

Nature vs. Nurture: The Gut Microbiome and Genetics in the Development of Gastrointestinal Disease

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Abstract

Emerging investigation into the physiologic role of the gut microbiome continues to yield new evidence for significant microbial influence across numerous gastrointestinal diseases. Integrating this new knowledge with the existing understanding of disease pathogenesis will be a critically important aspect of medicine in the near future. Furthermore, the gut microbiome and host genetics likely share significant functional overlap, the extent of which we are only beginning to understand. For instance, evidence suggests that starting even before birth the gut microbiome influences immune system development. In the early years of life, the gut microbiome also functions to establish proper metabolic functions of the gastrointestinal tract. Alterations of the normal gut microbiome can even lead to disease development in infancy and early childhood. Later in life, dysbiosis has been shown to be a commonality in inflammatory bowel disease, potentially serving an etiologic role as well. More importantly, there appears to be a significant interaction between the gut microbiome and certain genetic polymorphisms in inflammatory bowel disease, which may help to identify future therapeutic targets. Lastly, the scope of the gut microbiome continues to expand as we discover other microbial inhabitants such as archaea, fungi, and viruses, which all likely influence both normal gastrointestinal function and disease pathogenesis.

Keywords: Gut microbiome; Immune development; Microbial colonization; Inflammatory bowel disease; Archaea; Fungal microbiota; Viral microbio

Introduction

The gut microbiome is a dynamic facet of the gastrointestinal tract, representing a truly diverse ecosystem with between 500 to 1000 unique bacterial species; at the same time, the gut microbiome also represents a commonality with at least 160 species shared among individuals, predominately from the two main bacterial phyla, Bacteroidetes and Firmicutes [1]. This encompassing scope of the gut microbiome makes it difficult to ascertain its true importance across its numerous physiologic roles. In particular, when considering the essential role of genetics in disease pathogenesis, where does the gut microbiome fit in? A critical notion when considering the importance of the gut microbiome in comparison to genetics is an understanding of the role of the gut microbiome in the development of the immune system and the pathogenesis of certain early childhood diseases. It is increasingly apparent that the gut microbiome is a critical modulator of immune responses to bacterial products present within the gastrointestinal tract. Fluctuations in the relative concentrations of bacterial species can result in alterations in normal immune and inflammatory signaling, leading to an exacerbation of particular disease states. It is important to understand that these changes are often times not transient; rather, there is increasing evidence to implicate a number of environmental and dietary factors starting at birth that influence not only the composition of an individual's gut microbiome, but also impact their susceptibility to certain diseases both during childhood and in adult life [2]. This article aims to analyze the current literature surrounding the development of the microbiome

from birth through infancy and explore the specific microbial alterations associated with the development of several disease states. In particular, it will examine the overlap between genetic polymorphism and the gut microbiome in the etiology and pathogenesis of inflammatory bowel disease. Lastly, the emerging role of the archaea, fungi, and viruses in the gut microbiome will be discussed to highlight the vast potentially unexplored sources of influence from the gut microbiome on gastrointestinal and immune function.

The Role of the Gut Microbiome in Immune System Development

When does our first exposure to microbial colonization begin? The conventional approach would state that we are first exposed to the outside world at birth, when we immediately begin microbial colonization. Our dominant microbes would therefore depend on the route of delivery; this was supported by evidence from 16S rRNA sequencing of rectal swabs from newborns which showed that vaginal deliveries resembled the vaginal flora of *Lactobacillus*, *Prevotella*, or *Sneathia* and caesarean-section deliveries were dominated by skin flora such as *Staphylococcus*, *Cornebacterium*, and *Propionibacterium* [3]. Despite this, numerous other studies have found that the composition of the meconium, not the mode of delivery, is the driving influence for microbial colonization [2,4,5]. In fact, one set of authors highlighted that the study supporting the influential role of delivery method used rectal swabs at birth, which mimic the mother's flora, rather than using the first intestinal discharge of the newborn that was entirely representative of their microflora [6]. Further, it was even noted that the similarities in the composition of the microbiota and meconium could persist into several months of life [6]. The important take away from these studies should be the strong possibility of *in utero* microbial

