The intestinal microbiome and health

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Purpose of review
A diverse array of microbes colonizes the human intestine. In this review, we seek to outline the current state of knowledge on what characterizes a 'healthy' or 'normal' intestinal microbiome, what factors modify the intestinal microbiome in the healthy state and how the intestinal microbiome affects normal host physiology.

Recent findings
What constitutes a 'normal' or 'healthy' intestinal microbiome is an area of active research, but key characteristics may include diversity, richness and a microbial community's resilience and ability to resist change. A number of factors, including age, the host immune system, host genetics, diet and antibiotic use, appear to modify the intestinal microbiome in the normal state. New research shows that the microbiome likely plays a critical role in the healthy human immune system and metabolism.

Summary
It is clear that there is a complicated bidirectional relationship between the intestinal microbiota and host which is vital to health. An enhanced understanding of this relationship will be critical not only to maximize and maintain human health but also to shape our understanding of disease and to foster new therapeutic approaches.

Keywords
gastrointestinal, health, immunity, metabolism, microbiome

INTRODUCTION

It has long been known that a wide array of microbes colonizes the human body and that the interaction between these organisms and their host may be critical in health and disease. However, it is only recently, with the advent of new molecular techniques, that the full diversity of this 'microbiome' has been appreciated. The human intestinal microbiome is among the most complex of the body sites: it includes 500–1000 species and several million genes. New research shows that there is a complicated bidirectional relationship between the intestinal microbiome and the host which is likely critical for human health as well as implicated in disease pathogenesis. The gut microbiome appears to be important in digestion of food and extraction of nutrients, in modifying the host immune response, in protecting against infection, in the metabolism of drugs, and in participation in and regulation of host metabolism. At the same time, the microbiome is itself modifiable by diet, host and environmental factors. In this review, we seek to outline the current state of knowledge on what characterizes a 'healthy' or 'normal' intestinal microbiome, what factors modify the intestinal microbiome in the healthy state and how the intestinal microbiome affects normal host physiology. We also provide a snapshot of disease implication.

WHAT CHARACTERIZES A 'HEALTHY' OR 'NORMAL' INTESTINAL MICROBIOME?

The majority of research on the intestinal microbiome, including the pioneering work done by the Human Microbiome Project to characterize the microbiome in healthy individuals, has focused on the large intestine (colon) and feces, and the diverse microflora of this distal gut region as characterized by stool studies will be the focus of our review as well [1]. However, the gastrointestinal system is certainly not limited to the distal gut, and it is important to note that the bacterial composition of the esophagus, stomach and small

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Curr Opin Infect Dis 2015, 28:464–470
DOI:10.1097/QCO.0000000000000196

KEY POINTS

- There is a complicated bidirectional relationship between the intestinal microbiota and host which is vital to health and likely promotes disease.
- What constitutes a 'normal' or 'healthy' intestinal microbiome is an area of active research, but key characteristics may include diversity, richness and a microbial community's resilience and ability to resist change.
- A number of factors, including age, the host immune system, host genetics, diet and antibiotic use, appear to modify the intestinal microbiome in the normal state.
- New research shows that the microbiome likely plays a critical role in the development of the healthy human immune system and host metabolism.

intestine appears to be significantly different from that of the colon both in diversity and in which microbes predominate. In the esophagus, for example, limited diversity and the presence of a few genera, such as streptococcus, appear to be associated with health, whereas the low pH of the gastric lumen creates an environment that selects for a limited number of acid-tolerant bacterial populations [2▪]. In addition, in the colon, there appear to be differences between the microbial populations found in the lumen (stool) and those found in mucosal tissue samples [3].

The healthy human distal gut microbiota is very diverse, likely encompassing over 1000 species. There may be a certain shared 'core microbiome,' which in healthy individuals appears to be dominated by the phyla Firmicutes and Bacteriodetes, followed by Actinobacteria and Verrucomicrobia [2▪]. However, the relative proportions and species present within individual microbial communities may vary dramatically. Further studies characterizing microbial gene profiles suggest that there are shared common functional pathways across individuals (a 'functional core microbiome'), but these pathways may be fulfilled by different types or communities of bacteria in different people [4].

The gut microbiota has been further described by Arumugam *et al.* [5] to form three distinct enterotypes dominated by a relatively high representation of *Bacteroides, Prevotella* or *Ruminococcus*. This characterization has been somewhat controversial, with some finding that patients' microbiota cluster into only two *Prevotella* or *Bacteroides* dominated 'enterotypes' [6]. Others have proposed that rather than fitting into discrete 'enterotypes,' individual patient's intestinal microbiota relative to others can be conceptualized as existing along 'enterogradients,' that is along a continuum of microbial community structures [7].

