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Review

The Impact of the Gut Microbiota on Human Health: An Integrative View

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The human gut harbors diverse microbes that play a fundamental role in the well-being of their host. The constituents of the microbiota—bacteria, viruses, and eukaryotes—have been shown to interact with one another and with the host immune system in ways that influence the development of disease. We review these interactions and suggest that a holistic approach to studying the microbiota that goes beyond characterization of community composition and encompasses dynamic interactions between all components of the microbiota and host tissue over time will be crucial for building predictive models for diagnosis and treatment of diseases linked to imbalances in our microbiota.

Introduction

We have only recently started to appreciate that the human body is home to far more than human cells: we harbor at least 100 trillion (10^{14}) microbial cells (Whitman et al., 1998) and a quadrillion viruses in and on us (Haynes and Rohwer, 2011). Collectively, the microbial associates that reside in and on the human body constitute our microbiota, and the genes they encode is known as our microbiome. This complex community contains taxa from across the tree of life, bacteria, eukaryotes, viruses, and at least one archaeon, that interact with one another and with the host, greatly impacting human health and physiology. Only a small minority of these can be cultured, and recently, culture-independent high-throughput sequencing has greatly expanded the repertoire of known microbes both in our bodies and in the environment (Shendure and Ji, 2008; Whitman et al., 1998). Highly multiplexed studies have made it possible to characterize and compare many samples rapidly (Caporaso et al., 2010; Hamady et al., 2008), enabling the detection of spatial, temporal, and disease-associated patterns in our microbiota.

The microbiota plays a major role in health and disease in humans; indeed, it is sometimes referred to as our "forgotten organ" (O'Hara and Shanahan, 2006). The microbiota is involved in energy harvest and storage, as well as in a variety of metabolic functions such as fermenting and absorbing undigested carbohydrates (Gill et al., 2006), a trait that has probably acted as a strong evolutionary force toward the establishment of bacteria as human symbionts. Perhaps even more importantly, the gut microbiota interacts with the immune system, providing signals to promote the maturation of immune cells and the normal development of immune functions (Chow et al., 2010).

In this Review, we give an overview of the current understanding of the microbiota and its development in healthy individuals. To further characterize this "healthy microbiota," we also describe the interactions that typically occur in the human gut between viruses, eukaryotes, bacteria, and the host immune system. We then discuss how imbalances in the composition of the microbiota, and the induced changes in interactions with the host, relate to diseases such as obesity or Crohn's disease. In each section we highlight lessons that have been learned about various interacting parts of the microbiota and argue that adopting an integrated perspective will foster a deeper understanding of the many diseases that are related to disruption of the microbiota and lead to microbiota-based therapeutic options for treating these diseases. Finally, we detail the findings of some recent studies that do present an integrated view of the human microbiota in the context of human health and disease.

The Healthy Microbiota

Characterizing the Human Gut Microbiota: Who Is in There?

Determining what constitutes a healthy microbiota and the variability found across populations is a prerequisite for assessing deviations that are associated with disease states. Microbes colonize all of the surfaces of the human body that are exposed to the environment, with the majority residing in the intestinal tract, which is the focus of this Review. Distinct microbial communities are also found in the mouth (Nasidze et al., 2009), in the vagina (Ravel et al., 2011), and on the skin (e.g., Costello et al., 2009; Grice et al., 2009). Bacterial communities in a given body site resemble themselves more than those in different body sites, i.e., the oral bacterial communities across individuals are more similar than those of the skin and mouth of an individual (Costello et al., 2009). At each body site there is also considerable interindividual variability (Costello et al., 2009; Robinson et al., 2010).

The bacterial component of the microbiota has been the subject of intensive study in recent years, driven by large-scale projects such as the Human Microbiome Project (Peterson et al., 2009; Turnbaugh et al., 2007) and MetaHIT (Qin et al.,

2010). These studies have demonstrated a large variability in the composition of the community in healthy individuals, with twins sharing less than 50% of their species-level bacterial taxa (Turnbaugh et al., 2010) and even fewer viral sequences (Reyes et al., 2010). The factors that shape community composition are beginning to be understood. Host genetics play an important role in the establishment and shaping of the gut microbiota, as it has been demonstrated that composition of the bacterial community is influenced by specific host genomic loci (Benson et al., 2010; Spor et al., 2011), although this is not the case for viral communities (Reyes et al., 2010). Metagenomic studies have established that despite the extensive interpersonal variability in community composition, there is a shared core of functionalities in the microbiome (Burke et al., 2011; Turnbaugh et al., 2009a). Building upon this knowledge, the literature characterizing the microbiota (or at least the bacterial component) in disease is exploding (Table 1) (Pflughoeft and Versalovic, 2011).

The gut microbiota is typically dominated by bacteria and specifically by members of the divisions Bacteroidetes and Firmicutes (Turnbaugh et al., 2006). Although there is a huge range of variation in the taxa present in the gut and interindividual variability in microbial composition, it has been suggested that the microbiota of most individuals can be categorized into one of three variants or "enterotypes" based on the dominant genera (*Bacteroides*, *Prevotella*, or *Ruminococcus*) (Arumugam et al., 2011). These clusters may in fact be more appropriately characterized as a ratio of the abundance of *Bacteroides* and *Prevotella*, with the *Ruminococcus* enterotype folded into the *Bacteroides* group (Wu et al., 2011). These broad patterns are driven primarily by dietary effects (Wu et al., 2011); it remains to be seen how important they are in understanding overall community function.