colonization of the digestive tract. Supporting this concept is experimental evidence noting the detection of microorganisms in amniotic fluid, fetal membranes, umbilical cord, placenta, and meconium, all of which would support colonization prior to birth [6]. This was also shown experimentally by inoculating pregnant mice with a labeled *Enterococcus faecium* strain, which was detected in the meconium of offspring on the day prior to labor, but was not detectable in controls [7]. The mechanism of the colonization would likely then be due to increased bacterial translocation that occurs during late pregnancy. While this differs from the conventional approach, the notion of *in utero* colonization supports the complex immune and inflammatory interactions mediated by the gut microbiome, which results from localized levels of bacterial products and intestinal permeability. Although much remains to be clarified about the mechanisms of the initial microbial colonization, it appears evident that it is not solely dependent on the route of delivery, but also closely related to *in utero* factors. Continued research utilizing 16S rRNA technologies will undoubtedly help to expand our knowledge about the origin and very foundation of our gut microbiome.

Regardless of the mechanism of initial colonization, the gut microbiome undergoes marked remodeling over the next several months. The initial changes begin during the first weeks of life, when the predominating bacteria from birth begin to be overshadowed by bacteria from one of a few taxa, including *Escherichia*, *Clostridium*, *Bacteroides*, or *Bifidobacterium* [8,9]. This composition of the gut microbiome then changes over the remainder of the first year of life, heavily influenced by dietary intake. For example, breast-fed infants often have a predominance of *Bifidobacterium* and *Lactobacilli* in contrast to formula fed infants who had an observable increase in *Clostridium difficile* [8-11]. Following the introduction of solid foods into the diet, the composition of the gut microbiome once again changes, beginning to resemble the adult microbiome, which is dominated by *Bacteroidetes* and *Firmicutes* [8,9]. It is not until 3 years of age that the gut microbiome is finally established [12]. Importantly, these changes in the gut microbiome from birth through infancy reflect optimization of the gut microbiome for the metabolism of carbohydrates, vitamin biosynthesis, and xenobiotic degradation [6]. In this respect, it is quite phenomenal the gut microbiome exhibits significant flexibility, which is potentially an adaptive mechanism for the breast milk compositional changes from colostrum to mature milk and also the influence of maternal diet on breast milk composition. While it has yet to be studied, it is possible that the adaptability of the gut microbiome in these early months may be part of the mechanism behind some of the influential benefits of breast milk, ensuring optimal nutrient extraction and mediating appropriate immune responses to the bacterial products and immunoglobulins from breast milk.

Failure of appropriate microbial colonization of during early infancy can ultimately result in deleterious effects, which manifest as allergies, autoimmune diseases, or atopic conditions. For example, increased levels of *Clostridium coccooides* have been found in infants with an allergy to cow's milk protein [13]. Other food allergies and also atopy have been linked to alterations in the concentrations of butyrate producing species [13,14]. In addition to food allergies, increased *E. coli* and enteric bacteria are associated with an increased risk for eczema [6,15,16]. Interestingly, maternal smoking during pregnancy is not only associated with eczema, but also increased the levels of enteric bacteria, suggesting that this may be a potential mechanism explaining some of this increased risk for eczema [2]. Perhaps the most well studied bacterial alteration of infancy is a decrease in *Bifidobacterium* and *Lactobacilli*, which is associated with an increased risk of food

allergies and atopy. Despite supporting evidence from studies including the use of bacterial cultures, fluorescence in-situ hybridization, and 16S rDNA sequencing, two major prospective trials offer conflicting results [15,17-22]. Interestingly, only the studies carried out in Sweden or Estonia found a protective role of *Lactobacilli* against the development of atopy, suggesting that potential genetic or environmental factors, such as variability in the bacterial strain, may play an influential role in determining the microbiome of early infancy [6].

