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Role of the gut microbiota in defining human health

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Abstract

The human superorganism is a conglomerate of mammalian and microbial cells, with the latter estimated to outnumber the former by ten to one and the microbial genetic repertoire (microbiome) to be approximately 100-times greater than that of the human host. Given the ability of the immune response to rapidly counter infectious agents, it is striking that such a large density of microbes can exist in a state of synergy within the human host. This is particularly true of the distal gastrointestinal (GI) tract, which houses up to 1000 distinct bacterial species and an estimated excess of 1×10^{14} microorganisms. An ever-increasing body of evidence implicates the GI microbiota in defining states of health and disease. Here, we review the literature in adult and pediatric GI microbiome studies, the emerging links between microbial community structure, function, infection and disease, and the approaches to manipulate this crucial ecosystem to improve host health.

Keywords

gastrointestinal tract; inflammation; microneme; probiotics

Role of microbial community composition in defining host health

The application of culture-independent tools has dramatically improved our ability to interrogate the vast diversity of unculturable or fastidious microbial species present in disparate

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environments and has led to significant advances in our understanding of ecosystem functioning [1–17]. Many of the tools developed for environmental microbial studies have recently been applied to human samples, providing a more comprehensive view of our microbial inhabitants in a number of discreet host niches, including the respiratory, gastrointestinal (GI) and urogenital tracts [7,18,19–24]. Recent studies have demonstrated that bacterial community composition is dramatically altered in diseases such as obesity and periodontal disease, with healthy subjects typically exhibiting distinct, diverse and temporally stable bacterial consortia at these sites when compared with patients displaying disease symptoms [6,25,26,27–29]. A number of studies have also demonstrated that bacterial community structure plays a key role in defining its functionality; compared with lean individuals, obese subjects exhibit a dramatic tenfold shift in the ratio of Firmicutes to Bacteroidetes (from 3:1 to 35:1), two of the major phyla present in the human GI tract [7]. This altered community structure is associated with a shift in function, resulting in increased energy harvest from ingested food; unexpended excess energy is deposited as adipose tissue [17]. Diet is a complex confounding factor in such studies [30,31] in that it can dramatically impact the composition of the gut microbial community [31]. A high-fat diet has been associated with an increase in Firmicutes and Proteobacteria and a concomitant decrease in Bacteroidetes in both wild-type mice and in isogenic resistin-like molecule-β animals that are resistant to high-fat-induced obesity, indicating diet to be a key determinant of gut microbiome composition [31]. In humans, a high-fat diet resulted in a similar phylogenetic shift in the GI microbiome associated with obesity [6]; this restructuring is largely due to dietary selective pressure, which promotes organisms optimally poised to metabolize and import readily available carbon sources, particularly simpler sugars, such as glucose, fructose and sucrose [32]. In support of this, a separate study of C57BL/6J mice fed a high-fat/high-sugar Western diet exhibited GI microbiome domination by the class Mollicutes (within the Firmicutes phylum), which was associated with increased body fat and upregulation of metabolic pathways involved in the import and fermentation of simple sugars and host glycans [32]. Interestingly, a high-fat diet has also been shown to reduce the abundance of Bifidobacteria [33], which are traditionally thought of as beneficial species in the gut microbiome.

The shifts in microbiome elicited by diet and other factors (described later) are key to host health, particularly because the structure of the GI microbial population has been associated with protection against pathogens. Recently, Dong and colleagues demonstrated that aseptic mosquitoes (*Anopheles gambiae*), the natural vector for *Plasmodium falciparum* (the causative agent of malaria), were susceptible to this parasite once their natural gut microbiome was disrupted through antibiotic treatment [34]. This effect was ameliorated by feeding or injecting the insects with live bacterial species, which was associated with a reduction in the number of oocysts produced by *P. falciparum* [34]. The authors further established that this protective effect was elicited indirectly through microbiome-dependent manipulation of the insect's immunity, involving upregulation of several anti-*Plasmodium* factors and the antimicrobial peptides cecropins 1 (*Cec1*) and 3 (*Cec3*), Defensin 1 (*Def1*), as well as other basal immune response factors such as lysozyme c-1 [34]. Other murine studies have also examined gut microbiome destructuring (mediated by antibiotic administration) and infection by GI pathogens, and have also demonstrated a key role for native bacterial species in controlling the behavior and physiology of infectious agents [35,36]. Following administration of antibiotics that dramatically alter the composition of the gut microbiome, *Salmonella enterica* serovar Typhimurium or *Clostridium difficile*-infected mice exhibit a supershedder phenotype (shed $>10^8$ CFU/g of pathogenic cells in stool), resulting in rapid transmission of the infectious agent [35,36]. These studies demonstrate the complexity of the host–microbiome interaction, and the key role played by the microbial community in modulating the host immune response and controlling the behavior and outgrowth of pathogenic species.

The importance of microbial immunomodulation is further exemplified by a recent study demonstrating that mice mono-colonized with a murine gut commensal anaerobe, segmented filamentous bacterium (SFB), exhibited induction of $CD4^+$ T-helper cells producing a Th17 cytokine (IL-22 and IL-17) profile [37]. Expression analysis of SFB-colonized mice demonstrated an upregulation of serum amyloid A, which specifically induces a dendritic cell-mediated Th17 cell-inducing environment in the gut. In addition, SFB colonization also reduced the severity of *Citrobacter rodentium* infection; mice colonized with SFB did not exhibit penetration of the colonic wall by *C. rodentium* and had significantly less colonic inflammation when compared with noncolonized animals [37]. These findings are of particular interest given recent reports that aberrant Th17 populations are associated with a number of chronic diseases such as inflammatory bowel disease (IBD) [38], lupus, multiple sclerosis, psoriasis, and rheumatoid arthritis (reviewed in [39]), disorders that are believed to be linked to GI dysbiosis.

Prenatal microbial exposures & early immune development

Recent studies have suggested that predisposition to disease may, at least in part, be determined *in utero*. Maternal exposure to environmental stimuli, particularly microbes during pregnancy, appears to play an important role in postnatal immune functioning and, in particular, the subsequent development of allergic disease [40–42]. Schaub *et al.* demonstrated that mothers exposed to farms and farm animals during pregnancy were less likely to have children who developed allergies and asthma (Figure 1) [43]. These prenatal exposures impacted immune responses and were associated with increased number and function of cord blood T-regulatory (Treg) cells, which are linked to lower Th2 cytokine secretion (increased Th2 cytokine secretion is a characteristic of an allergic response). The authors speculated that maternal prenatal exposure to farm-associated microbes could provide a form of natural immunotherapy, potentially shaping the child's immune development during the gestational period. The work of Ege and colleagues [40] provides further support for this hypothesis, demonstrating that children whose mothers had been exposed to stables during their prenatal period, exhibited higher expression levels of the innate immune components, Toll-like receptor (TLR2, TLR4 and CD14), which specifically recognize and facilitate response to both Gram-positive and Gram-negative bacteria. Moreover, the authors demonstrated that this response was dose dependent; for every extra farm-associated species the mother encountered, expression levels increased by 1.16-fold [40].

The fact that these exposures mediate their effect via microbes is supported by the finding that bacterial species such as *Acinetobacter lwofii* and *Lactococcus lactis*, isolated from farming communities, have specifically been shown to reduce allergic responses in murine models [44]. Both *A. lwofii* and *L. lactis* isolated from cow sheds demonstrated the ability to polarize T-cell maturation towards a Th1 response by stimulating dendritic cell IL-12 production, resulting in abrogation of allergic inflammation and improved airway responsiveness [44]. Thus, there is evidence for the potential of these farm-associated microbial species to manipulate the immune response; however, whether they directly or indirectly (via maternal immune manipulation) impact the immune response of the developing fetus remains to be determined.