Knowledge about bacterial communities far outpaces that of viral and eukaryotic communities, although these agents are known causes of many disease states in the gut. In our discussions, we take a broad view of eukaryotes and viruses, including fungi and microbial lineages within eukaryotes and bacteriophages among the viruses. The eukaryotes and viruses that are associated with humans have typically been studied with a focus on specific pathogenic organisms (Bogitsh et al., 2005; Collier et al., 2011). However, increasing attention is being paid to elucidating the eukaryotic component of the microbiota (Ghannoum et al., 2010; Scanlan and Marchesi, 2008), and recent research is beginning to show that even healthy humans harbor a diverse consortium of viruses that make up the human virome (Breitbart et al., 2008; Haynes and Rohwer, 2011; Virgin et al., 2009). Most viral sequences currently being identified in mammals are novel, suggesting that we are just beginning to characterize the diversity within the human virome (Breitbart et al., 2008; Haynes and Rohwer, 2011; Virgin et al., 2009). This is likely an underestimate of total viral diversity, as nucleic extraction methods are known to be biased toward encapsulated viruses. Similar to gut bacteria, interpersonal variation in the virome is high (Minot et al., 2011; Reyes et al., 2010). The initial microbial eukaryotes found associated with the microbiota will likely be those known from parasitology, such as *Giardia* and *Entamoeba*, that can be identified through culture-independent microscopy methods. Elucidating the variability in community composition across individuals awaits comprehensive studies of geographically diverse populations. Changing the focus from individual agents that are implicated in disease toward a community view has already precipitated paradigm shifts in the view of the virome and disease (Virgin et al., 2009), and the same will likely be true for eukaryotic microbes.

Temporal Dynamics of the Microbiota

Initial work aimed at enumerating and categorizing human-associated microbial organisms (Costello et al., 2009; Eckburg et al., 2005; Ley et al., 2005) has been complemented by studies that describe the temporal dynamics of bacterial communities (Caporaso et al., 2011). Understanding the stability of the microbiota within an individual through time is an important step in enabling prediction of disease states and developing therapies to correct dysbiosis (imbalances in the microbial community). Time series data show that the composition of the microbiota is relatively stable within healthy adult individuals over time for bacteria (Caporaso et al., 2011; Costello et al., 2009), viruses (Reyes et al., 2010), and eukaryotes (Scanlan and Marchesi, 2008). However, this temporal consistency assumes that numerous variables, including diet, disease, and environment, are also being held constant.

Dietary changes in particular have been shown to have significant effects on the microbiota. For example, once established, the healthy adult human virome seems to be relatively stable, with >95% of viral sequences showing minimal variation over a time period of 1 year (Reyes et al., 2010). However, Minot et al. have recently shown that the viromes of healthy adults can converge to a more similar state due to dietary intervention (Minot et al., 2011). Additionally, it has been shown in mice that shifting to a high-fat, high-sugar "Western" diet from a low-fat, plant polysaccharide-rich diet can change the microbiota within a day (Turnbaugh et al., 2009b). In another study in humans, shifting from a high-fat/low-fiber diet to a low-fat/high-fiber diet caused notable changes in the gut microbiota within 24 hr (Wu et al., 2011). Interestingly, diet also correlates with enterotype, as individuals on a diet high in animal fat have a *Bacteroides*-dominated enterotype, whereas a carbohydrate-rich diet is associated with the *Prevotella*-dominated enterotype (Wu et al., 2011).

These longitudinal studies allow us to differentiate transient microbes that generally originate from the environment from permanent members of the microbiota and elucidate differences in the host interactions between these two populations. These studies have provided a baseline characterization of the variability of the gut microbial composition, laying the foundation for future studies to be able to detect differences in the microbiota that are characteristic of disease states and to be better able to predict these states.

Establishment of the Microbiota

As we develop and change from infancy to old age, so does our microbiota. In contrast to the studies described above, where either the timescale is relatively short or the subjects are already adults, work on the initial colonization of the intestinal tract of infants has opened a window into the succession dynamics of the gut (Figure 1). Traditionally babies have been considered sterile in utero and thus present a blank canvas for colonization by microbiota at each generation. There is countervailing

Table 1. Changes in the Gut Microbiota Associated with Disease

Implicated Microbiota[a]	Changes in Microbiota Presence/Function	References[b]
Allergies		
Lactobacillus spp. ↓	early colonization with *Lactobacillus* associated w/decreased allergies	Round et al., 2011
Bifidobacterium adolescentis ↓	early colonization with more diverse microbiota might prevent allergies	Round and Mazmanian, 2009
Clostridium difficile ↓		
Helicobacter pylori ↓	*H. pylori* tolerance mediated by Tregs that suppress asthma	Arnold et al., 2011
Celiac's disease		
Bacteroides vulgatus ↑	higher diversity (Shannon-Wiener index) in Celiac's disease patients versus controls	Elinav et al., 2011
Escherichia coli ↓		
Clostridium coccoides ↓		
Gastric Cancer		
H. pylori ↑	important element in carcinogenic pathway for developing gastric adenocarcinomas	Lathrop et al., 2011
Austim		
Bacteroidetes ↑	increased bacterial diversity in feces of autistic children compared to controls	Robinson et al., 2010
Proteobacteria ↑		
Actinobacteria ↓		
Firmicutes ↓		
Obesity		
Bacteroidetes ↓	significant changes in gut microbiota are associated with increasing obesity	Ley et al., 2005; Pflughoeft and Versalovic, 2011
Lactobacillus ↑		
Firmicutes/Bacteroidetes ratio ↓		Ley et al., 2005
Methanobrevibacter smithii ↓		Turnbaugh et al., 2009b
Anorexia		
Methanobrevibacter smithii ↑	Bacteroidetes, Firmicutes, and Lactobacillus similar to lean patients, though *M. smithii* significantly increased	Armougom et al., 2009; Pflughoeft and Versalovic, 2011
IBD—Crohn's Disease		
Bacteroides ovatus ↑	less diversity in patients with Crohn's disease compared to healthy patients	Dicksved et al., 2008
Bacteroides vulgatus ↑		
Bacteroides uniformis ↓		
IBD (General)		
Bacteroidetes ↓	IBD associated with overall community dysbiosis rather than single causal bacterial species	Spor et al., 2011; Perry et al., 2006
Lachnospiraceae ↓		
Actinobacteria ↑		
Proteobacteria ↑		
Clostridium leptum ↓		
Clostridium coccoides ↓		
Faecalibacterium prasnitzii ↓		
Firmicutes/Bacteroidetes ratio ↓		
Bifidobacteria ↓		
Type 2 Diabetes		
Firmicutes ↓	shifts in gut microbiota associated with increases in plasma glucose concentrations	Brown, 2000
Clostridia ↓		
Bacteroides-Prevotella ↑ versus *Clostridia coccoides-Eubacterium rectale* ↓		
Betaproteobacteria ↑		
Bacteroidetes/Firmicutes ratio ↑		

[a]Changes relative to healthy subjects. Increase: ↑. Decrease: ↓.
[b]References are exemplary rather than exhaustive and focus on studies that compare healthy versus diseased individuals.

