheet, where there is constant remodeling of the actin and turnover of FA. Furthermore, our data demonstrated that the bacteria-mediated ROS generation induced rapid and transient oxidative inactivation of target cysteines in the redox-sensitive tyrosine phosphatases, LMW-PTP and SHP-2. Both of these are known regulators of FAK phosphorylation, and therefore, FAK activity.⁶² Thus, these events resulted in increased phosphorylation of FAK. Consequently, phosphorylation of FAK substrate proteins, focal adhesion formation, and cell migration were all significantly enhanced in response to commensal bacteria in both *in vitro* and *in vivo* models of wound closure. These results supported that commensal bacteria regulate cell migration and restoration of intestinal barrier functions via induced generation of ROS in epithelial cells.

The NADPH oxidases (Nox) enzymes are widely conserved across the animal, and plant kingdoms. Cellular Nox enzymes often serves as the catalytic sources for ROS generation in response to bacterial stimuli. Activation of NOX2 is responsible for neutrophil and macrophage ROS generation. However, apart from the microbiocidal effect at a high concentration in mammalian cells, the physiological level of ROS

may also serve as critical secondary messengers in multiple signal transduction pathways. In humans, paralogs of NOX2 are found in many tissues, two of which are NOX1 and DUOX2. Both of these genes are mostly expressed in the colonic tissue. Recently, Leoni et al. demonstrated that FPR ligand, annexin A1, and its cleavage product Ac2-26, mediate activation of ROS by activating the intestinal epithelial NOX1.¹¹⁶ Furthermore, the study showed that epithelial cell migration was regulated by this signaling cascade through oxidative inactivation of the redox-sensitive regulatory phosphatases PTEN and PTP-PEST, with consequent activation of focal adhesion kinase (FAK) and paxillin.¹¹⁶ *In vivo* studies using intestinal epithelial-specific Nox1^{-/-IEC} mice demonstrated defects in intestinal mucosal wound repair, while systemic administration of ANXA1 promoted wound recovery in a NOX1-dependent fashion.¹¹⁶ Subsequently, we reported that specific members of the gut microbiota stimulate FPR1 on intestinal epithelial cells and generate reactive oxygen species (ROS) via enterocyte NOX1.⁵⁸ Ultimately, NOX1-derived ROS caused a rapid increase in the level of phosphorylated FAK and enhanced epithelial migration and promoted wound closure in mouse models.^{62,63} Thus, our work demonstrated that the enteric microbiota activates FAK and thereby enhances epithelial restitution and promotes epithelial repair of mucosal wounds in a redox-dependent manner.^{59,62,63,116} Interestingly, germ-free mice show diminished expression of NOX1 and DUOX2 genes along the intestinal tract in comparison with the conventionally raised mice, suggesting the microbiota can stimulate expression of these enzymes.¹¹⁸ Moreover, the promoter region for NOX-1 contains binding elements for AP-1, NFκB, CREB, STATs, and Interferon, indicating a role of cytokine-dependent regulation of gene expression. These studies support that microbiota has a crucial function in the regulation of the NOX-mediated and redox-dependent cellular signaling either by triggering receptor-mediated functional activation or by modulating expression of *Nox* genes.

Effect of microbiota on epithelial proliferation during wound healing

Epithelial proliferation is an integral cellular process for the repair of larger wounds. The intestinal epithelium is comprised of simple columnar

epithelial cells that are highly dynamic as they are continually renewed by stem cells residing in the crypt units. These cells are located in the crypts adjacent to the wounded area and undergo active proliferation to increase the number of proliferating progenitor cells within the crypts (Figure 2). Ultimately, they emanate out of the crypts as a monolayer of non-proliferating wound-associated epithelial cells (Figure 2), which migrate further and re-epithelialize the injured area, and thereby contribute to mucosal wound closure.^{17,63,119} Many studies demonstrated that various growth factors including transforming growth factor (TGF)- α and TGF- β , epidermal growth factor (EGF), insulin-like growth factor (IGF), hepatocyte growth factor (HGF), keratinocyte growth factor (KGF), fibroblast growth factor (FGF), the cytokine interleukin (IL)-1 β and IL-2, and trefoil peptides enhance proliferation of IECs.^{120–123} Remarkably, germ-free mice show a decreased

level of TGF- β expression in comparison with the conventional mice. Besides, several clostridial, bifidobacteria and lactobacilli species were shown to modulate TGF- β , KGF, and EGF-mediated cellular functions in the colonic epithelial cells.