Protective prenatal exposures do not appear to be restricted to farm animals; maternal prenatal exposure to household pets (cats and dogs) has also been shown to protect against allergic disease development [42,45–47] and it is hypothesized that this protection is, as with farm animal exposures, mediated via microbes. Pet exposure is associated with lower cord blood IgE levels [47], which is particularly pertinent given the crucial role that IgE plays in fetal immune system functioning [48] and that elevated cord blood IgE levels have previously been associated with subsequent development of allergic disorders [49–51]. Wegienka and

colleagues also demonstrated that exposure of nonallergic pregnant women to pets is associated with increased Treg cell numbers [42], suggesting that the protective effect of pet exposure against allergic disease development may be through the induction of Treg cell populations, which are known to play a central role in immune homeostasis [43]. The concept that microbial exposures are key to defining the developing immune response *in utero* is also supported by a study demonstrating that maternal prenatal exposure to antibiotics (which dramatically impact the human microbiome; see later) resulted in a dose-dependent increased risk for childhood asthma [52].

Again, whether such prenatal exposures directly (via exposure of the developing fetus to microbial products) or indirectly impact fetal immune response development is unclear; however, a recent study suggests that microbial exposure can occur *in utero* and can impact postnatal infant health. Amniotic fluid, which is typically sterile in healthy pregnant women [53], has recently been shown to be a source of direct microbial exposure for the developing fetus. *Leptotrichia* spp. and other related bacterial species were detected using culture-independent molecular approaches in the amniotic fluid of women in preterm labor with a strong dose-dependent relationship between bacterial abundance in the amniotic fluid and gestational age at delivery [54]. A separate study also detected bacterial species in the amniotic fluid of preterm pregnancies and further demonstrated that women in this study whose amniotic fluid was PCR-positive for bacteria exhibited elevated levels of IL-6, histological chorioamnionitis and funisitis, which were strongly associated with the development of neonatal sepsis [53]. The authors did not comment on the causative agent of sepsis in these neonates, making it difficult to determine whether prenatal exposures were directly or indirectly responsible for the subsequent infection. Nonetheless, the study provides evidence for direct *in utero* microbial exposure and the possibility that introduction of the developing fetus to microbial products may play a role in shaping postnatal immune development.

Postnatal GI microbial colonization & immune response development

Prenatal maternal exposures clearly influence early infant immune responses and therefore presumably also regulate postnatal microbial colonization, an area of research that has recently become the focus of intense study. Exposures that shape the gut microbial community composition have come under particular scrutiny (Figure 1). The diverse ecosystem of the human gut microbiome encodes genes for essential functions that the human host is incapable of performing, such as vitamin production and metabolism of indigestible dietary polysaccharides [55–57]. Thus, the host immune system must strike a balance between providing a favorable environment for this vital community while protecting against invasion or outgrowth of pathogenic species. Insights into how the immune system initially develops the ability to discriminate between harmful and beneficial microbial species is becoming more apparent and appears to be based, at least in part, on both prenatal maternal exposures (discussed earlier) and postnatal infant GI colonization events [56,58,59]. GI mucosal defense and homeostasis are typically mediated by two distinct mechanisms, immune exclusion – mediated by secretory antibodies at the mucosal surface – and immunosuppression to prevent inappropriate responses to ‘friendly’ antigens by recognizing both pathogenic and commensal bacteria via TLRs [60–64]. The ability to discriminate between microbial ‘friend’ and ‘foe’ is primarily dependent on postnatal immune development, which is increasingly associated with appropriate microbial colonization of the GI tract [59,65,66]. Studies of germ-free mice have demonstrated deficiencies in immune development in the absence of GI microbial colonization [67–69]. The importance of early microbial exposure is emphasized in a mouse study in which neonatal mice were exposed to lipopolysaccharides (LPS) or ovalbumin [70]. Mice exposed to LPS developed T-cell populations expressing $CD25^+$ and IL-10 upon antigen challenge and exhibited reduced airway hypersensitivity in comparison to those exposed exclusively to ovalbumin (who demonstrated a strong Th1-dominated response [70]). Several other animal

models and comparisons of human gut communities have reinforced the key role that appropriate microbial colonization plays in gut-associated lymphoid tissue (GALT) development [71], specific aspects of immune system development [14,72,73] and the integrity of the mucosal barrier [69,72,74]. Culture-independent studies have provided greater insight into the temporal fluctuations in bacterial community diversity as it develops during an infant's first year of life, including dramatic decreases in diversity upon antimicrobial administration [11]. However, by approximately 12 months of age, this gut community begins to resemble that of an adult-like microbiome, dominated by the bacterial phyla Firmicutes and *Bacteroides* and possessing members of the Proteobacteria, *Actinobacteria* and Verrucomicrobia, amongst others [11]. Thus, it has been suggested that this initial period of colonization, which coincides with immune response development, represents a crucial window during which aberrations in colonization patterns may impact appropriate immune maturation [75].

Much of the evidence for the link between early microbial colonization events in the GI tract and subsequent development of immune disorders come from studies of asthma and allergy, which are regarded as a failure in the development of a balanced immune response [42,75–78]. The hygiene hypothesis, originally based on the observation that children with older siblings exhibit a reduced incidence of allergic disease, was postulated to be due to exposure to viral infections [79]. However, a more recent evolution of this hypothesis is that a lack of exposure to microbes in early infancy due to improved living conditions leads to development of a skewed immune response [80]. Recent studies provide strong evidence for a link between early GI colonization events and the subsequent development of allergic disease [75,81]. A microbiological examination of almost 1000 stool samples from 1-month-old infants demonstrated that a high abundance of *Escherichia coli* was associated with the subsequent development of eczema, while infants colonized with large numbers of *Clostridium difficile* were associated with a higher risk for eczema, recurrent wheeze, allergic sensitization and atopic dermatitis [75]. These data suggest that microbiome deviations, particularly outgrowth of specific species even at this early stage of life, are associated with subsequent immune disease development.

Key factors that impact gut microbiome composition in the early stages of infancy and which have been associated with subsequent childhood asthma and allergy development include Caesarian delivery (rather than vaginal birth), formula-based diet (*in lieu* of breast milk), hospitalization, gestational age (preterm) and antibiotic administration [41]. These findings are supported by several other investigations. An independent epidemiological study associated antibiotic use in the first year of life with increased risk of atopy, the risk being greater in subgroups of children who were breast-fed for 4 months or longer or had two or fewer pets in the home [82]. The authors concluded that antibiotic use in infants could change the gut microbiota, which may negatively impact immune system development and increase the risk of atopy in specific groups of children [82]. A meta-analysis of studies on the mode of birth and allergic diseases demonstrated a 20% increase in the development of asthma and allergy in children delivered by Caesarian section compared with those delivered vaginally [81]. Vaginal delivery results in exposure to the maternal vaginal microbiome, typically composed of commensal organisms commonly found in the lower GI tract [83]. These infants typically possess higher abundances of certain *Bifidobacterium* and *Bacteroides* species, which have been associated with health-promoting effects, including downregulation of inflammatory responses [77,84,85]. In contrast, infants delivered by Caesarian section exhibit a delayed and deviant GI bacterial community, dominated by *Staphylococcus* spp., *Streptococcus* spp. [86, 87] and *C. difficile* [41] (associated with an increased risk of allergic disease development [78]). Indeed Penders and colleagues recently demonstrated that full-term infants born vaginally at home, who were exclusively breastfed, exhibited the most ‘beneficial’ gut

microbiota, characterized by high numbers of Bifidobacteria and reduced abundances of *E. coli* and *C. difficile* [41].