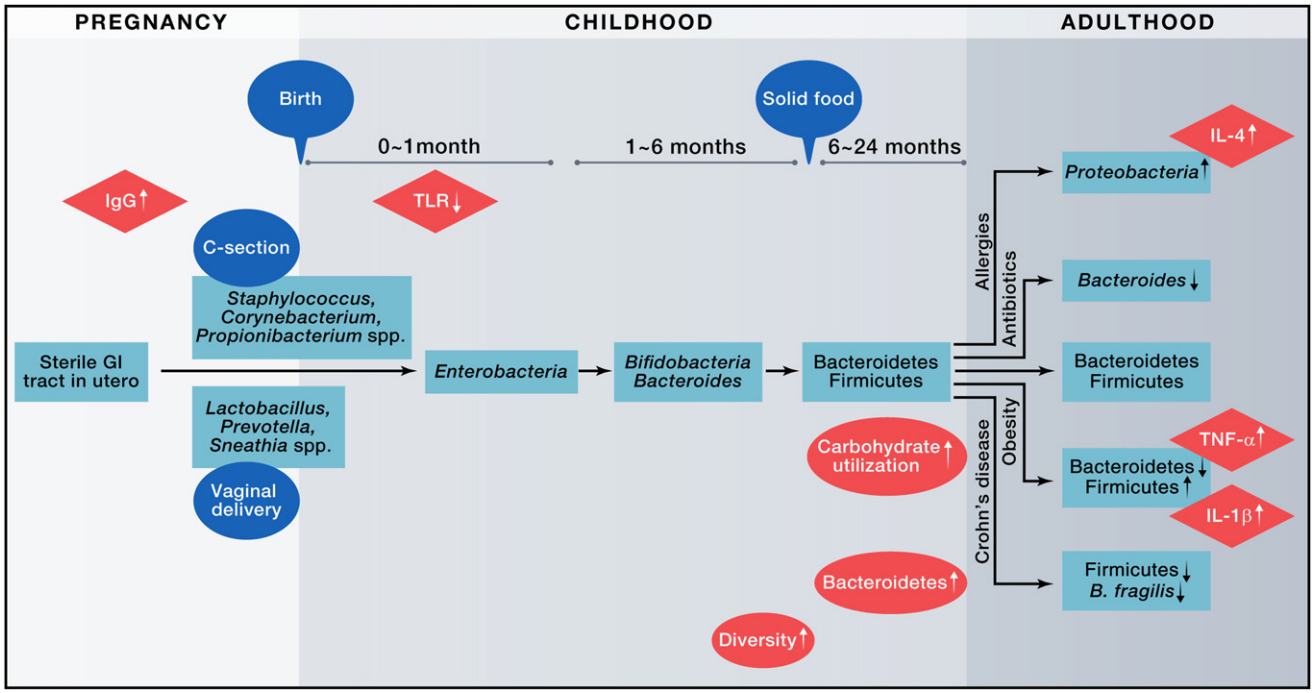

Figure 1. Development of the Microbiota

The gastrointestinal tract of the fetus is sterile until birth, after which the newborn is initially colonized. Depending on delivery mode, the initial communities tend toward a skin-like (caesarean section) or a vaginal-like (vaginal delivery) configuration. During the first weeks of life, there is a reduced activity of TLRs, potentially allowing the necessary formation of a stable bacterial community in the gut. As the infant grows, and with the introduction of solid foods, the microbiota diversity increases, and the community converges toward an adult-like state. At the same time, the immune system "learns" to differentiate between commensal and pathogenic bacteria. By adulthood, a relatively stable community composition (but varying between different individuals) is achieved, dominated mostly by Bacteroidetes and Firmicutes. Different diseases are characterized by significant changes in the microbiota and associated changes in the production of cytokines.

evidence that there are bacteria present in amniotic fluid in utero even in healthy neonates (Jiménez et al., 2008), but regardless both number and diversity of microbes are low. The earliest infant stool sample, the meconium, is free of detectable viral particles (Breitbart et al., 2008) and harbors very low diversity of bacteria (Koenig et al., 2011). Babies are exposed to a plethora of microbes from different environments immediately upon birth and are rapidly colonized by the microbes they first encounter, either from their mother's vagina or from skin microbes, depending on delivery mode (Figure 1) (Adlerberth and Wold, 2009; Dominguez-Bello et al., 2010). Infants born vaginally have communities resembling those found in the vaginal microbiota of their mothers. In contrast, those delivered by caesarean section harbor a microbiota characteristic of skin and dominated by taxa such as *Staphylococcus* and *Propionibacterium* spp. (Dominguez-Bello et al., 2010). Delivery mode has also been hypothesized to influence immunological functions during the first year of life via gut microbiota development, with babies delivered by caesarean section having lower bacterial cell counts in fecal samples and a higher number of antibody-secreting cells (Huurre et al., 2008).

