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The role of mucosal immunity and host genetics in defining intestinal commensal bacteria

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Abstract

Purpose of review—Dramatic advances in molecular characterization of the largely noncultivable enteric microbiota have facilitated better understanding of the composition of this complex ecosystem at broad phylogenetic levels. This review outlines current understanding of mechanisms by which commensal bacteria are controlled and shaped into functional communities by innate and adaptive immune responses, antimicrobial peptides produced by epithelial cells and host genetic factors.

Recent findings—Secretory IgA, which targets enteric bacteria, regulates the number, composition and function of luminal bacteria. Likewise, epithelial production of antimicrobial peptides help control enteric microbiota growth, translocation and perhaps composition. The developing role of innate signaling pathways, such as toll-like receptors and NOD2, is beginning to be studied, with dysbiosis following their genetic deletion. Inflammation and effector immune responses lead to decreased diversity and selective alterations of functionally active bacterial species such as *Escherichia coli* and *Faecalibacterium prausnitzii* that have proinflammatory and protective activities, respectively. Studies of humans, mice and comparative species indicate that both genetic and early environmental factors influence the development of a stable intestinal microbiota.

Summary/implications—Genetic and mucosal immunity strongly influence the composition and function of enteric commensal bacteria. This understanding should help develop strategies to correct dysfunctional altered microbiota in genetically susceptible individuals, better diagnose and correct potential dysbiosis in high risk individuals at a preclinical stage, and therapeutically target pathogenic bacterial species that help drive chronic inflammatory conditions.

Keywords

Microbes; Intestine; Immune system

Introduction

Humans have co-evolved with an incredibly complex group of interacting bacteria, fungi and viruses that populate their intestinal tracts in numbers that exceed mammalian cells by at

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least ten-fold. These enteric microbiota have fundamental physiologic roles in differentiation and maturation of epithelial cells and the mucosal immune response, as well as integral pathophysiologic roles in certain disorders, most notably inflammatory bowel diseases[1].

The composition of commensal enteric microbiota is defined by the host

Just as commensal enteric microbiota are important in shaping host development and inflammation, host factors also influence the luminal microbial composition. This was elegantly demonstrated in reciprocal microbiota transplantation experiments in which germ-free (GF, sterile) zebrafish and mice were colonized with intestinal microbes from conventionally-raised animals[2]. The authors showed that over time, at the phylum level, the transplanted bacterial community changed to reflect characteristic microbiota of the recipient host species. These findings suggest that luminal microbial populations do not grow in isolation, but rather are strongly affected by host-derived factors that are now beginning to be elucidated.

Chronic intestinal inflammation is associated with phylum-level changes in gut microbial communities

The gastrointestinal mucosal immune system has evolved to peacefully co-exist with a large burden of potentially inflammatory microbial components under normal circumstances. However, genetic and environmental insults can lead to dysregulated immune responses to commensal bacteria. Reciprocally, immune factors can also shape the composition and function of commensal enteric microbiota. Much of the data to support this notion comes from studies of differences in bacterial communities in patients or animals with intestinal inflammation compared to healthy controls. These studies are difficult to interpret in aggregate due to variations in animal models, human subjects, sampling methods of luminal contents, and techniques used to measure microbial composition. However, several general trends are apparent. Experimental murine models of chemically-induced or spontaneous colitis exhibit variable changes in overall bacterial abundance (Table 1). Microbial diversity is decreased in interleukin-10 deficient (IL-10−/−) mice. While Actinobacteria and Proteobacteria, especially *E. coli*, are increased in some colitis models, there are variable changes in Firmicutes and Bacteroidetes. Among the Firmicutes, within the class of Clostridia, the Clostridiales are increased whereas the Lachnospiraceae, particularly Clostridium groups XIVa and IV, are decreased.

Despite differences in the “core” microbiome between mice and humans, changes in microbial composition associated with intestinal inflammation are quite similar. For example, similar to IL-10–/– mice with colitis, microbial diversity is consistently decreased in humans with IBD (Table 2). Human IBD is also associated with increased Actinobacteria (except the Bifidobacteriales) and Proteobacteria, decreased Lachnospiraceae, and variable changes in Bacteroidetes. However, unlike experimental colitis, there are variable changes in Clostridiales in human IBD. Moreover, there are increased mucosal-associated bacteria in human IBD even though the luminal concentrations are decreased. Together, these findings indicate that dysregulated host immune responses are associated with broad changes in both the composition and distribution of commensal intestinal microbial communities. However, whether the microbial changes are a cause of, or result from, inflammation remains to be determined. Furthermore, the host-derived factors that change the microbiota as well as the downstream effects of broad microbial shifts on host disease processes are unknown and are areas of active investigation.

