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Regulation of the gut microbiota by the mucosal immune system in mice

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Abstract

The benefits of commensal bacteria to the health of the host have been well documented, such as providing stimulation to potentiate host immune responses, generation of useful metabolites, and direct competition with pathogens. However, the ability of the host immune system to control the microbiota remains less well understood. Recent microbiota analyses in mouse models have revealed detailed structures and diversities of microbiota at different sites of the digestive tract in mouse populations. The contradictory findings of previous studies on the role of host immune responses in overall microbiota composition are likely attributable to the high β-diversity in mouse populations as well as technical limitations of the methods to analyze microbiota. The host employs multiple systems to strictly regulate their interactions with the microbiota. A spatial segregation between the host and microbiota is achieved with the mucosal epithelium, which is further fortified with a mucus layer on the luminal side and Paneth cells that produce antimicrobial peptides. When commensal bacteria or pathogens breach the epithelial barrier and translocate to peripheral tissues, the host immune system is activated to eliminate them. Defective segregation and tissue elimination of commensals result in exaggerated inflammatory responses and possibly death of the host. In this review, we discuss the current understanding of mouse microbiota, its common features with human microbiota, the technologies utilized to analyze microbiota, and finally the challenges faced to delineate the role of host immune responses in the composition of the luminal microbiota.

Keywords: commensal, microbiota, mucosal immunity

Introduction

The mammalian body harbors trillions of microbes, including eubacteria, archaea, fungi and protozoa; these groups are composed of thousands of species (1). The majority of these microbes reside in the digestive tract, where rich nutrients foster the formation of well-organized microbial communities through interactions among microbes and host factors.

By coexisting as either symbionts or pathobionts within the host, these microbes bring about beneficial or detrimental impacts on the health of the host, respectively. However, because of the lack of knowledge on most of these microbes, many of them are simply regarded as commensals, while their effects on the health of the host remain largely unknown (1, 2). One of the most appreciated benefits to the host of the gut microbiota, which refers to the microbial community in the gastrointestinal tract, comes from metabolites of bacteria such as vitamins and short-chain fatty acids (SCFA), which are important for both systemic and intestinal development of the host (3). SCFA-producing colonic bacteria and certain types of colonic Clostridia, as well as ileal segmented filamentous bacteria (SFB), are critical in shaping the host immune balance in mice (4–7).

Given the significant impacts of commensals on host health, our next question is whether the host, in a reciprocal fashion, regulates the commensal microbes living inside its body. Understanding and identifying the host factors that control the populations and localization of symbionts and pathobionts is important for developing therapeutic treatments for human diseases that are affected by these microbes. To tackle this question experimentally, the host factors that affect microbial ecology can be manipulated. Murine models have great advantages for this type of study, due to the feasibility to genetically modify immune or metabolic components and the availability of well-established genetic knock-in and knock-out models. In addition, the microbiota in humans and mice share many common features. Here, we review the current understanding of how the host immune system regulates control of the microbiota; we base the article mainly on studies that have utilized genetically manipulated mouse models.

The mouse microbiota shares common features with the human microbiota but also has unique commensals that affect host immune responses and disease

We would like to first provide an overview of the mouse microbiota in the digestive tract to better understand its regulation. Post-weaning mice harbor 10^8, $10^{9–10}$, and $10^{10–11}$ commensal bacteria in the oral cavity, small intestine, and large intestine, respectively, whereas pre-weaning mice harbor $<10^8$ bacteria in whole digestive tract (8). Whereas the microbiota in these areas of the digestive tract in pre-weaning mice are relatively simple and uniform, the composition of microbiota in intestine of adult mice is complex and unique (8). After weaning, the microbiota in the upper digestive tract, namely the oral cavity and small intestine, continues to maintain its simple form as it is dominated by Lactobacillales, whereas the large intestine begins to harbor mostly Bacteroidales and Clostridiales that are able to digest more-complex carbohydrates (8, 9).

The oral microbiota in mice is characterized by a low α-diversity (i.e. the composition in each individual host) and a high abundance of proteobacteria and Lactobacillales, especially γ-proteobacteria and streptococci (9, 10). These features are common with human microbiota (11). Several commensal groups have identical species that colonize the human and mouse digestive tracts (e.g. Enterobacteriaceae species), but many commensals in humans and mice are not identical, even though they are similar. For example, although NI1060, the murine commensal that accumulates and induces periodontitis at the ligature-damaged gingival site, cannot be found in humans, a phylogenetically related bacterium is associated with human aggressive periodontitis (10).