Activation of specific cell signaling pathways is involved in intestinal epithelial wound repair. For example, TGF- α promotes enterocyte proliferation through the activation of extracellular signal-regulated kinase ERK1/ERK2 mitogen-activated protein kinase (MAPK).¹²⁴ Similarly, FPRs have been shown to be important for intestinal epithelial cell proliferation and wound closure via the activation of ERK1/ERK2 MAPK. Although the role of FPRs in microbial perception and effector function in phagocytes goes back decades, only recently have they been shown to promote wound closure in colonic, stomach, lung and retinal epithelial cells. Recently, FPR-deficient mice have been reported to show an exacerbated colonic epithelial injury

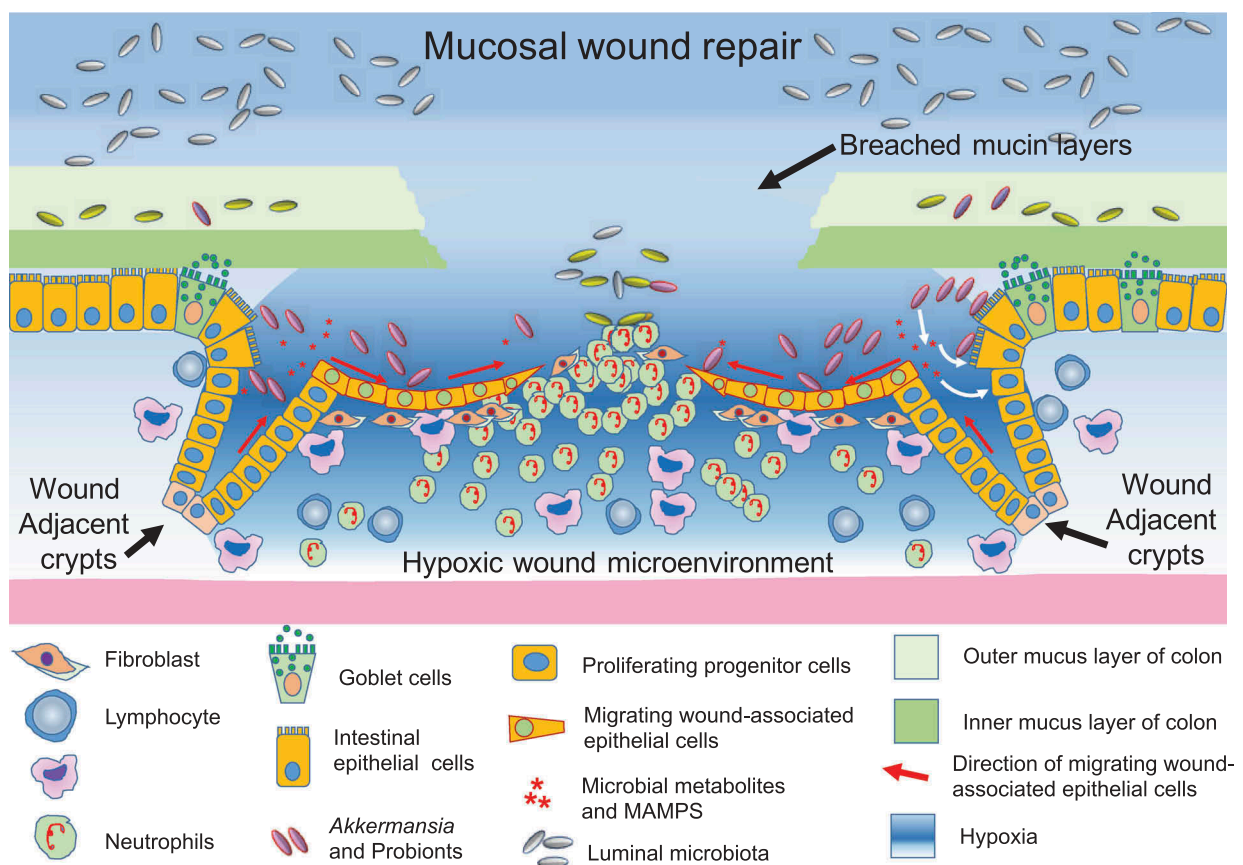


Figure 2. Host-microbiome interface of the mucosal wounds: dynamic changes in physiological and inflammatory responses of the wound microenvironment drive a spatiotemporal alteration of the microbial community structure resulting in the enrichment of a mucosa-associated microbial consortium, which in turn augments re-epithelialization of the mucosa, achieved by enhanced migration and subsequent proliferation of epithelial cells.