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Chronic intestinal inflammation affects the abundance of specific bacterial strains important in the pathogenesis of IBD

While experimental colitis and human IBD are associated with similar phylum-level changes in the enteric microbiota, recent data also suggest that inflammation alters the abundance of specific bacterial species. For example, numbers of *E. coli* are consistently increased in both human IBD and experimental colitis (Table 3). Specifically, the B2 and D phylogenetic groups of *E. coli,* and adherent-invasive strains of *E. coli* that bind to CEACAM6 on human epithelial cells are associated with Crohn's disease[17, 32, 33*]. Furthermore, mice engineered to express the human CEACAM6 receptor only develop intestinal inflammation when colonized with adherent-invasive *E. coli*[33*].

While the expansion of certain strains of *E. coli* may worsen intestinal inflammation, recent data indicate that increased numbers of a Firmicute in the Clostridiales order, *F. prausnitzii*, are protective. Interestingly, mice with TNBS colitis that do not harbor *F. prausnitzii* develop a dysbiosis, and administration of *F. prausnitzii* restores the intestinal microbiota to a normal state and decreases colitis[4]. Similarly, there are decreased numbers of fecal and mucosal-associated *F. prausnitzii* in ileal Crohn's disease[4, 15*, 29]. These findings suggest that the inflammation-associated changes in specific bacterial species may impact the composition of the entire intestinal microbial community and enhance or attenuate gut inflammation. Given the broad diversity of the commensal enteric microbiota, further studies will likely identify additional bacterial species affected by host inflammation.

The adaptive immune system shapes commensal microbial communities

Though much is known about the effects of intestinal inflammation on the general composition of the gut microbiome, relatively little is known about how specific components of the inflammatory milieu interact with the commensal luminal microbes. Among the multitude of mucosal adaptive immune responses, the impact of intestinal secretory IgA (sIgA) on luminal bacteria is the best characterized.

Secretory IgA serves as the first line of defense against intestinal microorganisms by limiting adhesion and entry into the epithelium, thereby facilitating clearance via the fecal stream—a process termed immune exclusion[34, 35]. By this mechanism, sIgA confines intestinal microbial populations to the lumen, mucosa, or mesenteric lymph nodes, and limits their systemic spread. Secretory IgA also maintains mucosal homeostasis through its effects on the composition of the commensal microbial community. This was originally reported by Fagarasan et al., who showed a 100-fold increase in the number of small intestinal anaerobes in AID−/− mice, which do not produce sIgA[36]. Similarly, others have demonstrated an expansion of luminal commensal anaerobic bacteria in Rag2−/− mice, which lack B- and T-cells[37]. Reconstitution of Rag2−/− mice with normal bone marrow restored the luminal microbiota to the normal state, confirming the importance of homeostatic adaptive immune responses in shaping commensal bacterial communities[37]. Interestingly, increased numbers of non-cultivatable anaerobes known as segmented filamentous bacteria (SFB) were detected in the small intestine of Rag2−/− and AID−/− mice and returned to normal levels upon reconstitution of the adaptive immune system using bone marrow transplant or parabiosis models, respectively[37]. The relevance of these findings is underscored by recent data suggesting that luminal SFB are critical for the development of normal intestinal immune function, including Th17 cell responses and antimicrobial peptide secretion[38**]. Furthermore, the presence of SFB enhances resistance to murine *Citrobacter rodentium*-induced colitis[38**]. Together, these results suggest that sIgA and other components of adaptive immunity alter gut microbial communities, which may in turn affect subsequent innate and adaptive intestinal immune responses.

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In addition to shaping the constituents of the enteric microbiota, sIgA also affects the function of certain commensal bacteria. Peterson, et al., elegantly measured the *in* vivo effects of monoclonal IgA on luminal bacteria in Rag1−/− mice selectively colonized with a commensal bacterial strain, *Bacteroides thetaiotaomicron*, and implanted with a subcutaneous monoclonal IgA hybridoma reactive to *B. thetaiotaomicron* capsular polysaccharide [39]. While luminal concentrations of *B. thetaiotaomicron* were similar between hybridoma-harboring mice and controls, the luminal bacteria in mice carrying the hybridoma downregulated genes encoding the capsular polysaccharide and genes involved in the metabolism of host-derived reactive oxygen and nitrogen species. These results suggest that commensal antigen-specific sIgA induces changes in luminal microbial gene expression that may allow the bacteria to exist in a non-inflammatory, symbiotic relationship with the host.