Despite these similarities, the mouse microbiota in the digestive tract has several unique features, including a low abundance of oral obligate anaerobes associated with major dental diseases, a high abundance of ileal SFB that induces T_h17-oriented immune responses, a low abundance of bifidobacteria which affects the susceptibility to infection of pathogens such as *Escherichia coli* O157:H7 (12), and a different abundance of Lachnospiraceae species (from the Clostridia class) which can control T_{reg} cells (4, 5, 8). The complexity in murine colonic microbiota is mainly associated with phylotypic α-diversity in mouse-specific Porphyromonaceae of Bacteroidales and Lachnospiraceae of Clostridiales, which represent about half of the murine colonic bacteria, and can be detected by denaturing gradient gel electrophoresis (DGGE) analysis despite being indistinguishable in 16S rRNA phylotype analysis of operational taxonomic unit (OTU) clustered at 97% nucleotide identity (8). Therefore, some immunological effects of microbiota in the digestive tract might be species-specific.

Difficulties that can complicate the assessment of the role of the host immune system in microbiota composition

The roles of the host immune system in the regulation of microbiota in the lumen of the digestive tract are still under debate. Contradictory results have been reported for the roles of individual host factors in the control of microbiota, many of which were based on comparisons between wild-type control mice and mice deficient in specific host factors (Table 1). For example, previous studies on the role of the innate immune receptors such as TLR5 and Nod2 in the regulation of intestinal microbiota composition have shown contradictory conclusions.

Comprehensive review of these studies underscores two main issues likely accountable for these discrepancies: (i) technical challenges in the determination of the microbiota composition and (ii) the limited knowledge of the high diversities and the dramatic changes in microbiota among individuals. For the former, the main problem is that many commensals are currently uncultivable. Non-biased and non-culture-based analytic techniques are essential to accurately assess the microbiota composition. Up to now, non-culture-based analyses commonly practiced include genomic hybridization, quantitative PCR (qPCR) using bacterial group-specific primers, DGGE, terminal restriction fragment length polymorphism (T-RFLP), species-specific microarray, random sequencing of amplified 16S rRNA gene libraries and meta-genomic pyrosequencing (45) (Table 1). In particular, the accuracy of microbiota analysis has been greatly improved by recent development of cost-effective next-generation sequencing techniques (46), although technical issues such as cross-hybridization and chimeric sequences may still potentially undermine the accuracy of 16S ribosomal RNA-based and meta-genomics-based analyses.

The high diversity of the microbiota composition in each individual host (α-diversity) and among individual hosts (β-diversity) also presents great challenges to deciphering the importance of specific host factors in regulating the microbiota. With great advances in analyzing the microbiota composition led by the Human Microbiome Project and other groups, studies have shown that microbiota compositions among human individuals are highly diverse (11). Moreover, the microbiota composition in murine intestine changes drastically with different diets, aging, and inflammatory states (8, 11, 47, 48).

Because of these high diversities and variations of the microbiota among individuals, even mice of the same genotype show different microbiota compositions if housed in separate cages within the same facility (49). In addition, different animal facilities and providers have reported unique profiles of microbiota compositions (4). Another issue is the presence of complexity among cryptic species in genomic DNA-based analysis. For example, the majority of the colonic bacterial population is composed of Bacteroidales and Clostridiales species that possess identical or very similar 16S rRNA phylotypes but have distinct metabolic profiles (8, 50). Therefore, abundance of these species is greatly affected by diet ingredients, but alteration in their abundance might not be reflected by 16S ribosomal RNA-based analyses (47, 50). Post-weaning mice possess extremely high diversities in these bacterial groups (8). Therefore, appropriate experimental controls must be incorporated and environmental contributions should be taken into account in studies to address the roles of host factors in regulating the microbiota.