and delayed repair in a mouse model of chemically induced mucosal injury. In addition, during the resolution phase of inflammation, there is a temporal increase in pro-resolving mediators including resolvin D1 and lipoxin A4, which activate resolution via the FPR2 receptor.^{63,113,116} Importantly, by using a mouse model of wound regeneration, we recently demonstrated that specific members of the gut microbiota promoted wound closure by stimulating enhanced proliferation of intestinal epithelial cells in the crypts which are located adjacent to the injured mucosa. Furthermore, our study demonstrated that the microbiota-induced proliferation of the crypt epithelial cells resulted from the rapid phosphorylation of ERK1/ERK2 MAPK, which requires activation of the FPR1 and enterocyte-specific NOX1-mediated redox axis.^{58,60,61,63,125} These studies support that commensal microbiota are important for efficient proliferation and regeneration of intestinal wounds.

Effect of microbial mamps on cytoprotection and control of epithelial proliferation during wound healing

The innate immune system perceives the microbial invasion or epithelial injury via PRRs and confers immediate cellular responses during epithelial reneration. For example, in 2004, Rakoff-Nahoum et al. have demonstrated that commensal bacterial recognition by TLRs plays a crucial role in the protection of intestinal epithelia.^{46,126} This was the first evidence of microbial effect, particularly the released-MAMPs, in preserving gut homeostasis, promoting tissue repair and host survival upon recognition of bacterial products through PRRs. In addition, this discovery was surprising because up until then, TLRs were implicated in the stimulation of inflammation. Furthermore, Fukata et al. later demonstrated that TLR4 and MyD88- dependent protection from injury required cyclooxygenase 2 (Cox2)-mediated generation of prostaglandin E2 (PGE2).¹²⁷ However, the MyD88-Cox2 signal that promotes regeneration is largely provided by macrophages which migrate toward the site of injury to stimulate the proliferation of epithelial progenitors.^{15,128} The mechanism for improved

epithelial repair may be through PGE2-dependent activation of epidermal growth factor receptor (EGFR). In addition, TLR2 signaling has been demonstrated to be required for the induction of Foxp3+ Tregs and IL-10 production by PSA produced by the *Bacteroides fragilis*. Epithelial TLR/MyD88 signaling is essential for the regulation of antimicrobial peptides and molecules, which are required to prevent microbial encroachments towards the intestinal mucosa.⁴⁷ Neal et al. recently demonstrated that intestinal stem cells (ISCs) also express TLR4 and microbial ligand-mediated direct activation of TLR4 on ISCs, especially Lgr5-positive ISCs, potently inhibits cellular proliferation in intestinal crypts.^{50,129} Furthermore, LPS-mediated direct activation of enterocyte-specific TLR4 resulted in the increased apoptosis of the ISCs in a mechanism that is dependent upon the activation of the p53-up-regulated modulator of apoptosis (PUMA) in the pathogenesis of NEC. Hence, further studies are needed to define the role of TLRs in intestinal stem cells. The cytosolic innate immune sensor Nod2 has been shown to confer a cytoprotective effect. The Intestinal epithelial Lgr5+ stem cells, which are located within the intestinal crypts, constitutively express the cytosolic innate immune sensor Nod2 at levels much higher than in Paneth cells. Moreover, muramyl dipeptide (MDP), a peptidoglycan motif common to all bacteria, stimulates stem cell survival of intestinal organoids. These events result in an efficient cytoprotection against oxidative stress-mediated cell death.¹³⁰ These findings are of special importance in the context of examining the crypt-microbiota interface during regeneration of injured mucosa. For example, Pedron et al. previously identified the presence of a “crypt-specific core microbiota” in both the ceacal and colonic crypts of mice.⁸⁸ The authors later postulated that the presence of the correct crypt-resident microbiota, particularly the released MDP, may have a protective effect on stem cells, making them more reactive to MDP itself and more resistant to death during injury.¹³⁰ Additionally, the presence of bacteria in the crypt had been shown in patients suffering from ulcerative colitis.¹³¹ Furthermore, our group reported that during intestinal wound regeneration, the epithelial cells remain in direct contact with the