Innate immunity influences the composition and function of the intestinal microbiota

In addition to adaptive immune responses, the innate immune system also affects luminal microbes. For example, mice deficient in Toll-like receptor (TLR) 5, a cell-surface receptor that recognizes bacterial flagellin, have altered intestinal microbiota and develop metabolic syndrome[40**]. Interestingly, the metabolic syndrome was transmitted to immunologically intact mice simply by transfer of the commensal enteric microbiota. Similarly, deficiency of T-bet, a transcription factor that orchestrates inflammatory responses, altered intestinal microbial composition in Rag2−/− mice that lack adaptive immunity, and was associated with colitis that can be transmitted to genetically intact hosts by luminal microbes[41]. In parallel, NOD2 deficiency resulted in selective expansion of Bacteroides species and an opportunistic pathogen*, Helicobacter hepaticus*, in the ileum, but not feces[42]. The mechanisms by which TLR5, NOD2 and T-bet deficiency impact luminal microbes are currently unknown.

The influence of antimicrobial peptides on the intestinal microbiota

Another component of innate immunity, the antimicrobial peptides (AMPs), are evolutionary ancient molecules used by host organisms to control potentially dangerous enteric microbes. Their incredible diversity, with more than 1500 AMPs reported to date[43], allows for a broad spectrum of antimicrobial activity against bacteria, fungi, viruses and protozoan organisms[44]. In the mammalian gastrointestinal tract, AMPs are produced primarily by epithelial cells. Both enterocytes and Paneth cells are capable of synthesizing AMPs, and secrete them into the intestinal crypts and mucus layer overlying the epithelial surface[45]. This results in high levels of antimicrobial activity at the mucosal surface, which allows for defense against pathogenic organisms and likely modulation of commensal microbiota.

Although regulation of intestinal microbiota by AMPs is mechanistically appealing, this concept has been difficult to verify due to the enormous diversity of the AMP molecules and the complexity of the mammalian microbiota. However, recent work using *Drosophila melanogaster* provides important insights into the role of AMPs in regulating the intestinal microbiota. Only seven distinct AMPs have been described in *Drosophila* [46], whose intestinal tract hosts a mere 10–20 bacterial phylotypes (as compared to 500–1000 in humans)[47]. Furthermore, induction of AMP expression in the *Drosophila* gut is entirely dependent on the immune deficiency signaling pathway[46], while the transcription factor Caudal suppresses AMP transcription by binding to regulatory sequences of the AMP genes[48]. This relative simple system makes *Drosophila* an ideal model to study AMP regulation of gut microbiota. Ryu and colleagues reported that inhibition of Caudal by RNA interference (RNAi) led to over-expression of all tested AMPs and gut epithelial cell apoptosis by day 18 [49]. Interestingly, apoptosis was not observed in mutant flies that were

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housed in GF conditions, implicating the intestinal microbiota as a key mediator of the phenotype. Specifically, there were decreased numbers of Acetobacteraceae strain EW911 (A911), but increased levels of Gluconobacter sp. strain EW707 (G707) in Caudal-RNAi relative to wild-type (WT) flies. Monoassociation of WT GF flies with G707 resulted in high levels of gut cell apoptosis compared to WT flies monoassociated with other gut commensals. This study demonstrates that dysregulation of AMP expression can profoundly change the commensal microbiota, with important downstream effects on gut physiology.

New understanding of AMP regulation in the mouse has extended these studies to the mammalian host. Cryptdins are mouse AMPs produced by Paneth cells, and are similar in structure and function to a group of human AMPs known as α-defensins[50]. In mice, matrilysin 7 (Mmp7) is necessary to cleave pro-cryptdin precursors into their active forms. Mmp7-deficient (Mmp7−/−) mice lack mature cryptdins and succumb more rapidly to *Salmonella* infection than their WT counterparts[51]. In contrast, transgenic mice that over-express a human α-defensin, human defensin 5 (HD-5), are markedly resistant to *Salmonella* challenge[52]. These two mouse models provide another experimental system to study the effects of AMPs on the intestinal microbiota.

Salzman and colleagues have extensively characterized the intestinal microbiota in the ileum of both Mmp7−/− and HD-5 transgenic mice using molecular methods[53**]. Consistent with previous studies, the Firmicutes and Bacteroidetes phyla were predominant in both mouse groups. However, Mmp7−/− mice had higher levels of Firmicutes and lower levels of Bacteroidetes than their WT counterparts, while reciprocal findings were observed in the HD-5 transgenic mice. Further analysis of specific bacterial changes revealed that the HD-5 transgenic mice had significant depletion of SFB in their intestinal microbiota and fewer lamina propria Th17 cells relative to WT mice, consistent with prior observations that SFB are critical for Th17 development[38**]. These findings support the concept that AMP levels have profound, biologically relevant effects on the intestinal microbiota.