The relationship of these bacterial alterations to the development of allergies and atopic conditions appears to be correlated with the role of the developing immune system. Under normal conditions, regulatory T cells (Treg) have an active role in suppressing inflammatory responses and controlling the secretion of certain immunoglobulins [23-26]. In addition these Treg cells can influence the differentiation of naïve T cells into specific helper T cells (Th), which each have different cytokines profiles and can alter the activity of other Th cells themselves [6]. The impact of the gut microbiome on the immune system becomes apparent when considering that certain bacterial species, including *Lactobacillus*, *Bifidobacterium*, *Bacteroides*, *Clostridium*, and *Streptococcus* have all been shown to induce Treg cells. Further, excess activation of Th1 or Th2 cells can result in chronic inflammatory, autoimmune, or allergic disease suggesting that alterations in the gut microbiome can influence the development of both the immune system and disease processes [6,27]. Additionally, the gut microbiome exerts an influence over the levels of non-secretory IgA, which impacts pathogen and allergen exclusion in the intestinal epithelia, mucus, and lumen [6,21,28,29]. This is in contrast, however, to the level of secretory IgA, which is positively associated with increased levels of *B. infantis* from breastfeeding [30]. Whereas the nonsecretory IgA is involved in the development of immunotolerance, secretory IgA and the associated increase in *B. infantis* may promote the expression of tight-junction proteins and confer an anti-inflammatory benefit [31]. The increase in *Bifidobacterium* seen in breast-feeding may be due to the prebiotic-like effects of human milk oligosaccharides (HMOs), which are fermented in the colon to release short-chain fatty acids, thereby altering the colonic microenvironment and promoting the growth of particular bacteria such as *Bifidobacterium* [32]. In fact, attempts to mimic these prebiotic effects of HMOs with plant-derived or synthetic oligosaccharide supplemented formula significantly reduced the incidence of allergic manifestations and infections during the first two years of life [33,34]; furthermore, this prebiotic supplemented formula was generally well tolerated in a systematic review of randomized control trials [35]. While these prebiotic supplements do improve outcomes for formula fed infants, there are still not capable of matching the benefits of HMOs from breast milk; this may partially be related to the ability of HMOs to preferentially select for the growth of *B. infantis* and *B. bifidum*, in contrast to a much wider species array seen with the plant-derived or synthetic oligosaccharides [36]. Regardless, the role of the gut microbiome in the early development of immunity highlights the importance of environmental and dietary factors in early infancy. Furthermore, the gut microbiome represents a potential target for therapeutic interventions with antibiotic, prebiotics, or probiotics. This is especially true given that alterations in the gut microbiome during childhood can manifest as chronic adult diseases; therefore identifying and correcting these imbalances early in life may have a considerable impact on long-term health.

As mentioned previously, the gut microbiome typically begins to resemble that of an adult around three years of age [12]. Any alteration

in the established gut microbiome, such as the administration of antibiotics, has the potential to decrease the microbial diversity and increase the risk for certain diseases. Given the wide spread use of antibiotics in the outpatient pediatric population, it is important to discuss some of the association between childhood antibiotic exposure and alterations of the gut microbiome. Antibiotics can induce an atopy-prone state by promoting an increase in the Th2 phenotype [37,38]. Even particular antibiotics show certain associations in relation to the gut microbiome, such as metronidazole, which decreases mucin secretion and intestinal barrier integrity by reducing the expression of the MUC2 gene [39]. Other examples include an increased risk for Crohn's disease among children treated with antibiotics during the first five years of life [40]. Asthma shows a dose-dependent association with early life exposure to antibiotics, in particular for broad-spectrum antibiotics [41-44]. Even prior to the establishment of the adult microbiome, a diversified gut microbiome is also essential for normal gastrointestinal function; reduced microbial diversity has been associated younger gestational age, increased duration of antibiotic therapy, and parenteral nutrition during infancy [45]. This reduced microbial diversity can be clinically significant and is associated with impaired barrier integrity, which increases bacterial translocation and the subsequent risk for catastrophic outcomes such as neonatal sepsis or necrotizing enterocolitis [46-48]. Taken together, these associations highlight that the gut microbiome is influential in the development of childhood disease, including allergies, autoimmune disease, and atopy. While there are countless other environmental and genetic factors that may contribute to disease pathogenesis, further understanding of the gut microbiome may help aid in discovering mechanisms to prevent or treat certain diseases during infancy and early childhood.

Genetic and Microbial Interactions in Inflammatory Bowel Disease

The gut microbiome and the host immune system have a dynamic relationship, which is constantly adapting to changes, undoubtedly based on mutualistic relationships that have developed over several millennia. Until recently, these relationships were relatively unstrained by external influences; however, modern medicine and societal changes have introduced a plethora of new medications and dietary changes, which are impacting the gut microbiome. Importantly, these influences may be uncoupling these evolved mutualistic relationships, predisposing the host to a variety of disease states [49]. Among these, inflammatory bowel disease (IBD) has shown particular susceptibility to alterations in the gut microbiome. Ultimately, it appears quite evident that patients with IBD are prone to dysbiosis, an effect that may influence disease development and progression. This section aims to further explore the role of the gut microbiome and host genetics in the etiology of IBD.