specific “probiotics”, or bacteria that promote pro-restitutive effects.¹⁹ These studies, along with the findings of PRR expression in ISCs, are important in determining potential microbiota-ISC interactions, which might be mediated through direct contact or release of products and/or metabolites during regeneration of injured mucosa.¹³⁰ Presence of the correct crypt/wound-associated bacteria and their products may dictate the fate of the wound regeneration. Overall, these findings support that microbial activation of PRRs-dependent signaling affects proliferation and survival of intestinal epithelial cells and thereby plays a pivotal role in the repair of intestinal wounds.

Microbiota-mediated immunomodulation and mucosal wound healing

Gut microbiota is integral to the activation of immune response and repair of intestinal damage. For example, gut symbionts serve a critical role by augmenting Interleukin-22 (IL-22) dependent mucosal wound repair. Recently, it has been demonstrated that IL-22 promotes intestinal stem cell-mediated epithelial regeneration. The study demonstrated that the recombinant IL-22 directly targeted ISCs, augmenting the growth of both mouse and human intestinal organoids, increasing proliferation and promoting ISC expansion. It has been previously described that the interactions between gut commensals, a range of lymphoid cells, and other environmental signals ensure the fine-tuning of IL-22 levels, such that they maintain the barrier function and repair without provoking overt inflammation.¹³² Remarkably, commensal microbiota potently induces retinoic acid production (a metabolite of vitamin A) by dendritic cells (DCs), which can, in turn, induce IL-22 production by innate lymphoid cells type 3 (ILC3s) and $\gamma\delta$ T-cells.^{133,134} ILCs are derived from the common lymphoid progenitor. ILCs participate in the control of pathogens, tolerance of commensal bacteria and also help repair of the intestinal injuries. These cells express signature transcription factors, including T-bet (ILC1), GATA3 (ILC2), and ROR γ t (ILC3). ILCs also include T-bet+ natural killer cells and Id3+ regulatory ILC.¹³⁵ Importantly, enteric commensals have been proposed to have a direct influence on IL-22 mediated

response by producing specific metabolites, which are ligands to the aryl hydrocarbon receptor (AHR). AHR functions as a ligand-dependent transcriptional factor, which senses environmental and endogenous compounds generated by commensal, dietary, or also cellular metabolism. Importantly, it has been shown that AHR is a key regulator of ILC3, which produces IL-22, a crucial mediator of intestinal wound repair.¹³⁶ Furthermore, many gut microbiota, including certain lactobacilli, produces ligands of AHR from bacterial metabolism and may activate AHR signaling pathways. Microbial AHR ligands include tryptophan, indole-3-aldehyde, and indole-3-acetic acid. Additionally, tissue-resident intraepithelial lymphocytes (IELs) express high levels of the AHR. A subgroup of IELs, termed $\gamma\delta$ T cells, also express AHR. The intestinal epithelial surface is the home of $\gamma\delta$ IEL, which can be activated by AHR ligands resulting in augmented mucosal healing following acute injury.¹³⁷ This tissue repair function of $\gamma\delta$ IEL has been linked to the upregulated expression of keratinocyte growth factor (KGF), which stimulates proliferation of colonic epithelial progenitors.¹³⁸ Activation of the $\gamma\delta$ IEL during DSS-induced colonic injuries of the mucosa mounts a coordinated transcriptional program involved in the regulation of cytoprotective and immunomodulatory factors. Furthermore, studies in germ-free mice indicated that commensal microbiota modulates critical components of this transcriptional program, thus delineating an interaction between gut bacteria and $\gamma\delta$ IEL during repair of the injured mucosa.¹³⁸ Similarly, the microbial fermentation product SCFA induces functional colonic Treg pool via epigenetic upregulation of the *Foxp3* gene, which efficiently suppresses inflammation and enhances mucosal tissue repair.¹⁰⁹