One way to circumvent the obstacles to comparing the composition of the microbiota is co-housing the wild-type

Table 1. Microbiota composition in genetically modified mice

Mice	Microbiota difference (⇧, increased; ⇩,decreased)	Samples	Methods	Co-housing	Reference
MyD88$^{-/-}$/TRIF$^{-/-}$	No difference	Skin, oral, fecal	Pyrosequencing	Littermates	(13)
MyD88$^{-/-}$	No difference	Ileal, cecal	Pyrosequencing	Littermates	(14, 15)
MyD88$^{-/-}$NOD	Porphyromonadaceae ⇧, Lactobacillaceae ⇧, Rikenellaceae ⇧	Cecal	PCR	Littermates	(16)
MyD88ΔIEC[a]	Bacteroidetes ⇩, Proteobacteria ⇧, some Firmicutes ⇧	Fecal	Pyrosequencing	Littermates	(17)
TLR2$^{-/-}$	No difference	Cecal	Pyrosequencing	Littermates	(15)
TLR4$^{-/-}$	No difference	Cecal	Pyrosequencing	Littermates	(15)
TLR5$^{-/-}$	No difference	Cecal	Pyrosequencing	Littermates	(15)
	Species-level difference, phylum-level no difference	Cecal	Pyrosequencing	Littermates	(18)
	Enterobacteria ⇧ in colitic but not non-colitic mice	Fecal	Pyrosequencing	Littermates	(19)
TLR9$^{-/-}$	No difference	Cecal	Pyrosequencing	Littermates	(15)
Rip2$^{-/-}$	No difference	Ileal, cecal, fecal	Pyrosequencing	Littermates	(20)
Nod1$^{-/-}$	No difference	Ileal, cecal, fecal	DGGE, pyrosequencing	Littermates	(20, 21,22)
Nod2$^{-/-}$	Bacteroidetes ⇧, Firmicutes ⇧ (terminal ileum), no difference (feces)	Ileal, fecal	PCR	Littermates	(23)
	Bacteroidetes ⇧, Firmicutes ⇧ (both ileum and feces)	Ileal, fecal	Pyrosequencing	Littermates	(24)
	Bacteroidaceae ⇧, Rikenellaceae ⇧, Prevotellaceae ⇧	Fecal	Pyrosequencing	Unspecified	(25)
	No difference	Ileal, cecal, fecal	DGGE, pyrosequencing	Littermates	(20,22)
ASC$^{-/-}$	No difference	Fecal	DGGE, pyrosequencing	Yes	(26, 27, 28)
	TM7 ⇧, Prevotellaceae ⇧, Lactobacillus ⇩	Fecal	Pyrosequencing	No	(26)
NLRP3$^{-/-}$	Citrobacter ⇧, Proteus ⇧, Shigella ⇧, Mycobacterium ⇧	Fecal	T-RFLP	Littermates	(29)
NLRP6$^{-/-}$	TM7 ⇧, Prevotellaceae ⇧, Lactobacillus ⇩	Fecal	Pyrosequencing	No	(26)
	No difference	Fecal	Pyrosequencing	Yes	(26)
IL-18$^{-/-}$	TM7 ⇧, Prevotellaceae ⇧, Lactobacillus ⇩	Fecal	Pyrosequencing	No	(26)
	No difference	Fecal	Pyrosequencing	Yes	(26)
Rag1$^{-/-}$	Lachinospiraceae ⇧, Porphyromonadaceae ⇩ (feces)	Skin, oral, fecal	Pyrosequencing	Littermates	(13)
	Neisseriaceae ⇧, Streptococcaceae ⇩ (oral), no difference (skin)				
Ig μ$^{-/-}$	No difference	Oral, ileal, fecal	Culture-based	Littermate	(30)
AID$^{-/-}$	Bacteroidaceae ⇧, Peptostreptococcus ⇧, Bifidobacterium	Ileal	Culture-based	No	(31)
	SFB ⇧	Ileal	PCR	Littermate	(32)
pIgR$^{-/-}$	Pasteurellaceae ⇧, Lachnospiraceae ⇧	Fecal	Pyrosequencing	Littermate	(33)
PDCD1$^{-/-}$	Erysipelotrichaceae ⇧, Prevotellaceae ⇧, Alcaligenaceae ⇧, TM7 ⇧	Cecal	Pyrosequencing	unspecified	(34)
IL-22$^{-/-}$	Bacteroides ⇩, Porphyromonadaceae ⇧, Prevotellaceae ⇧	Fecal	Pyrosequencing	No	(35)
	Clostridiaceae ⇩, Lactobacillus ⇩, Rikenellaceae ⇧				
	No difference	Fecal	Pyrosequencing	Yes	(35)
RegIIIγ$^{-/-}$	No difference in lumen	Ileal	Pyrosequencing	Littermates	(14)
	Eubacterium rectal ⇧, SFB ⇧ groups in mucosa	Ileal	PCR	Littermates	(14)
STAT3$^{-/-}$	No difference	Fecal	DGGE	Yes	(36)
IRF9$^{-/-}$	Variation ⇧	Fecal	DGGE	Yes	(36)
PPARγ$^{-/-}$	No difference	Fecal	PCR	Littermates	(37)
DEFA5 Tg[b]	Bacteroides ⇧, MIB groups ⇧	Fecal	PCR	Yes	(38)
MMP7$^{-/-}$	Firmicutes ⇧	Fecal	PCR	Yes	(38)
C1galt1ΔIEC[a]	Lactobacillus ⇧, Clostridium ⇧, Lachnospiraceae ⇧, Ruminococcus ⇧	Fecal, cecal	Pyrosequencing	Unspecified	(39)
	Bacteroidetes ⇧, Firmicutes ⇩	Fecal	Pyrosequencing	Littermates	(40)
B4galnt2$^{-/-}$	Helicobacter spp ⇩	Ileal, cecal, fecal	Pyrosequencing	Littermates	(41)
βGalT1 Tg[b]	Bacteroidetes ⇩, Firmicutes ⇧	Fecal	PCR	Yes	(42)
TMF$^{-/-}$	No difference	Fecal	Pyrosequencing	Yes	(43)
	Ruminococcaceae , Roseburia , Lactobacillus	Fecal	Pyrosequencing	No	(43)
Fut2$^{-/-}$	Bacteroides ⇧, Parabacteroides ⇧, Parasutterella ⇧, Eubacterium ⇧, Clostridiales ⇩	Fecal	Pyrosequencing	Littermates	(44)