Overall, inflammatory bowel disease is characterized by an unstable microbiome. Dysbiosis has been illustrated in both patients with Crohn's disease (CD) and ulcerative colitis (UC) during remission [50-52]. Even standard IBD therapies can significantly alter the gut microbiome, evidenced by nearly a 50% reduction in the bacterial load with mesalazine (5-aminosalicylic acid, mesalazine) administration and also an amplification of the dysbiosis with antibiotics [52,53]. Additionally, the level of dysbiosis correlated with disease severity when using the Pediatric Crohn's Disease Index [53]. When directly taking mucosal samples, IBD patients also appear to have increased mucosa-associated microbiota [54,55]. Complicating matters, it is

difficult to interpret whether the observed changes in the gut microbiome are causative changes, the result of inflammation, or due to genetic or environmental causes. There is contrasting evidence when comparing microbial alterations between IBD patients and their relatives. One study found no consistency between microbial changes in UC patients when compared to their unaffected twin, whereas another reported decreases in *Faecalibacterium prausnitzii* in both UC patients and their first degree relatives [56,57]. Since inter-individual difference are much greater than inter-disease differences, it is difficult to establish common microbial alterations that may be used to characterize a particular disease [54,58]. Still, a number of studies investigating the gut microbiome in IBD have consistently found decreased microbial diversity, which is associated with some specific bacterial alteration [54]. A common alteration seen in IBD is a decrease in Firmicutes, in particular *Faecalibacterium prausnitzii*, and an increase in Proteobacteria [58-63]. While there have been reported alterations among other major bacteria such as *Enterobacteriaceae*, *Bacteroides*, *Bifidobacteria*, *Lactobacillus*, these findings have not been consistent. This may be potentially related to variations in sample source, location, or method as well as patient factors such as age, diet, smoking history, and disease severity [54,64,65]. Overall, it appears evident that dysbiosis is a common feature of IBD; however, it is unclear whether this dysbiosis is the result of inflammation, genetics, or environmental factors.

Even though there are significant phenotypic differences between them, CD and UC share 30-40% of the genetic risk loci [66,67]. Among these genetic loci, however, there is also significant variation, as only 14% of the total phenotypic variations of CD can be explained by these genetic loci, suggesting a number of other influences driving disease development [68,69]. In fact, both diseases are also significantly influenced by environmental factors, although the contribution may be greater in UC than CD [70]. In total, 21 genetic loci have been identified for conferring an increased risk of developing CD, with the majority of genes involved with the signal transduction in immune function [71]. For example CD patients have shown increased expression of a tumor necrosis factor ligand TNFSF15, which is a co-stimulator of T cells and also causes proinflammatory cytokine production. The TNFSF15 ligand shares overlap in receptor function with the closely related TNFSF14, which functions to activate STAT3 signaling in response to pathogenic bacteria [72]. Furthermore, STAT3 is an important effector of T cell differentiation through the binding of the interleukin (IL)-23 receptor and the RAR-related orphan receptor C (RORC), both of which are IBD risk alleles. Given the increased expression of TNFSF15 in the gut of IBD patients, this highlights yet another area when alterations of the gut microbiome may act in conjunction with genetic factors to influence disease. In particular, the genetic loci involved in CD appear to affect immune signaling, which may be further affected by changes in the gut microbiome.

Importantly, when looking at dysbiosis in CD patients, it was found that dysbiosis was associated with the NOD2 gene status [73]. NOD2 functions as an intracellular receptor for muramyl dipeptide (MDP), a component of gram-positive cell walls, to induce the release of antimicrobial peptides (AMPs) from Paneth cells and also to stimulate the production of anti-inflammatory cytokines, namely (IL)-10 [54]. Other functions of NOD2 include activation from the binding of viral ssRNA to increase expression of interferon (INF)- β and also modulation of Toll-like receptor (TLR) signaling [74]. There are nearly 60 variants of NOD2, with 3 polymorphisms involved in 27% of CD, primarily in patients with ileal disease [71]. NOD2 deficiency is associated with decreased production of both AMPs from Paneth cells