As in adulthood, mode of nutrition also plays a key role in shaping the developing GI microbiome over the first year of life. In addition to the nutritional support breast milk provides, it also facilitates transfer of bioactive agents, for example, maternal secretory IgA, which provides passive immunoprotection [88,89]. In addition, IgA has been shown to sequester commensal species in the neonatal intestine and promote biofilm formation [88,89], an aspect that has been argued to facilitate colonization by protective native gut bacteria (immune inclusion) and prevent colonization by pathogenic species (immune exclusion) [90,91]. In mouse studies, a reciprocal relationship between the concentration of maternal IgA and bacterial colonization has been previously demonstrated, suggesting that breast milk IgA may delay the development of a diverse gut microbiome [92], providing a window for the development of protective biofilms of commensal organisms along the GI mucosa. Other components of breast milk modulate the developing mucosal immune response, while the presence of indigestible oligosaccharides promotes the growth of specific bacterial families such as the Bifidobacteriaceae and may act as decoy ligands for pathogens, preventing their mucosal attachment [93]. Thus the components of breast milk serve to both directly and indirectly enhance mucosal barrier function and shape immune development [93]. In addition to protection against allergic disease development, breastfeeding has also been associated with defense against neonatal diarrhea [94], necrotizing colitis [95], obesity (meta-analysis is provided in [96]) and Type II diabetes [97]. The fact that an association between early nutritional status (which clearly impacts the developing microbiome) and subsequent development of chronic adult diseases exists, argues strongly for the role of GI microbes in the development and modulation of these disorders. It also demonstrates that predisposition to these diseases may originate from initial GI colonization events and immune system programming during the early years of life, suggesting a role for early GI microbial colonization events in determining subsequent inflammatory disease outcomes.

Infants who are exclusively formula-fed exhibit altered GI microbiota with higher levels of *E. coli* and *C. difficile*, two species that have previously been linked to the development of eczema in infants [75]. Although it has been reported that there is no significant difference in the numbers of Bifidobacteria detected in stools of exclusively formula or breast milk-fed infants [41,98], the functional gene expression and type of Bifidobacteria present in infant stools has been shown to differ according to feeding mode [99]. Klaassens and colleagues used a *Bifidobacterium* mixed-species expression microarray to examine the differences between infants who were exclusively breast- or formula-fed. They demonstrated that diet explained 44% of the expression profile variation in Bifidobacteria in formula- or breast-fed infants and that each treatment group exhibited specific expression profiles [99]. For example, glycobiome-associated enzymes were more highly expressed in breast-fed infants who consume more diverse and complex breast milk oligosaccharides, providing these infants with the increased potential to metabolize these sugars [99]. These results suggest that not only is the presence and abundance of a specific microbial species or family important, but that microbiome expression profiles are strongly influenced by external influences, such as mode of feeding.

These and other studies have indicated that Bifidobacteria are beneficial to human health, which is currently a contentious issue. Nonetheless, several *Bifidobacterium* species including *Bifidobacterium bifidum, Bifidobacterium breve* and *Bifidobacterium infantis* have demonstrable anti-inflammatory properties that protect the epithelial cells from toxins [77, 100]. These three Bifidobacteria species have been shown to induce the anti-inflammatory cytokine IL-10 [100]. In addition, soluble factors from *B. breve* inhibit LPS-induced TNF-α secretion by immune cells [101] and reduce chemokine, other proinflammatory molecule

release and epithelial chloride secretion putatively by targeting serine/threonine kinase activity [102]. Sequence analysis of the commensal bacterial species *Bifidobacterium longum*, which is highly abundant in the breast-fed infant gut, revealed the blueprint of an organism specialized to competitively utilize the indigestible sugars in breast milk [103]. Possessing a high abundance of Bifidobacteria also appears to be beneficial in old age. In a recent study, *Bifidobacterium* species were isolated from Chinese centenarians from Bama, a village known for having a substantially higher than normal proportion of residents over 100 years old who exhibit a low occurrence of age-related inflammatory diseases. Compared with control animals, mice that received daily supplementation with a *Bifidobacterium adolescentis* strain isolated from the centenarians exhibited altered intestinal morphology, including a significant increase in villus height and crypt depth, features believed to enhance digestive efficacy. In addition, duodenal secretory IgA was significantly increased in animals receiving a high dose of this strain (2×10^{10} CFUs daily) compared with controls [104]. In a separate study, Sjögren and colleagues examined the relationships between mucosal and systemic immune responses and a number of species that have been implicated in protection against, or development of, allergic diseases [105]. They demonstrated that Bifidobacteria diversity was associated with accelerated maturation of the mucosal secretory IgA system and that increased abundance of *Bacteroides fragilis* at the early stages of GI colonization reduced LPS responsiveness [105]. Although the mechanism by which these species provide protection is not fully understood, what is clear is that their loss, reduction or altered gene expression in the GI tract during the critical early stages of immune maturation leads to the subsequent development of disease [73,75,106,107]. Based on these and other observations, it is becoming increasingly clear that early events in GI colonization, particularly those events that may alter this process, play a crucial role in the development and maintenance of the host immune system and predisposition to subsequent development of disease.

Diseases & disorders due to aberrations in the human gut microbiome

As discussed earlier, development of the GI microbiota over the first year of life appears to be intimately linked to subsequent disease susceptibility. Aberrations in the adult gut microbiota have also been associated with a number of diseases and disorders including allergic disease development [73,75], colon cancer [14], and even progression and severity of HIV (Table 1) [108]. Disruption of the gut microbiome, termed dysbiosis [18], is frequently accompanied by overgrowth of pathogenic bacteria or fungi, in conjunction with significant loss of microbial diversity or key functional groups [9,14,109–123] and an inflammatory response by the host [9,39,115,121,123–125], which contributes to disease development [108]. Dysbiosis has been associated with an imbalance between populations of inflammation-mediating T-helper cells (Th1, Th2 and Th17) and anti-inflammatory Treg cells. Prolonged overproduction of Th1- and Th17-associated cytokines has been linked with IBD (overproduction of Th1 for Crohn's disease [CD] [126] and Th17 for both CD and ulcerative colitis [UC] [38,127]) and autoimmune disorders such as lupus, multiple sclerosis, psoriasis and rheumatoid arthritis, while a Th2 skew is linked with asthma, allergic disorders and UC (Figure 2) [39,128,129]. Such chronic inflammatory responses set up a ‘vicious circle’, disrupting the GI microbiota [115], eliminating subsets of beneficial bacteria and permitting opportunistic colonizers, typically pathogens, to compete in this niche [115] and maintain a persistent inflammatory state. Members of the Enterobacteriaceae, which includes *Salmonella enterica* serovar Typhimurium, a bacterium linked to gastroenteritis [109,115,121], appear to use this strategy. In an attempt to eliminate the pathogen, the host response disrupts the native gut microbiota, providing the pathogen with the opportunity to proliferate [109], emphasizing the key role microbiome homeostasis plays in protection against pathogen overgrowth.

Many of the diseases and disorders associated with adult gut microbiome dysbiosis exhibit an overall reduction of bacterial diversity (which may, in part, be attributed to disease-related

diarrhea) and appears to be a characteristic shared by the majority of chronic inflammatory diseases (Table 1). To date, only colon cancer patients exhibit a significantly higher microbial diversity compared with that of healthy individuals; however, these assemblages exhibit dramatic temporal instability [14], an emerging characteristic of chronic inflammatory diseases [25,26]. Scanlan *et al.* emphasized that although the microbial diversity was increased in colon cancer patients, it was unclear which species proliferated, and suggested that the community may be composed of a multitude of organisms capable of toxin production that outcompete the native beneficial microbes [14].

It is beyond the scope of this review article to present an in depth discussion of all of the diseases and disorders associated with gut microbiome dysbiosis. Instead we have presented a summary in Table 1 and focus here on one, IBD, which encompasses many of the issues associated with chronic inflammatory diseases. Characterized by chronic or recurrent inflammation of the GI mucosa [130], the GI tract of the IBD patient appears unable to reprogram towards a noninflammatory state even after depletion of the primary pathogen [131]. This suggests that low levels of certain microbial species maintain a proinflammatory state, the microbiome is irreversibly altered and incapable of appropriate reassembly, or a combination of these factors contribute to this chronic inflammatory state. Interestingly, a recent study has proposed a role for the appendix as a 'safe-house' for commensal bacterial species, which exist in protected biofilms that may detach and serve to repopulate the GI tract following perturbation of the natural microbiome [132]. Although it has previously been suggested that there are no discernable long-term effects of appendectomies in westernized countries [132], appendectomy has been associated with progression and severity of IBD [133]. The impact of appendectomy (or indeed appendix colonization by inappropriate microbial species) on the ability to 're-seed' the GI microbiome, facilitating community reassembly following perturbation, particularly in patients with inflammatory GI disorders, is an intriguing concept that has not been comprehensively studied.