[a]ΔIEC, specifically deleted in intestinal epithelial cells. [b]Tg, transgenic.

and genetically deficient mice, since murine commensals are transferable to mice of different genotypes during co-housing (26, 35, 50). Up to now, no particular bacterium is known to be absolutely untransferable among cohabiting mice. The microbiota in mice of different genotypes eventually equilibrates after several weeks of co-housing unless deficiency in the host factor of interest intrinsically alters the microbiota composition (4, 26, 35).

Location determines whether the host targets pathogens and commensals for elimination

While much more work is still required to reach a consensus on the regulation of overall lumenal microbiota by the host immune system, there has been increasing understanding of the importance of host factors in the controlling of particular bacteria, including pathogenic bacteria, in the mucosa. Pathogenic bacteria possess unique ways to colonize in the host and induce host complications. Therefore, colonization by pathogenic bacteria in many ways is subject to natural selection during evolution and the host must acquire defense strategies to eliminate pathogens that do not naturally inhabit the host.

Many pathogenic bacteria cannot colonize in the presence of commensals, although the precise mechanisms in which commensals prevent colonization of pathogenic bacteria are not still well understood. For example, overgrowth of *Salmonella enterica* species, *Enterococcus faecalis* and *Clostridium difficile* in intestine requires dysbiosis caused by antibiotics (51–53). However, some pathogens including enteropathogenic *E. coli* (EPEC) and a related rodent pathogen, *Citrobacter rodentium*, have systems to attach to the host epithelium and obtain nutrients from the epithelium even in the presence of colonic commensals (54) (Fig. 1). Importantly, elimination of *C. rodentium* is mediated by $CD4^+$ T cells and IgG (55–57). However, germ-free mice that are monocolonized with *C. rodentium* do not eliminate *C. rodentium* that have turned off virulence genes responsible for attachment to the host (58). These facts suggest that the host immune system targets pathogenic bacteria only when they locate near the epithelium and thereby pose threats to the host.