Attempts to describe what features represent a 'healthy' or 'good' microbiome have characterized the intestinal microflora in terms of certain core genera which appear to be associated with health, microbial diversity, the relative abundance of certain microbes, microbial gene richness and/or resilience of microbial populations. Greater diversity of the intestinal microbiota appeared to be associated with better health (and better diet) in an elderly cohort, whereas decreased diversity was observed, for example, in patients with recurrent *Clostridium difficile* infection [8,9]. Another study suggested that greater bacterial richness may also be beneficial, as individuals categorized as having a low stool bacterial gene count (<480 000 genes) had more overall adiposity, insulin resistance and dyslipidemia as well as a more pronounced inflammatory phenotype as compared with high gene count individuals. Furthermore, the relative proportions of certain kinds of bacteria or the metabolic profile generated by certain microbial communities may be important to health [10,11]. In the absence of marked diet changes, studies show that a normal individual's gut microbiota may be quite stable over the long term [12]. Given this, a key feature of a healthy microbiome may be both its ability to resist change in the setting of stress (resistance) as well as its ability to return to an equilibrium state following stress (resilience) [7]. A recent study found that human gut microbes from all dominant phyla are resistant to high levels of inflammation-associated antimicrobial peptides. In Bacteroidetes, one mechanism for this resistance appeared to be related to lipopolysaccharide modification. *Bacteroides thetaiotaomicron* mutants which lacked this mechanism were displaced from the microbiome of mice during inflammation triggered by *Citrobacter rodentium* infection. This may represent at least one mechanism which helps to determine the stability of important members of the healthy microbiota during perturbation [13▪▪].

WHAT FACTORS MODIFY THE INTESTINAL MICROBIOME IN THE NORMAL STATE?

Age, the host immune system, host genetics, diet and antibiotic use all appear to modify the intestinal microbiome.

Age

The microbiome evolves quickly in early life, and is initially characterized by relatively low diversity and instability. Most colonization of the infant

gut begins at delivery, although bacteria can be found in meconium. Infants born vaginally are initially colonized with vaginal microbiota, such as *Lactobacillus* and *Prevotella*, whereas those born by caesarian section are initially colonized by skin microbes. In the first year of life, breastfed infants have an increased proportion of Bifidobacteria and a decreased proportion of anaerobic organisms relative to formula-fed infants. Some Bifidobacteria have specialized enzymes that allow them to metabolize human milk oligosaccharides, furthermore they have been linked to a variety of potentially beneficial effects such as increasing the production of immunoglobulin A (IgA) and fortifying the gut mucosal barrier. The infant microbiome interestingly appears to be enriched in certain genes that facilitate the acquisition of nutrients – for example those that encode the de-novo synthesis of folate. Adults in contrast possess a greater number of genes for dietary folate use. The microbiome shifts again with the introduction of solid foods and, by around age 3, children have a relatively stable, adult-like intestinal microbiome [2▪,14▪].

If the infant microbiome is significantly different from that of the healthy adult, so too there have been some changes, albeit more subtle, observed with advancing age. It appears that with age, the proportions of many members of the Firmicutes phylum, Bifidobacteria and *Faecalibacterium prausnitzii* decrease, whereas proportions of *Escherichia coli*, other Proteobacteria and staphylococci increase. It has also been suggested that elderly individuals have more variation in the evenness (or relative proportion of different bacterial species) in their microbiotas, and further that their microbiomes may tend to represent a proinflammatory phenotype, based on decreased potential for B12 synthesis, increased potential for DNA damage, stress response and immune system compromise [2▪,15].

The host immune system

In the gut, a very dense community of microbes is in constant close proximity to the epithelial cell surface, a situation which has the potential to lead to significant immune stimulation and deleterious inflammation. However, the healthy intestinal immune system has multiple mechanisms of tolerance that prevent excessive inflammation. First, there are two mucus layers in the colon. The first is a loosely adherent layer that is colonized by bacteria, but the second is a dense, strongly adherent mucus layer which is devoid of bacteria. This dense inner mucus layer covers the epithelial surface and provides a physical barrier that prevents direct contact between the microbes and the immune system [16]. Second, in the small intestine, antimicrobial peptides secreted by the host help to maintain distance of bacteria from the epithelium. Finally, T-cell responses to microbes in the intestine are suppressed, further helping to prevent inappropriate inflammation. The healthy immune system serves to modify the microbiome. Insights into its actions come principally from mouse studies in which mice with certain immune deficiencies (such as mutations in IgA or the function of innate lymphoid cells) appear to lead to alterations in the composition of gut microbiota, and the accumulation of potentially pathogenic organisms such has *Klebsiella*, *Proteus* and *Helicobacter* [17▪].