Evolving Development of the Microbiota

The diversity of both bacteria and viruses in the infant gut is initially very low, then climbs through early development (Adlerberth and Wold, 2009; Breitbart et al., 2008; Koenig et al., 2011; Vaishampayan et al., 2010). Early colonizers are generally aerotolerant, as the gut initially contains oxygen, and then are replaced by anaerobes that are typical of the adult gut microbiota (Palmer et al., 2007). One study found that the virome changes quickly, as a majority of the sequences (56%) found in the first week of like were not present after the second week, and the repertoire expands rapidly in diversity and number over the first 3 months (Breitbart et al., 2008). This contrasts with the stability seen in adults where 95% of viral types are conserved over time (Reyes et al., 2010). Viral particles were not found in the formula or breast milk, although transmission of specific viruses, such as HIV, is known to occur via this route. Overall, this suggests that the infant is predominately acquiring these viral particles through environmental and maternal contact (Breitbart et al., 2008). These initial colonizers are replaced by phylotypes that by 11 months are specific to the infant and different from those found in the mother (Vaishampayan et al., 2010), although deeper sequencing might reveal that some of the initial taxa are present at abundances below the current detection threshold. A detailed time series of a single infant demonstrates that phylogenetic diversity increases gradually over time (Koenig et al., 2011). This pattern is punctuated by major shifts in taxonomic composition of the microbiota associated with life events such as antibiotic use and the introduction of solid foods (Koenig et al., 2011). The extent to which this added diversity develops

from the initial inoculum or comes from ongoing exposure to parents, diet, other environmental sources, etc. remains an exciting area of study as quantitative estimates are not yet available.

Metagenomic analysis revealed how the microbiome is enriched early in life in genes to facilitate lactate utilization when the infant's diet is breast milk and formula (Koenig et al., 2011). The microbiome of infants harbors the functionality found in mothers despite having a different taxonomic composition (Vaishampayan et al., 2010). Most interestingly, the functional capacity to utilize plant-derived glycans is present before the introduction of solid food, suggesting that the infant gut is ready to switch to a diet not exclusively based on milk before the actual change in diet takes place (Koenig et al., 2011). The bacterial composition begins to converge toward an adult-like microbiota by the end of the first year of life (Palmer et al., 2007) and fully resembles the adult microbiota by 2.5 years of age (Koenig et al., 2011). Once the microbiota has reached maturity it remains mostly stable until old age. The ELDERMET consortium studied the microbiota of the elderly, finding a characteristic composition different from that of young adults, particularly in the proportions of *Bacteroides* spp. and *Clostridium* groups (Claesson et al., 2011). Variability in community composition is greater in this age group than for adults, which could be related to the greater range of morbidities associated with age and the subsequent use of medications to treat them.

Effects of Antibiotics on the Microbiota

Although the microbiota is generally stable within individuals over time, the composition can be altered due to external perturbations. One of the major factors that can perturb the composition of the microbiota is antibiotic use. Antibiotics have a profound effect on the microbiota, and their overuse is linked with an increase in antibiotic-resistant pathogens. There is now compelling evidence of major alterations of the microbiota following treatment with antibiotics (Dethlefsen et al., 2008; Jernberg et al., 2007; Sullivan et al., 2001). Although the particular taxa affected vary among individuals, some taxa do not recover even months after treatment, and in general, there is a long-term decrease in bacterial diversity. As the established gut bacterial community reshapes after treatment with antibiotics, there is a reduced resistance to colonization, allowing foreign microbes that can outgrow commensal bacteria to cause permanent changes in the structure of the microbiota and varying states of disease. The repeated use of antibiotics in humans has been hypothesized to increase the reservoir of antibiotic-resistant genes in our own microbiome (Sommer et al., 2009). In support of this hypothesis, some European countries have observed a reduction in the number of antibiotic-resistant pathogens following a reduction in the number of prescribed antibiotics (Goossens et al., 2005).

Interactions between the Host Immune System and the Microbiota

Mammals have coevolved over millions of years with our microbiota (Ley et al., 2008). Thus it is not surprising that our immune system, and especially the mucosal immune system, has developed an intricate connection with our associated microbiota. It appears that both the innate and adaptive immune systems have evolved to require microbial interactions during their development (Chow et al., 2010; O'Hara and Shanahan, 2006). Germ-free mice have reduced gut secretory IgA, defects in development of gut-associated lymphoid tissues, and smaller Peyer's patches and mesenteric lymph nodes (Round and Mazmanian, 2009). IgA in particular plays a fundamental role in mucosal immunity, as it is induced in response to colonization by specific commensal bacteria to protect mucosal surfaces and contribute to host-microbiota mutualism (Bouskra et al., 2008; Macpherson et al., 2011; Peterson et al., 2007).

Recognition of Commensal Microbes by the Immune System

The innate immune system recognizes general microbe-associated molecular patterns (MAMPs) that are present across diverse lineages of bacteria, such as components of the bacterial cell wall (lipopolysaccharide and peptidoglycan) and flagellin. Toll-like receptors (TLRs) are one of the several proteins that the host uses to recognize such antigens. When TLRs are not present or are mutated, the gut and mucosal immune systems do not form normally (O'Hara and Shanahan, 2006). The commensal bacteria appear to be important in suppressing inflammatory response and promoting immunological tolerance, and this interaction also occurs through TLRs (O'Hara and Shanahan, 2006; Round et al., 2011). NOD-like receptors (NLRs) also recognize microbial molecules and can form oligomers (inflammasomes) that serve as sensors of damage-associated patterns. Deficiency of NLPRP6, for example, results in reduced IL-18 levels, an altered composition of the microbiota, and intestinal hyperplasia (Elinav et al., 2011).

Microbial Modulation of the Adaptive Immune System

The adaptive immune system is also programmed by the commensal microbiota, and microbes have been shown to impact the differentiation of T cell populations, which can be not only determined by self-/non-self-discrimination mechanisms but also educated by commensal microbiota (Lathrop et al., 2011; Lee and Mazmanian, 2010). Commensal bacteria are also capable of modulating the host innate immune system to promote their own fitness in the intestinal niche. For instance, *Bacteroides thetaiotaomicron* has been shown to induce peptides with bactericidal activity that target other intestinal microbes (Hooper et al., 2003). Being able to elicit low amounts of IgA by modulating the immunodominant determinants has also been proposed as a mechanism by which commensal bacterial gain an advantage in the gut environment (Peterson et al., 2007). One intriguing question is whether reduced eukaryotic parasite load is increasing autoimmune disease incidence by allowing immunological activity to be redirected, in a disruptive way, in the absence of a coevolved engagement with microbes (see discussion of the "hygiene hypothesis" below). These examples demonstrate the impact of microbial interactions with the immune systems on host health.

