

The gut microbiota and inflammatory noncommunicable diseases: Associations and potentials for gut microbiota therapies

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Activity Objectives

1. To understand the role of the gut microbiome in health and disease.
2. To understand the developmental factors that influence acquisition of a subject's gut microbiome.
3. To understand the strengths and limitations of current genetic methods used to study the human gut microbiome.
4. To understand current therapeutics available for modulating the gut microbiome for the prevention and treatment of noncommunicable diseases (NCDs), including their limitations.

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Rapid environmental transition and modern lifestyles are likely driving changes in the biodiversity of the human gut microbiota. With clear effects on physiologic, immunologic, and metabolic processes in human health, aberrations in the gut microbiome and intestinal homeostasis have the capacity for multisystem effects. Changes in microbial composition are implicated in the increasing propensity for a broad range of inflammatory bowel diseases, such as allergic disease, asthma, inflammatory bowel disease (IBD), obesity, and associated noncommunicable diseases (NCDs). There are also suggestive implications for neurodevelopment and mental health. These diverse multisystem influences have sparked interest in strategies that might favorably modulate the gut microbiota to reduce the risk of many NCDs. For example, specific prebiotics promote favorable intestinal colonization, and their fermented products have anti-inflammatory properties. Specific probiotics also have immunomodulatory and metabolic effects. However, when evaluated in clinical trials, the effects are variable, preliminary, or limited in magnitude. Fecal microbiota transplantation is another emerging therapy that regulates inflammation in experimental models. In human subjects it has been successfully used in cases of *Clostridium difficile* infection and IBD, although controlled trials are lacking for IBD. Here we discuss relationships between gut colonization and inflammatory NCDs and gut microbiota modulation strategies for their treatment and prevention. (J Allergy Clin Immunol 2015;135:3-13.)

Key words: Fecal microbiota transplantation, gut microbiome, inflammation, noncommunicable diseases, prebiotics, probiotics, short-chain fatty acids

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The health of our modern society is being threatened by a plethora of chronic inflammatory noncommunicable diseases (NCDs) that have in common an underlying low-grade inflammation. These include early-onset NCDs, such as allergy, asthma, and some autoimmune diseases, and later-onset NCDs, including cardiovascular disease, metabolic disease, and neurodegenerative disorders, which also appear to share common environmental risk factors¹ and genetic risk variants.² Although inflammation and the pathways to disease are multifactorial, the altered gut colonization patterns associated with decreasing microbial diversity are a central theme and increasingly implicated in the physiologic, immunologic, and metabolic dysregulation seen in many NCDs.

The adult gut harbors as many as 100 trillion resident microbes called the microbiota, and the corresponding genome (microbiome) has been estimated to contain 150-fold more genes than the host genome.³ These complex communities have a symbiotic relationship with the host and are involved in many aspects of host physiology. Generally, the 2 main phyla in adults are Bacteroidetes, comprising gram-negative bacteria, and Firmicutes, comprising gram-positive bacteria.⁴ The cell walls of gram-negative bacteria contain LPSs, which induce a strong host inflammatory response to protect against infection. Under normal conditions, finely tuned regulatory responses restrict excessive inflammation and maintain tissue equilibrium.⁵⁻⁸ The pattern of microbial exposure in early

Abbreviations used

CS: Cesarean section
FMT: Fecal microbiota transplantation
IBD: Inflammatory bowel disease
NCD: Noncommunicable disease
q-PCR: Quantitative PCR
SCFA: Short-chain fatty acid

life appears to be important for the development of robust host immune regulation, and disruption in either the microbiota or the host response can lead to chronic inflammation. In this context reduced exposure to commensals, helminths, and other infectious agents in affluent countries is a likely contributor, at least in part, to the increasing propensity for chronic low-grade inflammation and impaired immune regulation.⁹⁻¹¹

Our classical understanding of inflammation is of a normal physiologic response to an infectious threat or tissue injury. Inflammation leads to tissue repair, resolution, and restoration of the homeostatic balance in tissues. However, the chronic inflammatory states of asthma, allergy, obesity, diabetes, atherosclerosis, and other NCDs do not appear to fit this model. There is usually no acute or immediate threat, and there is no resolution. Instead, there appears to be a chronic tissue malfunction and a shift of the normal homeostasis or “balance” to adapt to new physiological or metabolic conditions.¹²