Host genetic regulation of the intestinal microbiome

The relative influences of host genetics vs. environment in shaping the gut microbial composition and function are not yet definitively established. Human, mouse and comparative species studies indicate that both genetic and environmental influences contribute to formation of the intestinal microbiota.

Human studies—Comprehensive pyrosequencing analysis of fecal samples from young adult female twins demonstrated that microbiota from family members are more similar than from unrelated individuals. However, there was no significant difference in the degree of similarity of the gut microbiotas between monozygotic and dizygotic twins, although monozygotic twins trended toward greater similarity. Importantly, a core microbiome was more evident at the level of shared bacterial gene functions rather than composition of bacterial species[54**]. In deep sequencing analysis of stool specimens from a single monozygotic twin pair, 36–49% of bacterial phylotypes were shared among both twins[55]. Molecular analysis of fecal and mucosal biopsies from ten monozygotic twin pairs either concordant or discordant for Crohn's disease demonstrated decreased microbial diversity with disease and decreased *F. prausnitzii* and increased mucosal *E. coli* concentrations in ileal Crohn's disease[20, 29]. Frank et al.[56*] reported that NOD2 composite genotype, ATG 16L1 genotype and disease phenotype (ileal involvement) were associated with shifts in mucosally associated microbial composition in Crohn's disease patients. In familial Mediterranean fever patients, the presence of mutated alleles of the *MEFV* gene that encodes Pyrin was associated with significant changes in fecal bacterial community structure[57].

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Mouse studies—Reproducible strain-specific variations in relative concentrations of the 8 bacterial species that compose the defined Altered Schaedler Flora (ASF) in 23 inbred mouse strains indicated an important role of host genetics in determining the intestinal microbiome, although a significant cage effect demonstrated local environmental influences[58]. Stable strain-specific fecal eubacterial profiles in six mouse strains supported a strong genetic influence, although uterine implantation of two different mouse strains in the same foster mother showed no differences in fecal microbiota profiles in the offspring with different genetic background[59]. Relocation studies showed that environmental changes prior to four weeks of age affected fecal microbiota, although bacterial profiles were stable in adult mice. These studies indicate that host genotype helps shape intestinal microbial composition, but early life environmental influences alter the ultimate microbiota profile.

Studies in knockout mice indicate that Crohn's disease-related genes affect commensal microbiota. Mice deficient in the multidrug resistance gene (Abcb-1 transporter or P. glycoprotein) have differences in cecal microbiota at 12 weeks that precede onset of colitis[60*]. In preliminary studies, we demonstrated that NOD2 deficiency in mice results in decreased mucosally-associated *F. prausnitzii* in both the ileum and cecum[61], consistent with previously observed regulation of ileal Bacteroides concentrations[42]. Although not known to be related to IBD, genetic deletion of the apolipoprotein a-I gene, which leads to impaired glucose tolerance and increased body fat), affected gut microbiota, although dietary changes had a greater effect (calculated 12% genetic effect vs. 57% dietary effect on structural variation of gut microbiota)[62].

Comparative vertebrate studies—Studies of broadly divergent vertebrates in zoos and free-living environments indicate that the composition of intestinal microbiota is heavily influenced by diet, structure of the GI tract (foregut, hindgut and cecal anatomy) and species phylogeny[63, 64]. In broad terms, the human commensal bacterial community is typical of an omnivorous primate. In parallel studies, the phylogenetic structure and pattern of virulence genes in mammalian *E. coli* diverged in carnivores, omnivores and herbivores[65].

Conclusion

A coordinated interplay between commensal microbiota and mucosal immune responses is reciprocally regulated by each partner. Both innate and adaptive immune responses strongly influence enteric bacterial composition and perhaps function, which is further molded by activated effector immune responses during inflammation. Luminal alterations of decreased bacterial diversity and expansion of selected species, most notably *E. coli*, occur during both acute and chronic inflammation of multiple causes, suggesting that these changes are nonspecific. Firm evidence implicates sIgA, whose primary target is luminal bacteria, innate bacterial signaling pathways and AMPs in shaping enteric microbiota profiles. Likewise, host genotype, in conjunction with environmental influences including diet and microbial exposure early in life, shape enteric microbial composition, which is remarkably stable once fully developed. Future research needs to better explain the role of T lymphocytes in regulating microbiota, microbial alterations in hosts with disease-related genetic polymorphisms, primary vs. secondary effects of inflammation, functional consequences of dysbiosis in intestinal inflammation and the homeostatic and pathogenic immune responses on enteric bacterial gene expression and function.