Crohn's disease and UC, the two main disorders associated with IBD, typically require care for the length of the patient's life. Patients with CD suffer from patchy inflammation that may occur anywhere along the GI tract and is characterized by large transmural ulcerations [134]. UC patients typically possess more superficial ulcerations restricted to the colonic mucosa and inflammation that characteristically extends continuously from the rectum throughout the large intestine [134]. Like other chronic inflammatory disorders, there are several risk genes (*IL23R*, *IL12B*, *HLA*, *NKX2–3* and *MST1*) associated with increased susceptibility to both CD and UC [135]. Specific additional genetic components exclusive to CD such as *NOD2/CARD15, FcGRIIIA, ATG16L1* and *IRGM* [136–141] have been identified, however, risk genes exclusive to UC remain poorly defined [138,142–145]. Several environmental factors have been shown to impact progression and severity of the disease, including smoking, diet, drugs, stress, appendectomy, geography and social status [133]. While aberrations in the gut microbiota have been associated with IBD [131], its etiology and whether the associated dysbiosis is a cause or result, remains to be determined [146,147]. Nonetheless, patients with IBD typically exhibit lower GI bacterial diversity compared with healthy individuals [5,9, 14], an increase in *E. coli* [123], and lower counts of specific Firmicutes [9,112] and *Bacteroides* species [111,118,148]. However it should be noted that some species of *Bacteroides* have been shown to increase in relative abundance in CD patients [5], suggesting that specific functions associated with particular species or strains may contribute to the niche-specific pathophysiology of UC and CD.

At a functional level, the butyrate-producers, which belong to the *Clostridium leptum* group of the Firmicutes, are less abundant in the GI microbiome of IBD patients. Butyrate is an essential regulator of gene expression, inflammation, differentiation and apoptosis, and is a major energy source for the mucosa-associated microbial community [149–151].

Faecalibacterium prausnitzii, a butyrate-producing bacterium with anti-inflammatory properties [122,123,147], is significantly reduced in abundance in the ileum of CD patients [111,116,123,152] in parallel with increased numbers of *E. coli* [123]. This emphasizes the key role a number of distinct bacterial species play in maintaining inflammatory homeostasis and that loss of key functional organisms leads to pathogen overgrowth associated with chronic inflammatory illness.

To further determine the basis for host health and disease, several groups have turned to high-resolution functional profiling of microbial communities. Metabolomic studies are becoming more prevalent in dysbiosis-related diseases; several metabolic pathways implicated in CD have now been identified [153]. In addition to better understanding the pathogenesis of this disease, these pathways are also useful for differentiating between patients and healthy subjects. A total of 2155 discriminating phenotypes for ileal-specific CD, 3113 for colon-specific CD and 2650 that were characteristic of healthy individuals were identified in this study [153]. An independent study using metabolic profiling demonstrated lower levels of butyrate, methylamine and trimethylamine in CD patients in comparison to UC patients [10]. In addition, elevated levels of amino acids in fecal samples were detected in IBD patients, which implicated malabsorption (presumably a consequence of mucosal inflammation), as an additional component of the disease [10]. In a separate functional profiling study, *in vitro* high-resolution proton magnetic resonance spectroscopy was used to measure levels of amino acids in the colonic mucosa of patients with active or inactive IBD and demonstrated that decreased amino acid levels were associated with reduced carbohydrate and protein metabolism [154]. Probably due to a shift in GI microbiota composition, this aspect of active IBD may result in lower energy and altered mucosal integrity. In comparison, samples from inactive IBD patients exhibited comparable amino acid concentrations with healthy controls [154]. These studies serve to highlight the complexity of IBD and the key role of microbial communities, functional gene expression and host responses in inflammatory disease exacerbation and remission.

Manipulation of the GI microbiome: implications for host health

Antibiotic administration

While antibiotics have revolutionized our ability to combat infectious diseases, their widespread use has led to a dramatic rise in the prevalence of antibiotic-resistant microbes, and recent concerns have been raised regarding the potential for adverse effects on host microbiota. In the process of eliminating the pathogenic agent, antibiotic administration dramatically impacts the native microbial community, leading to an unintentional state of dysbiosis [18]. The impact of these therapeutics appears to be more pronounced in infants 1 year of age or less, likely due to their influence on microbial colonization of the gut during the initial stages of immune response development (discussed earlier). A study of clamoxyl administration to mice demonstrated altered microbial colonization of the gut with the near depletion of *Lactobacillus* species coinciding with a reduction in the total aerobic and anaerobic bacteria, notably Enterobacteriaceae spp. and Enterococcus spp. [15]. In addition, administration of the antibiotic downregulated expression of genes involved in antigen presentation and the innate immune host defense [15], presumably because of changes in the microbial colonization pattern of these animals. Palmer and colleagues demonstrated dramatic decreases in GI bacterial community diversity upon the administration of antibiotics to infants in the first year of life [11], which has specifically been associated (in a separate study) with lower numbers of Bifidobacteria and *Bacteroides* [41]. These two bacterial genera appear to be critical for immune development, and their loss has been associated with the development of allergic disease, an aspect attributed to the antibiotic's effect on the gut microbiota [155].

Another aspect of gut microbiome manipulation by antibiotics is antibiotic-associated diarrhea (AAD), which affects 11% of children in the USA, is more frequent in those who are less than

2 years of age and appears to be dependent on the type of antibiotic received [156]. Surawicz reported that the administration of broad-spectrum antibiotics that target anaerobic bacteria is associated with a higher risk of AAD development [157]. Interestingly, a recent study examining factors predictive of development of *Clostridium difficile*-associated diarrhea (CDAD), including gut microbiome profiles, found that the resident microbiota and not the class of antibiotic administered provided the highest predictive power for CDAD development [4]. Despite the dramatic impact antibiotic administration has on the gut microbiota, the community in healthy adults appears to be relatively resilient; once administration of antibiotics has ceased, the gut microbiome largely returns to a pretherapy consortium after approximately 4 weeks [19]. However, subtle persistent changes were evident – a number of bacterial species had not returned to preantibiotic levels 6 months post-treatment [19]. The impact of the loss of these species on host health and the potential for long-term issues are currently unknown [19]. In a murine study, following treatment with the antibiotic enrofloxacin, changes in three key functions were associated with depletion of the bacterial gut community: loss of acetate due to reduced microbial metabolism of sugars and polysaccharides; decreased trimethylamine-N-oxide due to deficient microbial catabolism of choline (also associated with CD [10]); and an increase in creatine due to a lack of microbial enzyme degradation [13]. In addition, a loss of amino acids produced by microbial proteases, the reduction of metabolites from lactate-utilizing microbes and an increase in urea from the depletion of microbial ureases were also detected [13]. Therefore, although the microbial community may appear to return to a community that largely resembles the pretreatment consortium, the possibility remains that the impact of the loss of even a small number of specific microbial species may be more profound at the functional level. Determining the impact of such subtle alterations necessitates the use of high-resolution microbial community profiling tools, such as phylogenetic microarrays, high-throughput sequencing and metabolomic or metaproteomic approaches to determine the microbial community structure, function and host response.