Disease and Dysbiosis

The characterization of the gut microbiota and its connections to the host described so far have provided us an initial picture of what might be a healthy state from a microbial perspective. The study of diseases, however, has been classically

approached from a "one microbe-one disease" viewpoint. Viruses, eukaryotes, and bacteria were studied under conditions in which they were believed to cause disease. However, just as the "one gene-one enzyme" outlook proved to be an oversimplification that failed to explain complex phenotypes, we are now starting to appreciate the fact that humans are colonized with myriad viruses, eukaryotes and bacteria, and that some diseases might result from dysbiosis rather than the presence of a single disease-causing microbe. Examples of diseases associated with microbial dysbiosis include autoimmune and allergic diseases, obesity, inflammatory bowel disease (IBD), and diabetes (Table 1).

The Obese Microbiota: Interactions with the Immune System

Obesity is a physiological state that has emerged as a major health concern in populations that have adopted a Western diet and is closely tied to the microbiota (Ley, 2010). In animal models of obesity, the interplay between the dominant gut phyla, Bacteroidetes and Firmicutes, is shifted with a significant reduction of the former and a corresponding increase in the latter (Ley et al., 2005). The same trend is observed within individual humans on weight-reduction diets (reviewed in Ley, 2010), although a study with human twins showed that in obese individuals, the decrease in Bacteroidetes was accompanied by an increase in Actinobacterium rather than in Firmicutes (Turnbaugh et al., 2009a). The observed shift in the relative abundances of these phyla results in an increased capacity for harvesting energy from food and produces low-level inflammation. Several changes in host genetics and environmental factors have been used to induce obesity in animal models and thus provoke a change in microbiota composition. Remarkably, the energy harvest phenotype is transmissible simply by transplanting the obese microbiota into healthy, lean donors (Turnbaugh et al., 2006, 2008). Zhang et al. suggest that increased energy harvest in obese individuals is related to hydrogen transfer between taxa as they observed a concurrent increase in both hydrogen-producing Prevotellaceae and hydrogen-utilizing methanogenic Archaea (Zhang et al., 2009).

Obesity results in a chronic state of low-level inflammation very distinct from classical inflammation (Gregor and Hotamisligil, 2011). This pattern includes moderate induction of inflammatory cytokines such as TNF-α, IL-1β, and CCL2, as well as an increase in mast cells, T cells, and macrophages (Gregor and Hotamisligil, 2011). An increase in *Bifidobacterium* spp. has also been shown to modulate inflammation in obese mice by increasing the production of glucagon-like peptide-2, which reduces intestinal permeability and thus reduces the translocation of lipopolysaccharides (Cani et al., 2009). Although the elucidation of the exact mechanisms responsible for obesity is still an open and complex problem, these studies demonstrate the link between an imbalanced gut microbiota and diseased states and suggest hypotheses to be tested in future research. The importance of the interaction between the microbiota and the immune system in obesity was demonstrated in a study with genetically modified mice lacking TLR5, which recognizes flagellin and is one of the major microbial receptors of the innate immune system (Vijay-Kumar et al., 2010). These mice develop characteristics of metabolic syndrome along with significant changes in their gut microbiota, and it is hypothesized that the alterations in the gut flora induce a low-grade inflammatory signaling that eventually results in the development of metabolic syndrome. Moreover, this obesity phenotype is transmissible to wild-type mouse simply by transferring the microbiota (Vijay-Kumar et al., 2010). A recent study, however, was unable to reproduce some of these results, suggesting colony-specific effects (Letran et al., 2011).

Crohn's Disease

Crohn's disease is a chronic gastrointestinal disorder with an unknown etiology characterized by an inflammatory response of the intestinal mucosa (Sartor, 2006). The development of Crohn's can only partly be described by genetics, with many of the more than 99 polymorphisms associated with the disease linked to the immune system and the recognition of microbes (Anderson et al., 2011). Studies of twins discordant for Crohn's suggest that an environmental aspect is required for the development of the disease (Halfvarson et al., 2006; Loftus, 2004). An influential study by Cadwell et al. sheds light on how the virome, genome, and microbiota might be interacting in the development of Crohn's disease (Cadwell et al., 2010). In this study, Cadwell et al. used mouse lines with a hypomorphic (HM) *Atg16L1* gene (Cadwell et al., 2008), a gene associated with susceptibility of developing Crohn's (Hampe et al., 2007; Rioux et al., 2007). It was found that the Paneth cells, epithelial cells linked with mucosal immunity, of *Atg16L1*^{HM} mice displayed abnormalities when infected with murine norovirus, but wild-type mice maintained normal Paneth cells despite infection. This suggested that it was the combination of a virus and a susceptible gene that resulted in Paneth cell abnormalities (Ouellette, 2006; Vaishnava et al., 2008). Thus, the gut microbiota, in addition to the virome and host genetics, were shown to play an important role in the etiology of a complex mammalian disease.

Connections between Autoimmune Disorders and the Microbiota

Further evidence for the importance of the microbiota in normal immune function comes from the correlation between autoimmune diseases and the microbiota. In mouse models of type 1 diabetes, it has been shown that the interaction of the gut microbiota with the innate immune system modifies predisposition toward developing diabetes (Wen et al., 2008). Compared with non-autoimmune individuals, children at high genetic risk for diabetes exhibit a distinct composition of the gut microbiota, with decreased diversity over time and higher relative abundances of *Bacteroides ovatus* and firmicute strain CO19 (Giongo et al., 2011). Early results in animal models of multiple sclerosis and rheumatoid arthritis also suggest a pronounced influence of the gut microbiota. In animal models, these autoimmune diseases do not develop in germ-free mice (Lee and Mazmanian, 2010). In the case of multiple sclerosis models, the disease phenotype is restored when germ-free mice are colonized by specific bacterial taxa (Lee and Mazmanian, 2010). These autoimmune diseases all result from inappropriate action of the adaptive immune system mediated by the gut microbiota.