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Table 1

Changes in enteric microbiota during experimental colitis

	Method	Model	Abundance	Diversity	Actinobacteria	Firmicutes	Bacteroidetes	Proteobacteria
Lupp et al.[3]	FISH on feces, 16S clone libraries	*C. rodentium*	↓		↑	↓ (Lachnospiraceae)	↓	↑
Lupp et al.[3]	FISH on feces, 16S clone libraries	DSS	↓			↑ (Clostridiales)	↓	↑ (incl *E. coli*)
Sokol et al.[4]	Targeted 16S qPCR	TNBS	↑			↑ (*C. leptum, C. coccoides*) → (Lactobacillus) ↓ (*F. prausnitzii*)	↑	→ (*E. coli*)
Schuppler et al.[5]	FISH, 16S clone libraries	IL2–/–						↑ (Enterobacteriaceae)
Ye et al.[6]	16S Clone Library, Culture	IL-10–/–				↑ (Clostridium, Ruminococcus, Lactobacillus) ↓ (Lachnospiraceae)	↑	
Lupp et al.[3]	FISH on feces, 16S clone libraries	IL-10–/–				↑	↓	↑ (*E. coli*)
Wohlgemuth et al.[7*]	DGGE, Culture, FISH	IL-10–/–	↑→	↓				↑ (*E. coli*)
Bibiloni et al.[8]	DGGE	IL-10–/–			↑ (Bifidobacteria)	↑ (Clostridium, Enterococcus)	↑	

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Table 2

Changes in enteric microbiota in human IBD

	Method	Abundance	Diversity	Actinobacteria	Firmicutes	Fusobacteria	Bacteroidetes	Proteobacteria
Qin et al.[9*]	Metagenomic library		↓					
Manichanh et al.[10]	16S screen of Metagenomic Library				↓ (Clostridium leptum)			
Baumgart et al.[11]	16S Library				↓ (Clostridiales)			
Frank et al.[12]	16S Library	↓		↑	↓ (Lachnospiraceae)		↓	↑
Gophna et al.[13]	16S Library				↓ (Clostridia)		↑	↑
Bibiloni et al.[14]	16S Library & DGGE						↑	
Sokol et al.[15*]	Targeted 16S qPCR			↓ (Bifidobacteria)	↓			
Takaishi et al.[16]	Targeted 16S qPCR						↓	
Kotlowski et al.[17]	RISA							↑ (Enterobacteriacea)
Nishikawa et al.[18*]	TRFLP		↓					
Andoh et al.[19]	TRFLP				↓ (Clostridia)		↑	↑ (Enterobacteriales)
Dicksved et al.[20]	TRFLP		↓					
Andoh et al.[21]	TRFLP				↓ (Lactobacillus)	↑		
Martinez-Medina et al.[22]	DGGE				↑ (Clostridium spp)			
Scanlan et al.[23]	DGGE			→ (Bifidobacter)	↓ (Clostridium spp)		↓ (*B. fragilis*)	
Seksik et al.[24]	TTGE							↑ (enterobacteria)
Swidsinski et al.[25]	FISH	↑ (mucosal)			↓ (*C. coccoides* in biofilm)		↑ (*B. fragilis* in biofilm)	
Mylonaki et al.[26]	FISH			↓ (Bifidobacteria)	↑ (mucosal Clostridia)			
Swidsinski et al.[27]	FISH, qPCR	↑ (mucosal)						
Sokol et al.[28]	FISH/flow cytometry	↓			↓ (Clostridium spp.)			

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Table 3

Changes in specific bacterial species during intestinal inflammation

E. coli	**Method**	**Abundance**
Baumgart et al.[11]	16S Library	↑
Willing et al.[29]	TRFLP	↑
Kotlowski et al.[17]	RISA	↑ (B2+D phylogenetic group)
Martinez-Medina et al.[22]	DGGE	↑
Mylonaki et al.[26]	FISH	↑ (mucosal)
Martinez-Medina et al.[30*]	Targeted culture of E. coli	↑
Petersen et al.[31*]	Targeted culture of E. coli	↑ (B2 phylogenetic group)
Darfeuille-Michaud et al.[32]	Culture	↑ (AIEC)
F. prausnitzii		
Sokol et al.[15*]	Targeted 16S qPCR	↓
Willing et al.[29]	TRFLP	↓
Martinez-Medina et al.[22]	DGGE	↓
Sokol et al.[4]	FISH	↓