Microbial/nutritional manipulation of the GI microbiome

Owing to the increased prevalence of diseases and disorders associated with gut microbiota imbalances (Table 1) and the fact that traditional treatments such as antibiotic administration appear to have the potential for long-term disruption, microbial manipulation of the host microbiome to treat chronic diseases has become the focus of recent renewed interest. Manipulation may be elicited through pro-, pre- or synbiotics. First suggested by Metchnikoff in 1907 [158], probiotic therapy represents alteration of the gut microbiota by supplementation with live microorganisms that function to inhibit pathogen adherence to the mucosa [159, 160], improve the intestinal epithelial and mucosal barrier function [161], produce bacteriocins [162,163], increase IgA production [164] and downregulate proinflammatory cytokine secretion [165–167]. Prebiotics, originally defined as nondigestible food ingredients that improve host health by stimulating the growth or activity of colonic bacteria [168], have now been reclassified to include components that are resistant to gastric acidity, hydrolysis by host enzymes and absorption by the upper gastrointestine, are fermented by the gut microbiota, and stimulate growth of microbial species beneficial to the host's health [169]. Finally, synbiotics are supplements composed of a combination of both probiotics and prebiotics [168]. An exhaustive review of the use of probiotics, prebiotics and synbiotics is beyond the scope of this review; instead, we refer the reader to other excellent recent reviews on this topic [170,171] and focus on their use in a limited number of cases to highlight efficacy and concerns associated with these therapies.

Recent studies have demonstrated a direct link between the development of IBD and GI bacteria [172,173], and probiotic therapies have become a focus for various GI dysbiosis-based diseases. Successful use of probiotics for treatment of IBD appears to be dependent on the bacterial strain used, the stage of disease progression and the type of pathology used for analysis

(Figure 3) [174]. Probiotics appear to be more successful for treatment of UC than CD, however, probiotic intervention studies of CD patients have typically been small and few have been randomized double-blind controlled trials [175,176], making it difficult to definitively characterize probiotic efficacy in this patient population. However, there may be a physiological basis for enhanced probiotic efficacy in UC patients. For example, defensins are typically downregulated in UC patients, a feature that can be ameliorated by probiotic species such as *E. coli* Nissle 1917 [177], but cannot be corrected in defensin-defective CD patients. In addition, the ability of probiotic species to colonize specific niches along the GI tract may be variable due to physiologically distinct features such as mucus coverage and the density of antimicrobial peptide producing Peyer's patches at specific sites, potentially impacting their efficacy in UC and CD patient populations.

A clinical trial testing the efficacy of probiotic preparation VSL#3 (a mix of eight probiotic bacterial species) on patients with mild to moderately active UC, demonstrated the probiotic treatment induced remission in significantly more patients than those treated with mesalazine (5-aminosalicylic acid; standard of care [178]). Furthermore, 53% of patients who exhibited persistent symptoms despite receiving mesalazine or corticosteroids for their UC exhibited remission of symptoms following VSL#3 administration [179]. Bibiloni and colleagues also reported that the bacterial species in the VSL#3 cocktail could be detected specifically at the site of inflammation by molecular methods [179], suggesting a direct role for the probiotic species in mucosal colonization and modulation of the local immune response. A separate study reported that patients receiving VSL#3 had a significant reduction in UC symptom severity following 6 weeks of daily supplementation and that more patients were in remission after 12 weeks compared with the placebo group [180]. A pilot study of UC patients receiving a *Saccharomyces boulardii*-based probiotic demonstrated that 68% of the patients receiving the fungal supplement reported remission of symptoms [181]. This probiotic also appears to be promising for individuals with CD, where it has been shown to induce remission in significantly more patients compared with those treated with mesalazine [182]. Probiotics also appear to be promising for preventing and diminishing severity of necrotizing enterocolitis (NEC) in neonates [183–189]. However, these trials have been criticized because the timing, dosage and type of organisms were not optimal [184,186,187] and there were insufficient data to examine the long- and short-term effects [184,186,189].

Insights into how probiotic supplementation may impact the gut microbiome has come from a recent study of infants in the Trial of Infant Probiotic Supplementation (TIPS) study, in which infants at high risk for asthma development are fed a probiotic species (*Lactobacillus casei* Rhamnosus GG [LGG]) or placebo [190]. The bacterial community composition of stool samples from a subset of infants in this trial were examined using a high-resolution microarray, the 16S rRNA PhyloChip (Affymetrix, CA, USA) [191], which can detect approximately 8500 bacterial taxa (defined as strains or species that share ≥97% 16S rRNA sequence homology) in a single assay. Although the investigators remained blinded to the identity of the samples, clear differences in the abundance of LGG were evident and analysis of the data demonstrated that a high abundance of the probiotic species was associated with a distinct bacterial community in which many other known beneficial species, including members of the Lactobacillaceae and Bifidobacteriaceae, were also present in high abundance [190]. These communities were composed of species that were phylogenetically related, suggesting a high level of functional redundancy, a characteristic previously associated with stable microbial assemblages resistant to pathogen outgrowth [2,16]. By contrast, communities with low levels of LGG tended to possess a more variable community composition and high abundances of species previously associated with allergic disease development. Hence, it appears that the beneficial effects of probiotic supplementation are not simply due to a high abundance of the probiotic species itself, but rather to a restructuring of the gut microbiome toward a commensal rich, functionally redundant consortium. Although not examined in this study, it is likely (based

on previous studies [13,16]) that this large shift in community composition underlies altered functional gene expression and impacts host immune response, a factor which may impact asthma development, one of the primary outcomes being measured in this trial.

Federal regulation has recently been mandated for probiotic supplements because some preparations do not comprehensively list the species present or contain additional species that are potentially pathogenic [186], an aspect that has led to supplement variation in trials [184]. Although it must be stressed that adverse events involving probiotic supplements are rare, these precautions are being implemented because their use has been linked to higher mortality rates for ICU patients with pancreatitis [192] and *Lactobacillus* sepsis in immunocompromised patients [193,194]. Negative effects have not been found in NEC probiotic trials, but this is probably because the power of these trials is too small to detect side effects [189].

Prebiotics also represent a promising approach for the management of inflammatory diseases. They have the advantage of promoting subsets of existing native GI bacterial community members (e.g., Bifidobacteria) capable of degrading them and can increase production of important anti-inflammatory compounds, such as butyrate, without the conventional caveats of probiotic competitiveness or colonization efficiency. For example, oligofructose (enzymatic product of hydrolyzed inulin) was found to increase butyrate production in both dextran sodium sulfate (DSS) mice and humans [195,196], as well as limiting murine DSS damage and accelerating the healing process [195]. Although the mechanisms remain unclear, prebiotic germinated barley foodstuff has also been shown to significantly decrease IFN-γ expression [197]. IFN-γ is known to induce colitis and the proinflammatory cytokine IL-6, and increase TGF-β production (excess TGF-β has specifically been associated with colitis pathology [197]). Thus, while there are clear functional effects of feeding prebiotic compounds, their mechanism of action, which probably involves structural and functional shifts in the GI microbiota due to the selective pressure of supplementation with complex carbohydrate nutritional sources, remains to be fully elucidated.

Use of synbiotic supplementation is still in its infancy for GI dysbiosis, but some promising signs are starting to emerge in colon cancer treatment. Synbiotic therapy enhanced apoptosis in carcinogen-damaged cells and to genotoxic carcinogens in animal models [198,199] and reduced the number of tumor and lesion occurrences [199–201] by moderating Peyer's patch immune functions [200]. In humans, Rafter *et al.* reported that there were significant differences in the levels of *Bifidobacterium* species in fecal samples from synbiotic-treated colon cancer patients, but they did not find any effect on apoptosis, colonic inflammation or tumor cell invasion [202]. Synbiotic approaches for IBD include a supplement and a fungal probiotic therapy. A pilot study using a combination of *B. longum* and fructo-oligosaccharide/inulin found that those subjects receiving the synbiotic had significantly reduced inflammation compared with the placebo-treated group [203]. Synbiotics also appear to be promising in the treatment of CD; a supplement of *B. breve, B. longum* and *L. casei*, and the prebiotic psyllium was examined in ten CD patients who were not responding to standard therapies [204]. Although patients were permitted to choose when and how much of the synbiotic they self-administered, six of the patients exhibited remission of symptoms with substantially reduced CD activity [204].