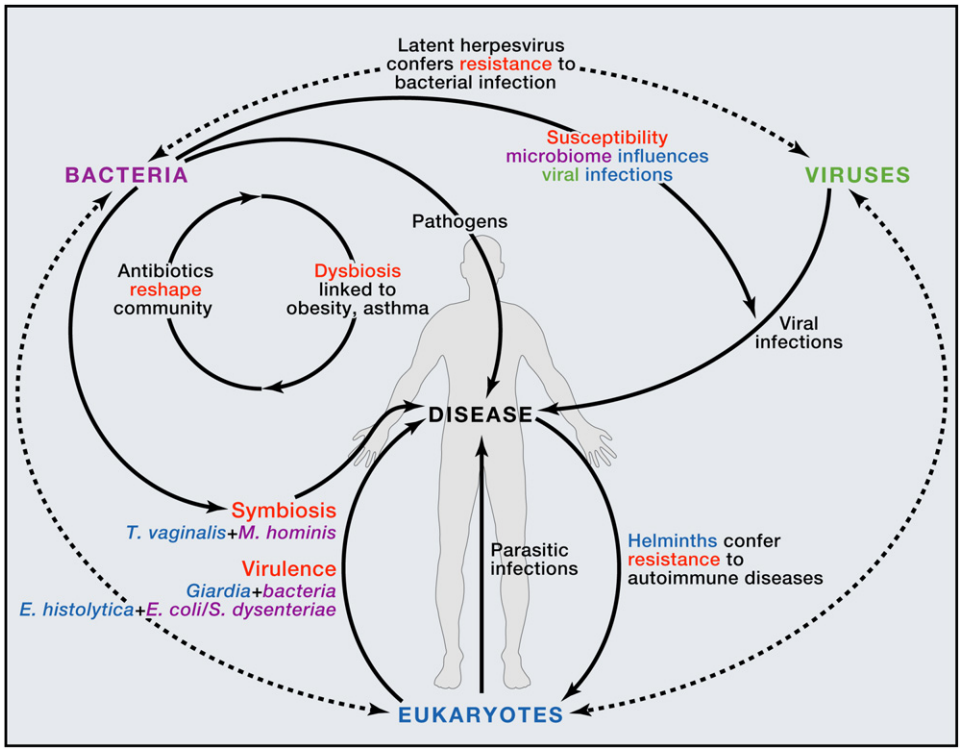

Figure 2. Effect of Interactions of Bacteria, Viruses, and Eukaryotes in Health and Disease
Diseases have been traditionally studied under a paradigm of "one microbe, one disease." However, a new understanding is emerging on how disease phenotypes are actually a result of complex interactions between bacteria, viruses, and eukaryotes, as well as their interactions with the host or with certain drugs. Virulence of some eukaryotes is, for instance, linked to the presence of certain bacteria, such as in the case of *E. histolytica* and *E. coli* or *S. dysenteriae*. The susceptibility of the host to viral infections is conditioned by the particular configuration of the microbiota, whereas herpesvirus infection can confer resistance to certain bacterial infections. Antibiotics can significantly reshape the composition of the microbiota. As a clear correlation has been observed between many diseases and dysbiosis, the widespread use of antibiotics may be linked to the dramatic increase observed in autoimmune diseases over the last years. Conversely, helminthes confer resistance to autoimmune diseases.

Introduction of bacterial polysaccharides from the commensal *Bacteroides fragilis* protects against the development of these autoimmune diseases (Lee and Mazmanian, 2010).

Complex Microbial Interactions in Disease

So far we have mostly described the effect of imbalances in the microbiota in health and some of the molecular mechanisms linking bacterial, eukaryotic, or viral communities with disease. However, complex diseases are rarely caused by a single factor, and understanding how different elements interact to induce a disease phenotype is crucial to facilitate prediction and treatment of such states (Figure 2). Recent studies have provided a number of examples of such complex, influential interactions. For example, although herpesvirus can cause acute disease, latent herpesvirus infection has been shown to protect mice against the bacterial pathogens *Listeria monocytogenes* and *Yersinia pestis* by stimulating increased levels of interferon-γ and tumor necrosis factor (Barton et al., 2007). The resulting basal level of immunity stimulation suggests that lifelong latency of herpesvirus might have been evolutionarily beneficial, resulting in a symbiotic host-virome relationship that promotes host health (Barton et al., 2007). Future therapies may thus involve the active modulation of viral and microbial communities in the context of genome susceptibility to combat disease states.

Helicobacter pylori provides a second example of how the interaction between members of the microbiota and the host can have a major impact in health. A Gram-negative pathogen linked with gastric ulcers and stomach cancer, *H. pylori* has been associated with humans for at least 58,000 years, although most of us carry it asymptomatically (Linz et al., 2007). Infection by *H. pylori* is intriguingly correlated with respiratory diseases such as chronic obstructive pulmonary disease (COPD) and pulmonary tuberculosis (Roussos et al., 2006). The evidence is, however, contradictory on the nature of this correlation: some studies show an increased risk of developing diseases in subjects infected by *H. pylori* (Roussos et al., 2002), but recent work by Perry and colleagues observed the opposite effect, where *H. pylori* confers protection against tuberculosis (Perry et al., 2010). In this study, *H. pylori* seropositive subjects with latent tuberculosis had higher tuberculosis antigen-induced IFN-γ responses and enhanced Th1-like responses than those not carrying *H. pylori*. Monkeys infected with *H. pylori* were less likely to develop tuberculosis than uninfected animals. Although the mechanism is unclear, infections early in life could result in differentiation of immature T cells to a Th1-like

phenotype, thus promoting a protective immune response. Alternatively, *H. pylori* could induce a continuous innate response from the host, providing incidental protection against other pathogens.