Macrophages are important components of the resolution of inflammation and epithelial wound healing. Depending on the tissue microenvironment, macrophages polarizes to become classically activated M1 (pro-inflammatory) or alternatively activated M2 (pro-regenerative). Microbial metabolites or cell wall components facilitate polarization of macrophages towards M2 and also enhances migration and wound-healing properties in M2 macrophages leading to augmented wound

regeneration in mouse models of colitis.¹³⁹ In addition, Trem2 (triggering receptor expressed on myeloid cells 2) is a cell surface receptor that is specifically induced in macrophages by IL-4/IL-13. Activation of Trem2 signaling augments an efficient wound healing of colonic mucosal injuries by inhibiting cytokines that stimulate M1 macrophage activation, and by promoting cytokines that induce M2 macrophage activation.¹¹⁹ Interestingly, the indigenous ligands, as well as bacterially synthesized ligands for Trem2, have been identified in the anionic residues present on the surface of Gram-positive and negative bacteria, suggesting a potential role of the intestinal microbiota in regulating macrophage activation through the Trem-2 during mucosal wound healing. Interestingly, the macrophages also play important beneficial roles in wound healing by secretion of pro-resolution IL-10, activation of epithelial cAMP response element-binding protein (CREB) and by subsequent synthesis and secretion of the pro-repair WNT1-inducible signaling protein 1 (WISP-1) in a microbiota independent manner.¹⁴⁰ In addition, mice deficient in IL-36R exhibited defective recovery following DSS-induced damage and impaired closure of colonic mucosal biopsy wounds in the distal colon. Interestingly, germ-free mice failed to induce L-36 γ in response to dextran sodium sulfate (DSS)-induced damage, suggesting that gut microbiota is involved in its induction.¹⁴¹ These findings demonstrate a cross-talk between immune cells, intestinal microbiota, and their metabolites eventuating in an efficient mucosal epithelial repair.

The microenvironment of wound mucosa undergoing active repair

Intestinal microenvironment and physiological parameters of the injured mucosa are remarkably different from intact mucosa. Therefore, it is plausible that the microbial community structure-associated with the injured mucosa are distinctly divergent from the intact mucosa or the luminal bacteria. Indeed, recent studies provided evidence that this alteration in microbial community is driven by the altered microenvironment of the intestinal tract during inflammation and active mucosal regeneration. The biochemical and biophysical

components of the wound microenvironment are contributed by the damaged and regenerating epithelial cells, resident and transmigrating immune cells and mesenchymal cells as well as the wound-associated bacteria.^{113,142} Therefore, sites of intestinal mucosal inflammation and subsequent restitution are characterized by multifaceted alterations in the tissue microenvironment, including the appearance of pro-resolving mediators, antimicrobials, bacterial small molecules and metabolites, enzymes, cytokines, mucins and changes in reactive oxygen species and oxygen tension.^{21,22,143} The functional role of antimicrobials, IgA and ROS in the regulation of bacterial population have been studied for decades and recently discussed (For reviews, please see refs.144–146).^{144–146}

During inflammation of the gut, mucosal microenvironment is changed due to depletion of the oxygen and generation of the reactive oxygen species. Transmigrating neutrophils accumulate in the injured intestinal mucosa and play a key role in altering the physiological parameters of the wound microenvironment. Also, the expression levels of the mucins, which predominantly supply the glycan for the foraging microbiota, are altered in the mucosal wounds.¹⁴⁷ Recently, we have demonstrated that FPR and neutrophilic NOX2 are required for the rapid depletion of microenvironmental oxygen and compensatory responses, resulting in a dramatic enrichment of an anaerobic bacterial consortium.¹⁹ Similarly, Campbell et al. demonstrated that the NOX2-mediated oxidative burst rapidly depletes the oxygen in the inflamed epithelia within recruited neutrophils during trinitrobenzenesulphonic acid (TNBS)-induced colitis. Concurrently, the study showed that intestinal epithelial cells increased synthesis of a mucin protein, MUC3.²⁰ Recently, we performed high-throughput sequencing (HTS) sequence analysis of the Bacterial 16S rRNA genes (V4 region) to delineate the bacterial population colonized in mucosal wounds undergoing active repair in a mouse model of colonic wound regeneration.¹⁹ As expected the initial composition of phyla present in the wound bed most closely resembled that of the intact mucosa, though interestingly, the community structure markedly changed over the several day healing period, and the microbiota composition of the