Aside from genetic and environmental factors, given the broad interpersonal variability in the human host GI microbiome and the capacity for a wide variety of potential perturbations to this ecosystem, it is not surprising that a pre-, pro- or synbiotic therapy that demonstrates efficacy in one patient population may not exhibit the same effect in another. Although initial results are promising, randomized, large-scale studies with well-characterized patient populations who are supplemented with controlled dosages of specific well-characterized supplements, and whose samples are examined using high-resolution microbiome composition

and functional analyses, are necessary to fully appreciate the therapeutic effects of GI microbiota manipulation (Figure 3). In the current era where patient-tailored care is being touted as the future of medicine, understanding the complex interactions between microbes and immune response that underpin host health is fundamental to truly providing such a level of care.

The overall indication is that gut microbial communities play a key role in the development of inflammatory disease and that these communities are amenable to manipulation to prevent or abrogate disease development. As the GI microbiome and specific pathways responsible for improved host health continue to be identified with newly developed high-resolution culture-independent tools, our understanding of the delicate balance between the human host and its microbial inhabitants continues to unfold. With these insights, novel therapies may be developed to restore or promote beneficial microbial communities to help combat a myriad of chronic diseases.

Expert commentary

From the influence of prenatal and early postnatal microbial exposures on the developing immune response to gut microbiome aberrations in adulthood associated with chronic inflammatory diseases, recent studies of the human microbiome have revealed the complexity of our dynamic relationship with microbes. Relatively recent changes in lifestyle, diet and the use of antimicrobials are just some of the factors implicated in increased prevalence of a range of inflammatory disorders that have a demonstrated basis in altered GI microbial community structure and function.

Five-year view

Given the cumulative data and, in particular, the recent microbiome studies, the maintenance of a diverse, functionally redundant microbial community encoding a core set of functional genes appears key to human host health. Further mouse and human studies of microbial community composition in parallel with immunophenotyping and long-term outcome measures, in well-defined cohorts, are necessary to fully understand the role of the human microbiome in defining states of health and disease. Given the microbiome initiatives underway globally and the increasingly sophisticated tools to interrogate samples, a more comprehensive understanding of the impact of therapeutics and other microbiome-manipulating agents and their long-term effect on host health will be better defined. In parallel, the development of culture-independent diagnostics based on these findings and using these new profiling approaches will vastly improve diagnosis and permit surveillance of at-risk populations.

Key issues

- The gastrointestinal microbiome is associated with host health status.
- Structure and composition of the microbiome defines functional gene expression of the community, pathogen abundance and physiology, and the host response.
- Prenatal and early postnatal microbial exposures impact immune response development and define predisposition to the development of inflammatory diseases.
- Specific microbes have demonstrated roles in immune response modulation.
- Manipulation of the microbiome through pro-, pre- or synbiotic supplementation may prove an alternative approach for improving host health status.

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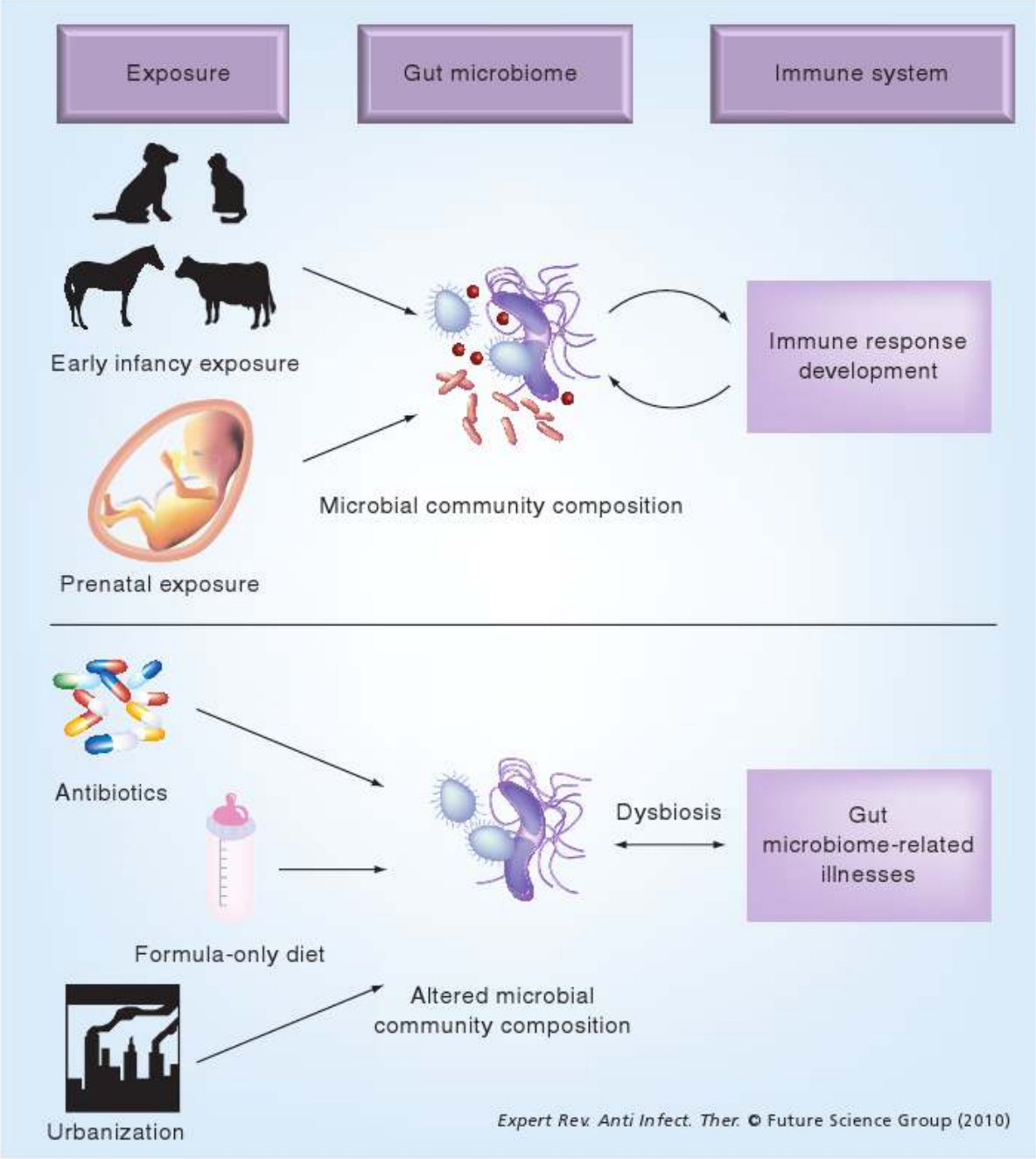

Figure 1. Factors that influence the infant gut microbiome and early immune development

Exposure to farm animals and pets, vaginal birth and breast milk, all of which have a potential microbial link, are putatively associated with a beneficial effect on the developing gut microbiome and host immune response. Factors such as urbanization (lack of microbial exposures), formula-only diet and antibiotic administration during the neonatal stage are associated with the development of subsequent chronic diseases such as asthma and atopy, putatively through the development of aberrant gut microbiomes.