Disruption in the interrelationships between nutrition and the microbiome and host metabolism are likely to be key elements in the disruption of normal homeostasis. Dysbiosis, “an imbalance in microbiota structure and/or function that disrupts host-microorganism homeostasis,” is an emerging feature of many NCDs.^{6,8} This concept is driving strategies to favorably affect the gut microbiota. However, a significant limiting factor is that the normal healthy gut microbiota is yet to be clearly defined, including the range and variant profiles and developmental windows that are likely to fall within this spectrum. To overcome this, large-scale investigations of the human microbiome, such as the Human Microbiome Project^{13,14} and MetaHit,³ are rapidly advancing our understanding by defining microbial compositions and their functions in large populations using coordinated strategies for project planning, analytic techniques, and education programs.¹⁵ Clearly, experimental models have also provided invaluable insights,^{6,8} but our emphasis here is on the human dimension, with a focus on recent observational studies that have assessed intestinal colonization patterns and their associations to NCDs and clinical trials that have explored gut microbiota modulation as a therapeutic strategy in both early- and late-onset NCDs.

METHODS FOR INVESTIGATION OF THE GUT MICROBIOTA AND MICROBIOME

Accelerating development of molecular biology methods to characterize the gut microbiome has rapidly advanced knowledge in this field. Next-generation sequencing, computer technology, and advanced analytic strategies for the handling of ever-expanding biological information (bioinformatics) can produce an unprecedented detailed description of both the genome and metabolic activity of bacteria without requiring growth or

TABLE I. Example of culture-independent methods used in studies assessing the role of the gut microbiota in NCDs

Method	Explanation	Benefits	Weaknesses	Insights from studies using the method in the context of NCDs
q-PCR	Amplification and quantification of 16S rRNA	Confirms phylogenetic identity, quantitative, quick	Cannot identify unknown species, PCR bias	Mucosal biopsy specimens from patients with IBD revealed abnormal gastrointestinal microbiota composition depleted of commensal bacteria (Frank et al, ⁵³ 2007). Delivery mode and exposure to older siblings affected gut microbiota composition assessed in infant stool samples and affected subsequent risk of allergic disease (van Nimwegen et al, ⁵⁰ 2011; Penders et al, ³⁷ 2013).
DGGE	Gel separation of 16S rRNA amplicons using denaturant	Semiquantitative, bands can be excised for additional analysis, quick	Cannot confirm phylogenetic identification, PCR bias	Studies combining DGGE with q-PCR reported reduced microbial diversity in stool samples at 1 and 12 months of age in children with sensitization, allergic rhinitis, and blood eosinophilia but not eczema or asthma at school age (Bisgaard et al, ⁴⁷ 2011) and reported compositional changes in stool samples of diabetic compared with healthy children. Numbers of bifidobacteria and lactobacilli and the Firmicutes/Bacteroidetes ratio correlated negatively with plasma glucose levels, whereas numbers of Clostridia displayed a positive correlation in diabetic children (Murri et al, ⁵⁸ 2013).
T-RFLP	Amplification of fluorescently labeled primers, followed by digestion of the 16S rRNA amplicon by restriction enzymes; separation of digested fragments by using gel electrophoresis	Semiquantitative, quick, cheap	Cannot confirm phylogenetic identification, PCR bias, weak resolution	Reduced gut microbial diversity in stool samples at 1 week of age was associated with increased risk of subsequent eczema (Ismail et al, ⁴⁹ 2012). Combining T-RFLP with q-PCR revealed that the concentration of Enterobacteriaceae was higher in stool samples of obese/overweight children compared with those of normal-weight children (Karlsson et al, ⁶⁶ 2012).
FISH	Hybridization of fluorescently labeled oligonucleotide probes with complementary target 16S rRNA sequences; fluorescence enumerated by using flow cytometry	Confirms phylogenetic identity, semiquantitative, no PCR bias	Dependent on probe sequences and cannot identify unknown species	Bifidobacterial species numbers in stool samples collected at 6 and 12 months of age were higher in children who retained normal weight at school age (Kalliomäki et al, ⁶⁷ 2008).
DNA microarrays	Hybridization of fluorescently labeled oligonucleotide probes with complementary nucleotide sequence; laser used to detect fluorescence	Confirms phylogenetic identity, semiquantitative, quick	Cross-hybridization, PCR bias, might miss species present in low levels	Children with eczema had higher total microbial diversity and reduced abundance of Bacteroidetes in stool samples at 18 months of age compared with healthy control subjects (Nylund et al, ⁵² 2013).
Sequencing of 16S rRNA amplicons	Massive parallel sequencing of partial 16S rRNA amplicons	Confirms phylogenetic identity but not at species level, quantitative, quick, identifies unknown bacteria	Labor intensive, expensive, PCR and amplification bias; need for computational expertise	Reduced total microbial diversity and diversity of Bacteroidetes in stool samples at 1 month of age were associated with increased risk of subsequent IgE-associated eczema (Abrahamsson et al, ⁴⁸ 2012). Reduced total diversity was also associated with increased risk of asthma but not allergic rhinitis at school age (Abrahamsson et al, ⁵¹ 2014). A low abundance of lactate- and butyrate-producing species in stool samples was associated with β -cell autoimmunity (de Goffau et al, ⁵⁷ 2013).
Metagenome sequencing	Massive parallel sequencing of the whole genome	Confirms phylogenetic identity, quantitative	Most expensive method, generates large amounts of data that need computational expertise	Compared with adults with high bacterial richness in stool samples, adults with low bacterial richness had more marked overall adiposity, insulin resistance, and dyslipidemia in association with a more pronounced inflammatory phenotype (Le Chatelier et al, ⁶⁸ 2013).