Host genetics

Family members have been observed to have more similar microbiotas than unrelated individuals, raising the possibility that genetics may shape the microbiome. A recent study examining fecal samples from 977 individuals including from both monozygotic and dizygotic twin pairs showed that microbiomes were more similar for twins than for unrelated individuals and more similar for monozygotic than dizygotic twins. They also found that the family Christensenellaceae appeared to be the most highly heritable taxon and was associated with low BMI [18▪].

Diet

One of the most important modifiers of the intestinal microbiome appears to be diet, both shaping its composition and functional metabolism [14▪]. De Filippo *et al.* [19] showed that the intestinal microbiota of children living in rural Burkina Faso differed significantly from children living in Florence, Italy. The microbiota of the children in Burkina Faso, who ate a high fiber diet rich in carbohydrates and non-animal protein, had greater microbial richness, greater abundance of *Prevotella* with lower abundance of *Bacteroides* and seemed to produce more short chain fatty acids – likely reflective of an enhanced ability to break down fiber, starch, oligosaccharides and carbohydrates from a diet of whole grains that escape digestion in the small intestine. The European children in contrast ate a diet high in sugar, starch, animal protein, fat and low in fiber. Another study comparing the fecal microbiota of children in Bangladesh versus the United States showed similar results [20]. Wu *et al.* [6] showed in a study in the United States that long-term consumption of a diet high in protein and animal fat was associated with a predominance of *Bacteroides*,

whereas a low fat, high carbohydrate diet was associated with a microbiota dominated by *Prevotella*.

Wu *et al.* [6] also conducted a controlled feeding experiment in which 10 individuals who were originally in the *Bacteroides* 'enterotype' were fed either a high fat/low fiber or a low fat/high fiber diet. Interestingly, there were changes seen in the microbiota within 24 h, but these were relatively modest and did not result in the patients switching from one enterotype to another. In a study by David *et al.* [21▪▪], 10 individuals were fed either an almost entirely animal-based diet or an entirely plant-based diet. Within 24 h, a relative increase in several types of bacteria resistant to bile acids was noted with consumption of the animal-based diet. The animal-based diet was also associated with increased expression of key genes for vitamin biosynthesis, the degradation of polycyclic aromatic hydrocarbons (carcinogenic compounds produced during the charring of meat) and the increased expression of β-lactamase genes. This diet decreased the levels of Firmicutes that metabolize dietary plant polysaccharides. Foodborne microbes from both diets including bacteria, fungi and viruses were noted to transiently colonize the gut. Interestingly, the microbial structures of each individual microbiome returned to baseline within 3 days of stopping the diet.

An intriguing article by Suez *et al.* [22▪] using data both from mice and limited data from humans suggested that consuming noncaloric artificial sweeteners led to an increase in glucose intolerance, possibly mediated by changes to the intestinal microbiota. Mice (and humans) fed large amounts of noncaloric sweeteners had a microbiome enriched in *Bacteroides*, and enriched in glycan degradation pathways previously linked to enhanced energy harvest in obese patients. Furthermore, differences in glucose intolerance between noncaloric sweetener drinking mice and controls were abolished after antibiotic treatment.

Finally, studies in populations in Japan suggest that genes encoding enzymes that metabolize marine red algae have been transferred from marine bacteria to specific bacteria in the human intestine, and that these bacteria and the genes they contain have then been widely disseminated in the population [23].

Antibiotics

Although antibiotics may not necessarily be considered part of the 'normal' human state, the ubiquitous nature of their use means that many (perhaps most) healthy adults will have taken at least one course in their lifetimes. Antibiotics have a significant short-term impact on the gut microbiota, with decreases in richness and diversity seen within 3–4 days after antibiotic administration. Changes in community membership and composition are also seen [24]. The extent of these changes, particularly on individual community members, differs by type of antibiotic administered. However, the impact of even short courses of antibiotics may have long-term effects on the intestinal microbiota. In one study, after short-term clindamycin administration, *Bacteroides* species composition was still significantly altered 18 months later [25]. In another study, three patients were given two 5-day courses of ciprofloxacin, 6 months apart. After the first administration, in two patients the intestinal microbiota largely returned to the preantibiotic state. However, after two doses of ciprofloxacin, all three patients had intestinal microbiota that was altered from the preantibiotic state, and these alterations persisted 2 months after the last dose of antibiotics [24]. Finally, patients with recurrent *C. difficile*, which is an antibiotic-associated infection, have been shown to have intestinal microbiota characterized by decreased diversity [9].