Consequences of Disrupted Host-Microbiota Interactions

In our modern environment, many people are not exposed to the microbiota of our evolutionary past. In the absence of appropriate microbial signals, the immune system does not develop normally (Round and Mazmanian, 2009). Autoimmune diseases in general, and allergies in particular, have significantly increased in developed countries over the last few years, which has been attributed to a burgeoning list of potential factors. The hygiene hypothesis postulates that lack of exposure to pathogenic and nonpathogenic microbial products early in life might result in an asthmatic phenotype due to an impaired development of the immune system (Strachan, 1989). Under this hypothesis, the fact that developing countries have lower rates of allergies could be due to larger family sizes, a larger percentage of the population living in rural environments with poor sanitary conditions, lower antibiotic use, and prevalence of helminths.

A potential mechanism that has been proposed to support the hygiene hypothesis is the counter-regulatory role of interleukin-10 (IL-10) (Wills-Karp et al., 2001). IL-10 is an anti-inflammatory cytokine that downregulates responses from both the innate and adaptive immune systems. Infection by microbial organisms results in the upregulation of IL-10, which subsequently suppresses inflammation and a predisposition to allergies. A reduced exposure early in life to infectious agents and human-resident mutualists could result in a weakened machinery for counter-regulation of inflammatory responses and therefore to an increase in the prevalence of allergies.

Although different genes can predispose individuals to allergic diseases (see, for instance, Moffatt et al., 2007 and references within), it is the interaction of these polymorphic genes with their environment that seems to be producing the most encouraging results. For instance, elevated serum IgE levels (a marker for atopy) have been associated with a single-nucleotide polymorphism (SNP) in the promoter region of *CD14*, a coreceptor for lipopolysaccharides (Vercelli, 2003). However, children carrying the polymorphism who have regular contact with pets have higher levels of serum IgE, whereas the opposite effect is observed for those in contact with stable animals (Eder et al., 2005). Parasites such as worms also induce the production of IgE, and it is suggested that the general absence of parasites in Western populations is a factor that contributes to the inappropriate immune response to allergens. In support of this hypothesis, there is anecdotal evidence that taking hookworm eggs reduces or eliminates allergic responses (Feary et al., 2011).

Evidence from *H. pylori* also points to consequences of disrupting the microbiota we have coevolved with over millennia (Blaser, 2011). Gastric colonization by *H. pylori* usually takes place within the first 10 years of life and can persist throughout life in the absence of antibiotics (Brown, 2000; Perry et al., 2006). Nearly all adults in developing countries harbor *H. pylori*, and this was likely true for humans in general for most of our evolutionary history. Interestingly, there is strong evidence linking the decreased prevalence of *H. pylori* in the Western world to increased antibiotic use and to the elevated rates of asthma and allergic disorders in this same population (Anderson, 2005; Banatvala et al., 1993). Moreover, increased prevalence of gastresophageal reflux disease (GERD), adenocarcinoma of the esophagus, and Barrett's esophagus has been linked to decreased prevalence of *cagA*$^+$ strains of *H. pylori* (reviewed in Atherton and Blaser, 2009). Overall, the strong link between *H. pylori* and modern diseases illustrates how the disruption of the human microbiota can have dire health consequences.

Interplay between Bacteria and Eukaryotes

The interplay between eukaryotes and bacteria in the gut is likely to be an important factor in understanding the variation in virulence that is observed across pathogenic taxa. Most diseases associated with eukaryotic parasites exhibit a range of pathogenicity in the human host, from severe symptoms or death to asymptomatic infections (e.g., Pritt and Clark, 2008). Some of this range may be due to variation in the microbial community of the host, in addition to host immune factors and variation between strains (Cox, 2001). The importance of such interactions has already been demonstrated for some species in the context of the overall bacterial community, though interactions between one eukaryote and one bacteria have received more attention thus far. The bacterial community in the gut is known to have an impact on the proliferation and pathogenicity of the *Giardia* parasite in mice and humans. For example, the presence of *Lactobacillus* slows the growth of *Giardia* (Müller and von Allmen, 2005). Community-wide differences in the microbiota result in isogenic strains of mice being either susceptible or resistant to pathogenic *Giardia* infections, and this difference is erased when the mice are treated with antibiotics (Müller and von Allmen, 2005). A further example of bacteria altering virulence comes from *Entamoeba histolytica* cultured with and without pathogenic strains of *Escherichia coli* and *Shigella dysenteriae* (Galván-Moroyoqui et al., 2008). *E. histolytica* lives in the lumen of the gut in 90% of cases and does not cause symptomatic infections, however the remaining 10% of infections are characterized by invasive amoebiasis (ameobic dysentery), and this fraction is the second leading cause of mortality due to eukaryotic infections after malaria. Thus, understanding the factors that result in epithelial invasion is crucial. *Entamoeba* was shown to be much more likely to become invasive and cause damage to epithelial cells in the presence of pathogenic *E. coli* and *S. dysenteriae* (Galván-Moroyoqui et al., 2008).

Some eukaryotic taxa have been shown to be intimately associated with bacteria in the form of endosymbioses, including *Trichomonas vaginalis* (Rappelli et al., 2001), *Giardia* (Nemanic et al., 1979), and *Entamoeba* (Ahmad, 1971). *Trichomonas vaginalis* is a major vaginal pathogen infecting 180 million people worldwide each year and causes vaginal inflammation as well as problems with pregnancy (Soper, 2004). In the case of *Trichomonas*, the bacterial endosymbiont is itself a pathogen, *Mycoplasma hominis* (Rappelli et al., 2001). This association leads to coinfection, and infected *Trichomonas* cells can transfer *Mycoplasma* directly to human epithelial cells (Rappelli et al.,

2001). Infection with *Trichomonas* also increases the likelihood of contracting other infections, including bacterial vaginosis and HIV (Soper, 2004), highlighting the importance of including eukaryotes in vaginal microbial studies. One study also hints that bacteria mediate the immune response to *Giardia* as only *Giardia* cells harboring bacterial endosymbionts are targeted and destroyed by Paneth cells, although the mechanism for this effect remains unclear (Müller and von Allmen, 2005).