resealing wounds differed distinctly from that of intact mucosa and the colonic luminal contents. Our study identified seven anaerobic and microaerophilic commensal bacterial genera that increased in abundance, specifically in the early regenerative mucosa. Most notably, the relative abundance of anaerobic mucinophilic *Akkermansia* (phyla: Verrucomicrobia) increased dramatically and additional bacteria that are represented in the wound-associated consortium includes the anaerobic taxa *Coprococcus*, *Mucispirillum*, *Odoribacter*, *Prevotella*, *Oscillospira* and aerotolerant lactobacilli. These findings indicate that, during repair of gut mucosal injury, temporally dynamic local environmental conditions in the wound favor the growth of specific taxa, and define a wound-associated microbiota consortium that preferentially thrives for several days and resolves to the original state as the wound repairs.¹⁹ Moreover, the dominant member of this wound-mucosa-associated microbiota, *Akkermansia muciniphila*, an anaerobic, mucinophilic gut symbiont¹⁴⁸, stimulated proliferation and migration of enterocytes adjacent to the colonic wounds.¹⁹ These findings thus demonstrate how wound microenvironments induce the rapid emergence of prorestitutive symbionts, designated pro-biont,' which contribute to an enhanced repair of mucosal injuries.¹⁹ Other workers have shown administration of the mucin utilizing *A. muciniphila*, to mice prevents the development of high-fat diet-induced obesity and ameliorates metabolic endotoxemia-induced inflammation through the restoration of the gut barrier.¹⁴⁸⁻¹⁵⁰ Some of these effects are the result of increased mucin secretion and intestinal tight junction proteins, which emphasize the dynamic role played by mucin utilizers in their interaction with the host. Also, the protective function of *A. muciniphila* could be recapitulated using *A. muciniphila* purified membrane protein or the pasteurized bacterium.¹⁵⁰

Overall, these results suggest that the microbiota can adapt to local environmental changes to form definable and transient consortia. Importantly, members of this consortium can stimulate prorestitutive signaling and increased migration and proliferation leading to enhanced epithelial wound healing. Mucosal wound environment provides a classic model of ecological succession, where the hypoxic environment, secondary to the influx of

transmigrating neutrophils, of the wound bed encourages the growth of *A. muciniphila* and several other types of anaerobic bacteria. Consequently, two to four days post-biopsy injuries in the colon, anaerobic *Akkermansia* and related consortium enrich to a peak of about 8% of the mucosa-associated bacteria, which in turn enhance epithelial regeneration, and then diminish in abundance along with the disappearance of neutrophils and alterations in the hypoxic condition. Thus, this study delineates a ecological feedback loop and cross-talk induced by the injured microenvironment and gut microbiota.¹⁹ Taken together, these findings support that commensal enteric bacteria play a crucial role in epithelial wound healing and restoration of barrier functions. These studies also highlight that discovery, development, and designing of the future therapies and interventions targeted for the inflammatory gastrointestinal diseases should be aligned with the mechanism of microenvironmental alterations and enrichment of beneficial microbiota during mucosal wound healing.

Concluding remarks

Numerous studies determined that the mammalian intestine houses taxonomically diverse and numerically vast prokaryotic microorganisms. However, mere identification of the microbial species and observation of their effect does not ensure deciphering the molecular mechanisms of the host-microbial interactions. The mammalian microbiota generates a diverse array of metabolites -metabolites are small molecules synthesized by the cells- in the intestine. Molecular functions of the microbial metabolites generated in the microenvironment to modulate the host cellular events are fundamentally crucial to determining the mechanism of diseases and development of potential therapeutics. Recently, there has been growing interest in the identification and functional characterization of microbial metabolites in the intestine. Future studies will delineate mechanistic functions of microbial products and small molecules in the pathogenesis of barrier disruption. Moreover, future research studies will be forging a multidisciplinary approach in computational, quantitative analytical chemistry and experimental

biology to systematically discover genes, pathways, and molecules essential for dissecting the novel etiology of diseases as well as will be pivotal in the development of the potential therapeutic application, diagnosis of a disease, or prognosis of an intervention.

Disclosure of potential conflicts of interest

No potential conflict of interest were disclosed.

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