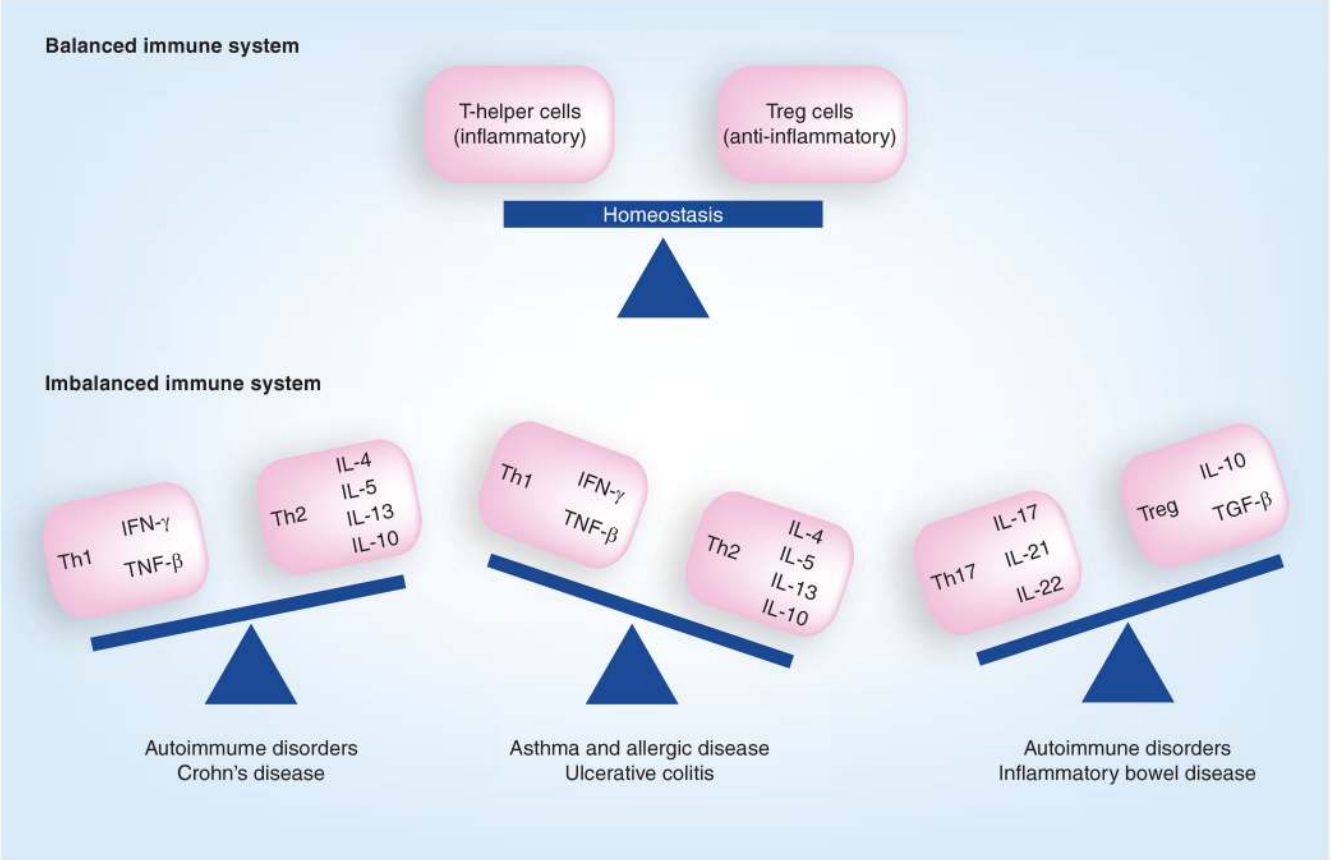

Figure 2. Consequences of immune system imbalances

Immune system homeostasis involves a regulated balance of inflammatory and anti-inflammatory cells; however, skewed responses putatively through lack of microbial exposure or outgrowth of pathogenic species may contribute to imbalances associated with the development of chronic inflammatory disorders.

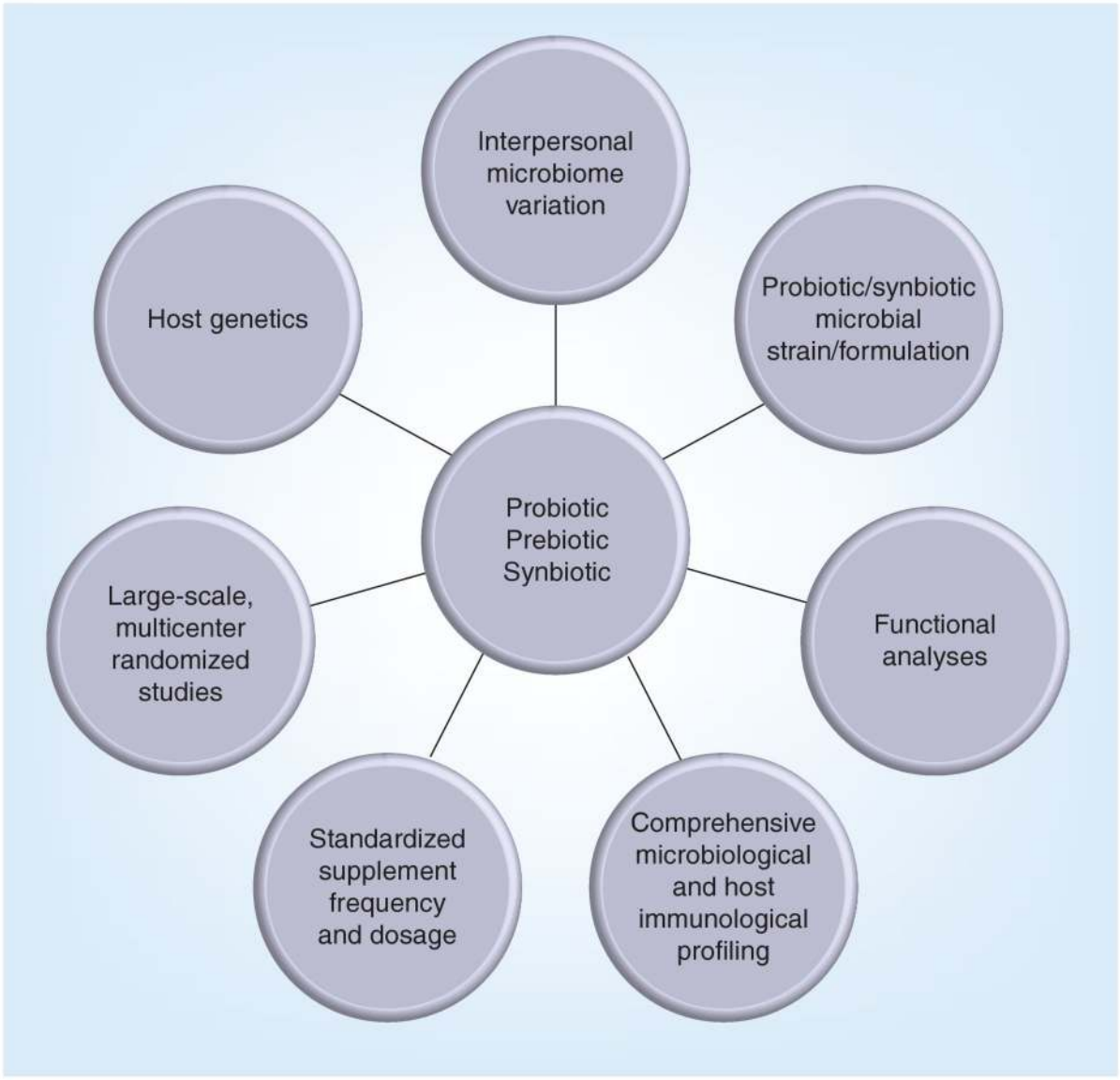

Figure 3. Factors critical for successful probiotic, prebiotic and synbiotic trials

Consideration of interpersonal genetic and microbiome variations, standardized trials with defined outcomes and characterized strains coupled with high-resolution microbial phylogeny, microbiome functional profiles and host responses, are necessary to fully understand the potential impact of microbiome manipulation through probiotic, prebiotic or symbiotic supplementation.