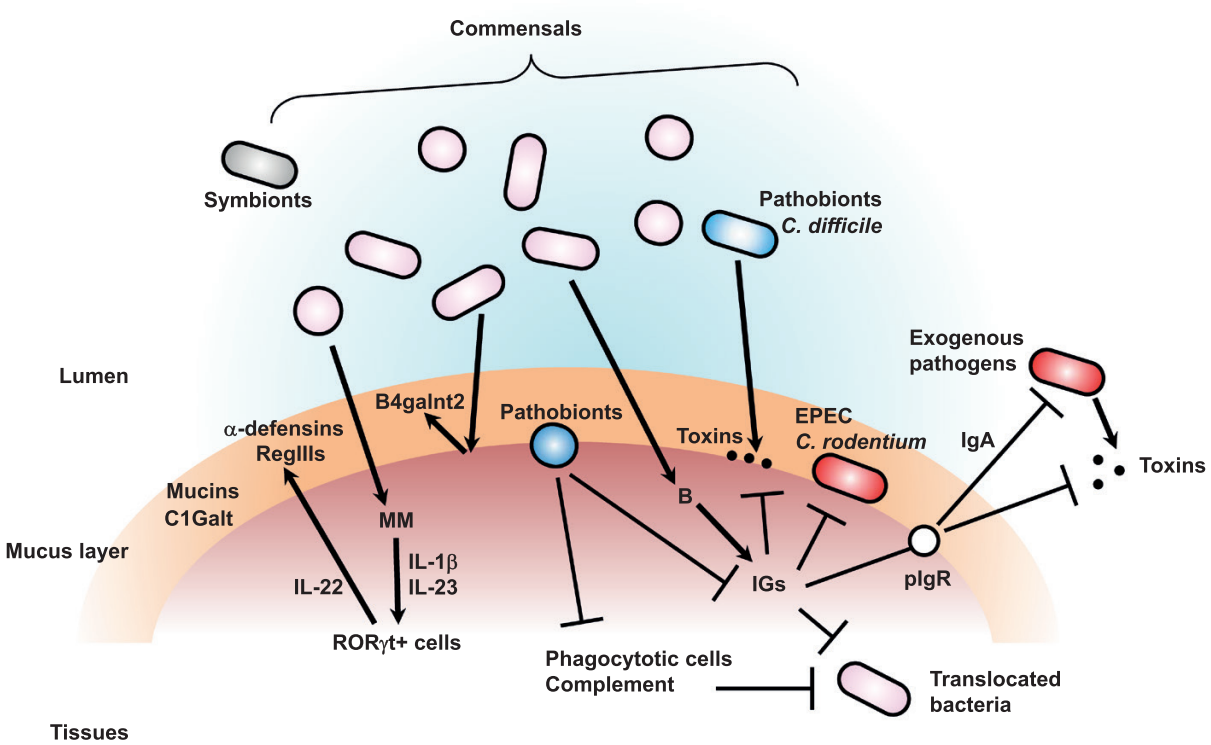

Fig. 1. Regulation of microbiota by the host immune system. Exogenous enteric pathogens and commensals including symbionts and pathobionts can colonize the lumen of the digestive tract. Host epithelium is protected by a mucus layer containing mucins and their glycosylating enzymes such as C1Galt. Commensals are segregated by the mucus layer and antimicrobial proteins (e.g. α-defensins and RegIII proteins) from epithelium. Commensals augment expression of immunoglobulins (IGs), of mucosal glycan-modifying enzymes such as B4galnt2 and of antimicrobial proteins. Toxin-secreting and non-toxin-secreting pathogens are eventually eliminated by immunoglobulins including IgA. Secretory IgA, which pIgR transfers to the lumen, is important to eliminate pathogens and their toxins. Immunoglobulins are also important for elimination of pathogens that either attach to the epithelium (i.e. *Citrobacter rodentium* and EPEC) or invade into host tissue. Furthermore, immunoglobulins neutralize toxins secreted from pathobionts such as *Clostridium difficile*. Translocated pathogens and commensals are also eliminated by multiple host defense mechanisms, including phagocytotic cells and complement components. However, some pathobionts, including periodontal pathobionts, subvert the host attack and induce diseases. Commensals also enhance their segregation from the host by stimulating a particular subset of myelomonocytic cells (MM) that induce IL-22 secretion from $ROR\gamma t^+$ lymphoid cells through IL-1β and IL-23. IL-22 induces expression of antimicrobial RegIII proteins for bacterial segregation.

In support of this hypothesis, T cell-independent IgA against toxin A of *C. difficile* was found to be protective against *C. difficile* infection independently of the polymeric immunoglobulin receptor (pIgR), which is required for translocation of secretory IgA to the lumen (59). In this case the immunoglobulins produced by the host likely neutralizes toxin A once *C. difficile* is detected in host tissues. In contrast to infections by pathobionts such as *C. difficile*, elimination of certain pathogens including *Vibrio cholerae* requires the pIgR (60), suggesting that secretory IgA controls only pathogens but not commensals. Of note, the host immune system removes the commensals only when they are translocated into tissues, which can be caused by loss of epithelial barrier. The translocated commensals are eliminated by complement components in a sepsis model (61) and phagocytotic cells, which are recruited upon Nod1- and IL-1β-mediated signaling in the *C. difficile* infection model (21, 27). Other immune responses mediated by multiple inflammatory signaling as well as adaptive immune responses also contribute to elimination of commensals in tissues, as extensively described by previous studies. Therefore, the host immune defense is activated against commensals only when they have escaped from where they are supposed to be (the lumen) and disseminate to host tissues.