Future Directions

Restoring Microbial Health

As we have seen in previous sections, disease states are often correlated with imbalances in the gut microbiota. Restoring a healthy microbial community by transplantation of a foreign gut microbiota has proven to be a valuable tool in the treatment of certain diseases (Bakken, 2009) and also allows the impact of the microbiota in determining phenotype to be tested. Transplant experiments that establish that the state can be recaptured by transmitting the microbiota can be used to further support the hypothesis that changes in the microbiota are a driving force in disease progression. For instance, obesity and several disease phenotypes induced by changes in host genetics cause dysbiosis in the gut. Remarkably, these phenotypes can be transferred to germ-free wild-type hosts simply by inoculating them with the microbiota from the diseased donors (Turnbaugh et al., 2006; Vijay-Kumar et al., 2010; Wen et al., 2008). Furthermore, transplantation from a healthy to a diseased individual can help in recovering microbial balance in the gut. Khoruts et al. showed how transplanting the microbial community from a healthy donor to a patient suffering from *Clostridium difficile*-associated disease (CDAD) significantly modified the bacterial composition in the patient (Khoruts et al., 2010). After 2 weeks, the microbiota of the recipient had dramatically changed from a Firmicutes- and Bacteroidetes-deficient configuration to a community highly similar to that of the donor, dominated by *Bacteroides* spp. This radical shift in the composition of the microbiota was also accompanied by the disappearance of the symptoms associated with CDAD in the patient.

The conditions for a successful transfer of an exogenous microbiota are, however, not yet well understood. The deep influence that antibiotics exert on the microbiota can be used to artificially manipulate the bacterial communities in the gut, in the hope that such manipulation will allow for a smoother transplantation of microbiota from a donor. Manichanh et al. tested whether the use of antibiotics prior to transplantation would facilitate the colonization process by reducing bacterial load (Manichanh et al., 2010). Somewhat counterintuitively, antibiotics did in fact not help increase the establishment of donor phylotypes, suggesting that the establishment of transplanted microbiota may be more difficult to achieve than initially thought. Antibiotic-manipulated mice models are attractive as an inexpensive substitute for germ-free animals, but additional work is required to determine whether they can play this role effectively. For example, uneven impacts of antibiotics on the gut microbiota may complicate the interpretation of studies that simulate germ-free mice by giving conventionally raised mice antibiotics. Determining how to facilitate transplantation, what donors are more compatible with the patient, or what risks might be involved in this procedure remains an open problem in this field.

Characterizing the Global Microbiota

Most studies of the microbiota so far have only characterized individuals under a Western diet (Peterson et al., 2009; Qin et al., 2010). Given the observed interpersonal variability in the composition of microbial communities, it is reasonable to assume that the microbiota of non-Western-diet populations will most certainly exhibit notable differences. For example, Japanese individuals are unique in harboring an enzyme acquired from a marine bacterium to help in the digestion of seaweed, which is prevalent in the Japanese diet (Hehemann et al., 2010). Differences in diet between children in Africa and Europe have also been shown to correlate with differences in the microbiota, with the African cohort being enriched in Bacteroidetes and depleted in Firmicutes, presumably to maximize energy uptake from their fiber-rich diet (De Filippo et al., 2010). It will therefore be important to characterize the full microbiota from geographically and socioeconomically diverse populations to establish the ''normal'' human microbiota. The global trend toward a Western-like diet, with its high intakes of red meat, high-fat food, and sugars, will likely result in a homogenization of the microbial communities harbored by different populations and perhaps the loss of ''endangered microbes'' that could play important roles in bacteriotherapy (Blaser, 2011).

Events that Alter an Individual's Microbiota

We also argue that there is a need for more complete time-series analysis of microbial communities at higher resolution and longer time ranges to assess normal variability and variation that is associated with disturbance events such as antibiotic use or diet shifts. Detailed time series of healthy individuals should yield better understanding of both the normal levels of variability through time and what amounts of change the microbiota can withstand before becoming diseased. These studies may include seemingly benign events such as cohabitation (does it change your microbiota, and if so does it affect men or women more significantly?) or fine-scale characterization to determine whether there is a ''morning'' versus ''night'' microbiota to inform the suggested timing of therapies.

Studies before and after events that perturb the microbiota, such as diet shifts, antibiotic use, surgery, drug treatments, or transplantations, will be key to assessing the impact of such events.

As discussed above, antibiotics have a noticeable, lasting effect in the composition of microbial communities (Dethlefsen et al., 2008; Jernberg et al., 2007; Sullivan et al., 2001). The microbiome can also interact with drugs and influence the way our bodies perceive drugs (reviewed in Gonzalez et al., 2011). Additionally, given our currently incomplete understanding of how the interactions of the microbiota can result in the development of diseases, it will be increasingly important to characterize all aspects of human-associated microbial communities, including multiple taxa and their dynamics, in disease phenotypes.

Conclusion

Taken together, the findings we have reviewed suggest that in order to further advance our understanding of health and

disease, we will require an improved characterization of the variability in the microbiota, a better understanding of how such variability can result in similar or different functional profiles, and more integrative studies that take into account the interaction between the microbiota, the host, and the environment to produce a phenotype. An increasing variety of disease states and disorders are being found to correlate with the host microbiota (recently reviewed in Virgin and Todd, 2011), including susceptibility to influenza (Ichinohe et al., 2011), retrovirus transmission (Kane et al., 2011), colon cancer (Kostic et al., 2011), autoimmune demyelination (Kostic et al., 2011), and even behavior (Heijtz et al., 2011; Vijay-Kumar et al., 2010). Large-scale sequencing projects such as the Human Microbiome Project (Peterson et al., 2009; Turnbaugh et al., 2007) and the Earth Microbiome Project (Gilbert et al., 2010) will ultimately be critical for providing a unified and all-encompassing view to better understand the link between the microbiota and health. Transforming the vast amounts of data that these projects will generate into useful and applicable knowledge requires novel approaches such as machine learning. The application of these methods will hopefully more accurately predict disease states and appropriate therapies by making use of larger numbers of samples across populations and over time (Knights et al., 2011).

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