and also IL-10 [75,76]. IL-10 production also shows an association with Mgl-1, a macrophage galactose-type C lectin, which is a marker of alternatively activated macrophages and that when bound by *Lactobacillus* or *Streptococcus* shows increased expression of IL-10; experimentally, Mgl-1 deficient mice show a more severe phenotype of colitis compared to wild type mice, along with decreased IL-10 levels [77]. Specific changes seen with NOD2 deficiency in animal studies include an increase in fecal-associated Bacteroidaceae along with an overall increased bacterial load in feces and the terminal ileum [78-80]. In human studies, NOD2 mutations are associated with increased *Bacteroidetes* and *Firmicutes* [79]. A potential explanation for this finding is the regulation of β -defensin 2 by NOD2, the expression of which is inducible by the presence of commensal bacteria [69,81]. Still, other animal studies have suggested these microbial changes seen in NOD2-deficient mice are dependent on housing conditions, as similar changes were noted in wild type mice when cohoused with the NOD2-deficient mice [82,83]. Clearly, there is plentiful evidence to suggest a relationship between the gut microbiome and NOD2; however, more research will be needed to further determine the extent of this relationship and its importance.

Another of the major polymorphisms that has shown increased susceptibility to CD is the ATG16L1 gene, which is involved in the regulation of autophagy. Autophagy is an essential cellular process for the degradation and recycling of proteins during periods of starvation [54]. ATG16L1 is also critical for mediating autophagosome formation in response to bacterial sensing by NOD1 and NOD2. Importantly, ATG16L1 and NOD2 share overlap in their function. When NOD2 is activated by MDP, it leads to ATG16L1 formation of autophagic vacuoles in dendritic cells and epithelial cells [84,85]. Therefore, NOD2 variants in addition to ATG16L1 polymorphisms can both lead to impaired autophagy. Among CD patients with NOD2 or ATG16L1 mutations, there also appears to be an increased frequency of abnormalities in Paneth cell size, distribution, and number of AMP-containing granules [86]. Two human studies have both shown that polymorphisms in ATG16L1 are associated with shifts in the composition of the gut microbiome [87,88]. Overall, the importance of genetic regulation appears to act in conjunction with the gut microbiome in respect to NOD2 and ATG16L1; certainly, future investigation will help to clarify the impact of these relationships and potentially reveal practical clinical applications.

Another aspect of disease pathogenesis related to the gut microbiome in IBD is maintenance of the intestinal barrier integrity. As discussed in other chapters of this book, the epithelial barrier is of critical importance in controlling the levels of pathogen exposure and immune system activation within the gut [46]. The H antigen is an oligosaccharide whose synthesis is regulated by the fucosyltransferase 2 (FUT2) gene and which acts as both an attachment site and a carbon source for intestinal bacteria [69]. Loss of function mutation to the FUT2 gene results in increased susceptibility to CD along with altered intestinal microbiota composition [89]. In particular, it was found that fecal samples showed decreased Bifidobacterial diversity and abundance when compared to controls [90]. This highlights yet another relationship between the overlap of the gut microbiome and host genetics that may impact disease development or progression in IBD.

Oxidative stress may also play a role in mediating inflammation in IBD patients, as metagenomic analysis has shown a decrease in genes for carbohydrate and amino acid metabolism with a corresponding increase in oxidative stress pathways [91]. This is an important

mechanism of inflammation in IBD, as the accumulation of unfolded proteins within the endoplasmic reticulum can induce cellular stress and the unfolded protein response (UPR) [74]. Three major pathways have been identified with the UPR responses; however, one gene in particular, XBP1, appears to be genetically associated with both UC and CD [92,93]. This is supported by evidence showing spontaneous small intestinal inflammation with crypt abscesses, neutrophil infiltration, and ulcerations in XBP1 deficient mice [93]. Additionally, these mice showed marked pro-inflammatory hyper-reactivity of intestinal epithelial cells towards microbial and cytokine stimuli, resulting in impaired handling of bacteria [93]. Furthermore, UPR signaling pathways interact with Toll-like receptors (TLRs) 3 and 4 to modulate immune signaling [74]. Importantly, the activation of TLRs by bacterial products has been implicated in a variety of metabolic changes that are associated with microbial dysbiosis [94]. Overall, it appears evident that there is an interaction between genetic regulation of UPR signaling and the host-microbial interactions on the epithelial surface of the intestine [74].