The mucosal epithelium segregates commensals from the host in bacterial stimulation-dependent and stimulation-independent ways

Spatial segregation of commensals and the host is essential for their mutually beneficial relationship as well as for the maintenance of a balanced homeostatic state in the host. The mucosal lumen is a non-protected area within the host where commensals are licensed to exist freely without interference from the host. The lumenal area close to the intestinal epithelium is protected from microbes by mucus and antimicrobial factors secreted from epithelial cells and particular specialized cells including Paneth cells in the small intestine (62) (Fig. 1).

Deficiencies of transglycosylases, C1Galt and B4galnt2, result in alterations of the microbiota composition (Table 1) (39–41). Antimicrobial peptides, including individual RegIII proteins and defensins, specifically regulate the abundance of certain bacterial types (63, 64). RegIIIγ is important in segregating bacteria such as *Eubacterium rectale* and SFB so that they are ≈50 μm away from intestinal epithelium, and the loss of RegIIIγ results in colonization of Gram-positive bacteria on the epithelium, although RegIIIγ deficiency is not associated with alteration in overall microbiota composition (14). Importantly, the expression of RegIII proteins and α-defensins, but not mucins, is enhanced in the presence of commensals [(62, 65), also see GEO GDS2968, GDS4319 and GDS640 for the global gene expression profiles in germ-free and conventional mice] (66–68).

The expression of all RegIII proteins is dependent on IL-22 (14), suggesting that the microbiota composition is not influenced by differential expression of RegIII proteins. However, the lack of B4galnt2, which is also induced by commensals and modifies mucosal glycans, alters the microbiota composition (40). Therefore, commensals indeed promote the spatial segregation of themselves from the host by providing stimulation to the host. From an evolutionary point of view, this may represent a strategy of immune evasion by commensals to favor their residence in the host.

Production of IL-22 in RORγt$^+$ innate lymphoid cells is dependent on IL-23 and IL-1β, which are produced by bacteria-sensing resident macrophages and a subset of dendritic cells (69–73). Since normal gut microbiota is found mice lacking ASC, an essential component of the IL-1β-producing inflammasome (74), segregation of commensals from epithelium likely does not affect the whole microbial population. However, particular commensals, including *Bacteroides thetaiotaomicron* and *Akkermansia muciniphila,* have the ability to interact with mucus components and utilize the intestinal mucus as an energy source (75). Thus, given the importance of the mucus layer and antimicrobial peptides in the spatial segregation between the host and gut microbiota, many ongoing studies are directed to delineating how alterations in these two key components of host defense contribute to dysbiosis and disease development.

Conclusion

In summary, our current understanding is that the host eliminates commensals and pathogens when they translocate into tissues or invade the lumenal site proximal to the epithelium, but at the distal site in the lumen the host exerts very little control over the bacteria. As discussed above, due to the high diversities and changes within and among individual hosts, rigorous and careful experimental controls must be included in studies to determine the ability of the host immune system to control the lumenal microbiota composition. For the same reason, discrepancies have arisen among previous studies that addressed the roles of various host factors in shaping the microbiota.

However, with the recent advances in technologies, more information and conclusive results are anticipated from future studies of the microbiota composition, commensal localization, and metabolomes. For example, whole-genome sequencing of many standard commensal strains is under way as a part of the Human Microbiome Project and other programs. This will greatly enhance the detection of more specific species related to the standard commensals by qPCR. Application of a long-through-type next-generation sequencing technique (45) to meta-genomic analysis will accelerate the assembly of more accurate contigs. On the other hand, there are currently no comprehensive searchable databases like NCBI GEO (76) that are available for microbiota composition, and the deposition of microbiota composition data to a public database is currently not mandatory for publication of the data. Such a public database, if available, will tremendously advance our understanding of host–commensal interactions and related diseases.

Finally, the ultimate goal is to translate the knowledge obtained from mouse-model studies to humans in order to understand dysbiosis-related diseases. Although the human microbiota has been extensively characterized and diet-based prebiotic/probiotic approaches are popular, the human microbiota is much more complex and diverse, and manipulations of the microbiota in human studies are subject

to many ethical considerations. Despite the caveats, active research in recent years on the role of the host immune system in mouse microbiota has provided valuable insights into the regulation of human microbiota and the pathogenesis of intestinal disorders.

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