Table 1
Diseases and disorders associated with human gut microbiome aberrations

Disease/disorder	Potential role of the microbiome	Recent findings (2004–present)
Atopy and asthma	• Pre- and postnatal microbial exposures appear key to appropriate immune development [40,43,44,76,105,205–213] • Mode of delivery and nutrient uptake are important factors for GI community development and protection against subsequent atopic disease development [11, 41,46,75,81,84,99,214–216]	• Mothers exposed to farm animals during pregnancy are less likely to have children who develop asthma and allergies [40,43,106,205,206,210] • Early exposure to cat(s) and/or dog(s) is protective against atopic diseases, presumed to be microbially mediated [42, 45–47,213,217,218] • Infants born through the birth canal are exposed to and colonized by specific Bifidobacteria and *Bacteroides* species [41,84,215,216] • Caesarian-delivered infants exhibit delayed GI microbiome development; communities are dominated by *Staphylococcus* spp., *Streptococcus* spp. [87], Enterobacteria [219] and *Clostridium difficile* [41] • A 20% increase in asthma prevalence is associated with Caesarian birth [81] • Breast-fed infants possess functionally distinct GI microbiomes compared with formula-fed children [11,41, 46,73,75,99]
Candida infection	• Depletion of gut microbiota permits *Candida albicans* proliferation and infection [114]	• Depletion of the gut microbiome through antibiotic administration is associated with increased *C. albicans* abundance and infection [114]
Celiac disease	• Celiac disease patients exhibit GI microbiome abnormalities compared with healthy individuals [117]	• Pediatric celiac disease patients have significantly higher numbers of total bacteria, in particular Gram-negative organisms, compared with asymptomatic patients and healthy subjects [117] • *Bacteroides* spp. and *Escherichia coli* were significantly higher in celiac disease compared with healthy subjects; abundance of these species returned to healthy levels in asymptomatic patients [117] • The ratio of *Lactobacillus*–Bifidobacteria species to *Bacteroides–E. coli* was lower for celiac disease patients [117] • Metabolomic study identifies signature metabolic pathways associated with celiac disease [220]
CC	• High abundance *of Clostridium leptum* and *Clostridium coccoides* subgroups in CC patient GI bacterial communities [14]	• Overall bacterial diversity increased for CC patients compared with healthy controls [14] • CC patients exhibited temporal GI bacterial community instability compared with healthy controls [14] • Microbial butyrate production is thought to reduce the chances of CC development [3]
Type I diabetes	• Interaction between the gut community and innate immune system may be a predisposing factor for diabetes [221] • Role in the development of insulin resistance [222] • Differential GI microbiomes are present in DP, DR and ADP mice [12,223]	• Metabolic profiling reveals a contribution of gut microbiota to fatty liver phenotype in insulin-resistant mice [222] • *Bacteroides* abundance higher in DP compared with ADP [223] and DR mice [12] • Higher abundance of *Lactobacillus* and *Bifidobacterium* species in the gut microbiomes of DR compared with DP mice [12]

Disease/disorder	Potential role of the microbiome	Recent findings (2004–present)
Type II diabetes	• Linked to obesity	• Type II diabetes is considered a comorbidity of obesity [224] • Studies that examine the effects of the gut microbiota on Type II diabetes are often included in those that study the relationship between obesity and gut bacterial community (e.g., [27,225,226–228]) • Gut microbiota may contribute to insulin sensitivity, causing low-grade systemic inflammation, which may be independent of obesity [229]
HIV	• Gut microbiome dysbiosis may be critical for pathogenesis [108]	• Dysbiosis and intestinal inflammation may be critical to impairment of the GI microbial structure and function in early stages of HIV infection [108] • Low abundance of Bifidobacteria and lactobacilli detected in gut microbiomes of early-stage HIV infection [108]
IBD	• Immune response to gut microbial community • Composition of GI microbiota contributes to inflammation [115, 121] • Treg-promoting organisms depleted; overgrowth of bacteria that induce proinflammatory Th17 cell populations [39]	**Crohn's disease (IBDC)** • Healthy individuals had higher bacterial diversity than IBDC patients [5,9] • Characteristics of GI dysbiosis include lower counts of *Clostridium leptum* [119], *Bacteroides uniformis* [5], Firmicutes [9,112] and *Bacteroides* [111,118], and higher abundances of *E. coli* [110,123], Proteobacteria [112] and *Bacteroides ovatus* [5] • GI microbial community in IBCD patients exhibits greater temporal instability compared with that of healthy individuals [14] • Lower number of *Faecalibacterium prausnitzii*, butyrate-producing bacterium, found [111,116,123,152] in parallel with increased *E. coli* abundance [123] • Novel strains of *E. coli* with disease phenotypes reported [110] • Excessive amounts of IL-21 detected in IBDC patients; may be a response to overinduction of Th1 cells by luminal microbiota [230] • Adherent-invasive *E. coli* found in higher abundance, diversity and in more IBDC patients than healthy individuals; higher variability of seropathotypes [116] • Metabolomic analysis identifies 2155 discriminating phenotypes for IBDC in the ileum, 3113 for IBDC in the colon and 2650 for healthy individuals [153] • IBDC patients exhibit differential tyrosine and phenylalanine, bile acid and fatty acid metabolism [153] • Metabolomic profile identified reduced levels of butyrate, acetate, methylamine and trimethylamine in IBDC patients [10] • Elevated concentrations of amino acids detected in fecal samples, implying malabsorption caused by inflammatory disease [10] • Lower levels of amino acids detected in patients with activated IBDC [154] **Ulcerative colitis (IBDU)** • Microbial diversity lower when compared with healthy individuals [119] • Higher counts of *E. coli* reported for IBDU patients in comparison to IBDC patients [119,231]

Disease/disorder	Potential role of the microbiome	Recent findings (2004–present)
		• Lower levels of Bifidobacteria [232] and *Clostridium coccoides* [233] reported in comparison to healthy individuals • Differential *Clostridium leptum* and lactobacilli profiles identified by DGGE analysis of IBDU and healthy subjects [234] • Lower levels of amino acids detected in patients with activated IBDU [154]
IBS	• Dysbiosis typically preceded by infection, change in diet or therapeutic administration (e.g., antibiotics) [18]	• Enteric infection may be one cause of IBS [18] • Abnormal detection of hydrogen and methane in patients' breath suggests changes in bacterial fermentation [18] • Absence of *Lactobacillus* spp. and reduced *Collinsella* abundance associated with IBS [113]
Gastroenteritis	• Pathogenic species take advantage of GI microbial community disruption to elicit infection [109, 115,121]	• Suggested that *Salmonella* capitalizes on host disruption of GI microbiome through immune response to this infectious agent to proliferate and infect host [109]
NEC	• Immature intestinal epithelial cells may lead to proinflammatory response upon microbial colonization [235] • NEC potentially due to abnormal bacterial colonization of the GI tract and lack of appropriate commensal bacteria in the gut [236]	• Bacterial community exhibits lower diversity in all preterm infants, particularly NEC infants [237] • NEC patients had higher abundance of Gammaproteobacteria in the GI tract [237] • Suggested that lower bacterial diversity may favor certain dominant organisms, which proliferate with the administration of antibiotics (standard of care for NEC infants) [237]
Obesity	• May be responsible for metabolic endotoxemia and consequential diseases from endotoxemia [27, 225] • Involved in food storage and energy harvest from food [2] • Regulates peripheral metabolism [1,2]	• Obese patients may depend on interspecies transfer of H_2 between Archaea and Bacteria to increase energy uptake [238] • Obese individuals exhibit lower abundance of Bacteroidetes and a higher abundance of Firmicutes compared with lean people [7] • Ratio of Bacteroidetes and Firmicutes reverts back to a composition that resembles that of lean subjects following a diet and exercise regime [7] • Absence or presence of specific functional groups and not bacterial species may be a more appropriate measure of the differences between obese and lean people [16]
Rheumatoid arthritis	• Treg-promoting organisms depleted; overgrowth of bacteria that induce Th17 cell populations, leading to inflammation [39] • Intestinal microbes associated with etiology [122]	• Patients with rheumatoid arthritis had significantly less Bifidobacteria and *Bacteroides–Porphyromonas–Prevotella* group, *Bacteroides fragilis* subgroup and *Eubacterium rectale–Clostridium coccoides* group species [122]

ADP: Asymptomatic diabetes prone; CC: Colon cancer; DGGE: Density gradient gel electrophoresis; DP: Diabetes-prone; DR: Diabetes-resistant; GI: Gastrointestinal; IBD: Inflammatory bowel disease; IBDC: Irritable bowel disease–Crohn's disease; IBDU: Irritable bowel disease–ulcerative colitis; IBS: Irritable bowel syndrome; NEC: Necrotizing enterocolitis.