Another potential microbial influence in the IBD is the role of bacterial by-products, such as butyrate and hydrogen sulfide, in the induction of intestinal inflammation. Butyrate is responsible for a number of physiologic roles within the gastrointestinal tract, including maintenance of intestinal barrier integrity, regulation of epigenetic gene expression, and mediation of anti-inflammatory responses to oxidative stress [95-97]. Alterations of butyrate producing species have been noted in both UC and CD patients with somewhat differing roles. Among UC patients, there is evidence for ulcer invasion by and increased concentrations of the butyrate producing species, *Fusobacterium varium* [98]. This potential etiologic role of *Fusobacterium varium* is supported by evidence suggesting efficacy from two weeks of combination therapy with amoxicillin, tetracycline, and metronidazole in UC patients for eradication of the bacteria [99]. In contrast, CD patients have shown a consistent decrease in *Faecalibacterium prausnitzii*, another predominant butyrate producer [65]. As a result, the decreased concentrations of butyrate may promote localized inflammation within the gut, furthering disease progression. Inflammation may also be induced hydrogen sulfide, which is a product of sulfate-reducing bacteria (SRB) that is toxic to intestinal epithelial cells [54]. These SRB have been found to be increased in some UC patients, suggesting another microbial influence in IBD. While more research will be needed to determine the significance of the butyrate producing species and SRB, at present they highlight another relationship between the host and the gut microbiome in IBD.

Overall, there is increasing evidence to highlight the role of the gut microbiome and dysbiosis in the etiology of IBD. While specific bacterial alterations may not be reproducible, there is still evidence for alteration of butyrate producing species and a reduced bacterial diversity. Additionally, there are a number of genetic factors, including polymorphisms in the NOD2, ATG16L1, and FUT2 genes, which impact disease pathogenesis in IBD and also exhibit interactions with the gut microbiome. Other effects of the gut microbiome on intestinal barrier integrity, oxidative stress pathways, and the production of bacterial products also likely influence the development of IBD. Given the multifactorial etiology of IBD, continued research on the gut microbiome will likely be of critical importance to advance our understanding of these disease processes.

Expanding the Microbial Spectrum: Archaea, Fungi, and Viruses

While the majority of the literature on the gut microbiome focuses on the role of bacteria, there are a number of other microbial inhabitants within the gut that also play critical physiologic roles. These microorganisms have long been present; however, we have been limited in our ability to study them due to technologic limitations. Fortunately, recent advances have expanded our understanding of these microorganisms and their complex relationship with the gut microbiome. For this reason, highlighting some of the recent research on archaea, fungi, and viruses within the gut microbiome is critical for establishing a comprehensive perspective on the impact of the gut microbiome in health and disease.

Archaea

Archaea are unicellular organisms most commonly known for their ability to survive in extreme environmental conditions, yet they also represent active members of the human microbiome. While there are a number of archaea present in humans, the dominant archaea species is *Methanobrevibacter smithii*, which plays a critical role in the regulation of methanogenesis within the digestive tract [100]. More specifically, *M. smithii* predominately controls hydrogenotrophic methanogenesis, during which it consumes hydrogen gas to reduce carbon dioxide; in addition to reducing the partial pressure of carbon dioxide gas present within the colon, this optimizes fermentation for a number of bacterial species present within the gut [100]. *M. smithii* can also produce a variety of adhesion-like proteins with substrate-related regulation, allowing it survive in a number of specialized microenvironments within the gut [101,102]. Alteration of metabolic homeostasis by archaea has also been cited as a potential mechanism increasing the risk for systemic disease. Notably, *M. smithii* can promote increased calorie intake from the diet by influencing the metabolism of *B. thetaiotaomicron*, which increases lipogenesis and host fat content [103]. Another archaea, *M. stadtmanae*, increases levels of tumor necrosis factor (TNF) in vitro and may influence inflammation in IBD. This is evidenced by increased immunoglobulin G (IgG) responses to *M. stadtmanae* in IBD patients [104]. These archaea may also be influential in colorectal cancer (CRC) where 80% of CRC patients are methane producers and methane production increases with the severity of the cancer; however, it remains to be seen if these archaea have any causative role or whether they are merely better suited for the harsher tumor microenvironment than normal intestinal flora and subsequently dominate the microbiome in this setting [105,106]. Undoubtedly, more research will be required before any definitive conclusions can be made on the role of archaea, as there are conflicting results from studies on colorectal cancer, obesity, inflammation, and irritable bowel syndrome [103]. Still, archaea are an influential population within the gut microbiome, which interact with the gut microbiome to influence disease outcomes in conjugation with a variety of environmental and genetic factors.