HOW DOES THE MICROBIOME AFFECT NORMAL HOST PHYSIOLOGY?

The microbiome appears to significantly impact both the immune system and host metabolism.

Immune system

The impact of the microbiome on the host immune system is an active area of research. Data principally from animal models imply that the microbiome has important effects on the structural development and function of gut-associated lymphoid tissues, T cells and B cells, although the details of these interactions remain under study. Interestingly, studies comparing germ-free mice with conventionally housed mice show that the germ-free mice seem to have impaired development of gut-associated lymphoid tissues, such as Peyer's patches and isolated lymphoid follicles. Early stages of B-cell development occur in the intestinal mucosa (as well as in the fetal liver and bone marrow), and it appears that editing of receptors on developing intestinal B cells is regulated by extracellular signals induced by gut microbiota. Further, IgA-producing B cells mature in Peyer's patches under stimulation from commensal organisms. Similarly, germ-free mice appear to have decreased numbers of T helper Th1 and Th17 cells. germ-free mice also have reduced numbers of T regulatory (Foxp3+) cells in the colonic lamina propria; this reduction can be reversed by colonizing

the mice with intestinal microbiota. In addition, specific types of *Clostridia* and possibly *Bacteroides* seem to have the ability to induce T-regulatory cells. Induction of T-regulatory cells by the microbiota appears to protect mice from colitis, infection with enteric pathogens and allergic diarrhea, suggesting that this interaction is important in gut homeostasis in the healthy individual. Whether innate lymphoid cells are affected by gut microbiota is, as yet, controversial, although some studies suggest that microbiota may be important for the differentiation of group 3 innate lymphoid cells and may play a role in their regulation as well. Finally, the secretion of both proinflammatory and anti-inflammatory cytokines by macrophages, dendritic cells and neutrophils appears to be regulated, at least in part, by intestinal flora [17▪].

The observation that there are decreased responses to certain oral vaccinations in children living in a number of developing countries as compared with children living in westernized countries further suggests that the intestinal microbiota may have important effects on the immune system, although these interactions are complex and likely affected by a number of factors, including malnutrition. For example, oral rotavirus vaccine elicits immune responses in only 49% of children living in Malawi as opposed to over 95% of children living in westernized countries. Populations with greater enteric disease burden experience lower efficacy of the cholera vaccine, and studies in children from Chile show that those with small bowel bacterial overgrowth respond less well to the oral cholera vaccine [26]. A recent study looking at immunoglobulin G (IgG) response to influenza vaccine in mice showed that both germ-free and antibiotic-treated mice had decreased short-term IgG responses to trivalent inactivated influenza vaccine as compared with conventionally housed mice. If germ-free mice were conventionalized by transfer to standard housing conditions, influenza-specific IgG concentrations recovered to levels comparable to the control mice. The mechanism of this observed effect was hypothesized to be related to sensing of the microbiota by toll-like receptor 5, a cell-surface receptor specific for flagellin. Interestingly, the same reduction in immune response in germ-free and antibiotic-treated mice was not seen with live attenuated vaccines (such as the yellow fever vaccine) [27▪].

Further evidence of the critical role that the intestinal microbiota plays in immunity comes from the oncology literature in which, in mouse models, it has been shown that antibiotic-treated or germ-free mice have a poorer response to chemotherapy drugs. Cyclophosphamide, for example, works in part by stimulating antitumor immune responses. Viaud *et al.* [28] found that cyclophosphamide alters the composition of the gut microbiome and induces translocation of some Gram positive bacteria into lymphoid organs, where the bacteria then stimulate a certain subset of 'pathogenic' T helper 17 cells and memory T helper 1 immune responses. Germ-free or antibiotic-treated mice exhibited a reduced T helper 17 response, and their tumors were resistant to cyclophosphamide.

Metabolism

The intestinal microbiota both dramatically expands the host's range of metabolic capabilities and helps to regulate host metabolism in a range of ways that we are only beginning to understand [29▪]. Colonic microorganisms play an important role in the synthesis of micronutrients such as vitamin K, B12, biotin, folic acid and pantothenate, as well as absorption of calcium, magnesium and iron [30]. Microbiota in the colon encodes a vast array of carbohydrate active enzymes which allow them to break down indigestible dietary residues, releasing short chain fatty acids which are important for health and immunity. Butyrate, for example, appears to be important in the differentiation of T-regulatory cells [2▪]. Patients with inflammatory bowel disease have fewer butyrate-producing bacteria in their intestine, which may be an important factor driving intestinal inflammation [29▪]. Colonic organisms also break down proteins into amino acids, which can then be converted, also by intestinal bacteria, into a variety of signaling molecules and antimicrobial peptides that promote both resilience and resistance to infection [2▪].