Fungi

The role of the fungal microbiota is poorly understood, largely due to their relatively low abundance and the difficulty in culturing these organisms. Of the previous attempts to characterize the fungal microbiome, only a small fraction of the species could be identified from intestinal samples and an accurate representation is therefore quite limited. Despite this, the advent of next-generation DNA

sequencing techniques has also greatly expanded our ability to study the fungal microbiome. In particular, attention has been focused on the potential role of fungi in the development of colorectal adenomas. Luan et al. compared the microbial composition between colorectal adenomas and the adjacent normal tissue of 27 patients by DNA sequencing [107]. Their results showed that the adenomas had significantly decreased fungal diversity compared to the adjacent normal tissue; however, no significant differences were seen between the adenoma and adjacent biopsy samples at the phylum, genus, or species level by t-test [107]. The decreased fungal diversity within the tumor microenvironment is likely secondary to changes in the tumor microenvironment that make it less suitable for growth. Similar to the increased concentrations of *M. smithii* seen in some colorectal cancers, this too may highlight a role for non-bacterial species in more extreme physiologic conditions, such as the tumor microenvironment. Further, certain fungal species changes were noted with tumor progression and suggest that particular fungi may have adaptive mechanisms for changes in the environment [107]. Although the clinical significance of these fungi is yet to be determined, it is evident that fungi are present and active within our microbiome. Our advances in sequencing technologies will only help to further our understanding of these organisms and their potential impact on disease.

Viruses

Another emerging focus within the study of the gut microbiome is that of the enteric virome. Similar to fungi, viruses are difficult to identify and use in research studies. Still, recent research has highlighted the role of these viruses and their interactions with the other members of the gut microbiome and host genetics. For example, experimental mice with mutation causing decreased expression of the autophagy gene ATG16L1 have been shown to have increased susceptibility to inflammatory bowel disease (IBD) [108]. Furthermore, this ATG16L1 mutation results in structural and functional abnormalities within paneth cells that are similar to those seen in IBD patients homozygous for ATG16L1 allele [109]. The development of these paneth cell abnormalities appears dependent on the presence of both an ATG16L1 allele and also infection with a murine norovirus (MNV). Studies done in ATG16L1 mutant mice placed in a MNV-free facility showed that these mice are resistant to the development of these paneth cell abnormalities [108]. Further, both MNV-infected control mice and uninfected ATG16L1 mutant mice are resistant to the development of Paneth cell abnormalities with chemically induced colitis [110]. Interestingly, infecting either germ-free or antibiotic treated mice with MNV leads to partial or complete reversal of certain abnormalities in intestinal and lymphoid function, which included thin villi, small crypts, paneth cell defects, reduced CD4 and CD8 cells, reduced IgA in intestine, reduced IgG in serum, and decreased expression of genes associated with immune system development [108,111]. Further research of the gut viral biome is essential for understanding the role of these viruses and their interactions with the host immune system and gut microbiome. It remains to be seen whether the viruses present within the gut serve a unique role or simply restore function to the commensal bacteria. Regardless, it appears evident these viruses likely impact disease in relation to other environmental and genetic factors.

Conclusion

The recent focus on the gut microbiome has illuminated the vast physiologic importance of the microbial community in maintenance of

normal gastrointestinal function and also the potential for disease development with alterations in the gut microbiome. In addition, host genetics are clearly of critical importance influencing disease pathogenesis and immune function. Emerging evidence suggests the gut microbiome is also important in the early development of the immune system, demonstrating dynamic changes from birth through infancy and into early childhood. Furthermore, alteration of the normal gut microbiome during this period can manifest in disease development, ranging from milder condition such as allergies and atopy to severe complications such as neonatal sepsis and necrotizing enterocolitis [6]. Later on in life, gut microbial alterations appear to act in concert with genetic polymorphisms in inflammatory bowel disease to influence disease development and progression [54]. Continued understanding of these complex relationships may help to better understand disease etiology and even develop potential therapeutic targets directed at this dysbiosis. Finally, the role of the gut microbiome continues to expand beyond simply the influence of bacteria. Recent evidence implicates archaea, fungi, and viruses as other inhabitants of the gut microbiome, which also interact to influence disease outcomes [103,107,108]. Overall, it is clear the gut microbiome and genetics are not separate entities; rather, they act in conjunction to affect a plethora of physiologic functions within the gut and also demonstrate how much remains to be explored.

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