The importance of the gut microbiota to metabolism may extend beyond regularly consumed dietary compounds to drug metabolism. One of the best known examples of this is digoxin. For decades, it has been clear that in a subset of individuals digoxin is inactivated and the inactive metabolite secreted. Broad spectrum antimicrobial therapy was shown to block digoxin inactivation and simultaneously resulted in increased serum levels of the drug. Finally, it was discovered that *Eggerthella lenta* was the gut bacteria capable of catalyzing the inactivation of digoxin. In mouse models, inactivation of digoxin was blocked by increasing protein intake (likely an inhibitory effect of arginine) [29▪].

It appears that the microbiome is critical in regulating host metabolism and may be an important factor in preventing (or promoting) obesity and insulin resistance. As mentioned above, there are notable differences in the microbiota of obese versus

lean individuals. Some studies have found differing ratios of Firmicutes to Bacteroidetes [10], whereas others have noted differences in bacterial richness and diversity. Other studies have noted that the gut microbiota of obese individuals has an increased capacity for energy harvest [31▪,32]. In mice, transfer of fecal material from obese and lean littermates also appeared to transfer each phenotype: germ-free recipients of feces from obese donors gained significantly more adiposity than those from lean donors [33▪]. Feces transferred to mice from obese and lean human twins also demonstrated a similar transfer of phenotype. Moreover, when the mice were fed a 'good diet' (low fat, high fruit and vegetable), the lean flora appeared dominant; that is when mice who had received feces from an obese human were cohoused with mice who received feces from a lean human, both exhibited a lean phenotype [34]. Intriguingly, a recent small study in which intestinal microbiota was transferred from lean donors to patients with metabolic syndrome led to increased insulin sensitivity in the recipients [35].

A SNAPSHOT OF THE DISEASE IMPLICATIONS OF THE MICROBIOTA

As summarized in this review, recent work has set the framework for understanding if and how the microbiota contributes to disease pathogenesis. This critical area holds promise for insights into new disease diagnostics and therapeutics. Because of its luminal association with the densest microbiota on the human body, colon diseases are a particular target of initial investigations into the microbiota contributions to disease. Our foray into this area grew from a recognition that the toxin secreted by a bacterium associated with diarrheal disease, enterotoxigenic *Bacteroides fragilis*, activated carcinogenic mechanisms in colonic epithelial cells [36▪]. Remarkably, upon the addition of this single bacterium to the complex colon microbiota in a susceptible mouse model, marked induction of distal colon adenomas was observed [36▪]. Initial translation of these laboratory findings to the bedside revealed that nearly all patients with colon cancer are colonized with enterotoxigenic *B. fragilis* and, in many cases, with more than one strain of enterotoxigenic *B. fragilis* whereas individuals undergoing screening colonoscopy are significantly less frequently colonized [37▪]. Nonetheless, ~50% of those undergoing screening colonoscopy were colonized with enterotoxigenic *B. fragilis* suggesting that exposure to enterotoxigenic *B. fragilis* is common. Importantly, additional studies suggest that right colon cancer displays a striking difference in microbiota organization compared with left colon cancer. Namely, the mucosa of right colon tumors (cancers and adenomas) and their normal tissues distant from the tumors are covered in a mucosally adherent polymicrobial and invasive biofilm that is further associated with activation of procarcinogenic colon tissue mechanisms. In this work, microbiome sequencing did not reveal these differential findings between right and left colon making it clear that to understand the contribution of the microbiome to disease requires the use of diverse approaches (e.g. microbiologic, biologic and genomic) [38▪].

CONCLUSION

In sum, it is clear that there is a complicated bidirectional relationship between the intestinal microbiota and host which is vital to health and likely disease. An enhanced understanding of these complex relationships will be critical not only to maximize and maintain human health but also to shape our understanding of disease and to devise novel therapeutics.

Acknowledgements

C.L.S. thanks the current and former members of her laboratory for the inspiration they provide and their contributions over time.

Financial support and sponsorship

S.T. acknowledges support for this article from NIH T32 grant 5AI007291-24. C.L.S. acknowledges support for this article from R01 CA151393 and R01 CA151325.

Conflicts of interest

There are no conflicts of interest.

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