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DGGE, Denaturing gradient gel electrophoresis; FISH, fluorescence in situ hybridization; T-RFLP, terminal RFLP.

isolation of bacterial species. Despite these advancements, differences in culture-independent DNA-based approaches and sequencing make direct comparison of studies difficult.^{4,16} This is an important consideration for clinicians when evaluating research in this field. Here we provide a short description of modern molecular biology approaches used in observational studies and clinical trials (Table I), and more detailed reviews can be found elsewhere.^{4,15-17} Classical culture techniques can still provide a detailed depiction of a microbial community. However, because the majority of gut bacteria are anaerobic and challenging to cultivate, it is estimated that less than 20% to 30% of the gut microbiota have been cultured.¹⁷ This does not mean that all uncultured organisms are unculturable *per se* but rather that optimal growth conditions have not been designed or discovered.¹⁷

Culture-independent techniques have the advantage of providing a more representative view of the gut microbiota and its diversity.¹⁷ They are based on extraction of DNA and amplification of portions of the 16S rRNA gene (16S), which is highly conserved among bacterial species but variable in other internal regions of the gene and thus allows species identification.^{15,17} In clinical trials methods such as quantitative PCR (q-PCR), denaturing gradient gel electrophoresis, terminal RFLP, fluorescence *in situ* hybridization, DNA microarrays, and next-generation sequencing of the 16S gene or its amplicons have been used.¹⁷ These methods not only provide qualitative and quantitative information on bacterial species but also on microbial diversity and ordination and are increasingly being used in clinical studies.^{15,17} Still, a level of imprecision in 16S-based microbial classification and the associated sequence data have currently hampered capacity to clearly define which specific microbes might be associated with specific diseases, although this is being developed further.^{15,18} It has also been argued that traditional measures of diversity in a microbial community are still relatively crude, and new diversity profiles are being developed to overcome this issue.¹⁹

The 16S sequencing approach is now the most widely used. Although there are many benefits, there are also limitations, mainly that the precision is dependent on whether the proportions of the resulting 16S gene sequences accurately reflect the proportion of bacteria in the original sample because this is affected by copy number variation, the PCR primer, and amplification bias.¹⁵ Cell lysis during DNA extraction can also lead to variation in taxa, such as bifidobacteria. The magnitude of these biases varies substantially between studies.^{15,20}

Another limitation is that 16S sequencing does not provide any information on functional capacity (eg, metabolic capacity). One way of addressing this is through predictive bioinformatics methods. Because the human intestine is a community with several available reference genomes, bioinformatics software packages can be used to predict an approximate metagenome.^{15,17,21} This is done by connecting functions of gene products encoded by the most closely related sequenced genomes with observed taxonomic profiles, thereby creating a functional profile.^{4,15,21}

Alternatively, metagenome or metatranscriptome sequencing is now being used to attain a functional profile and higher resolution of the composition of bacterial communities (identification to the species and also strain level). This is also known as shotgun sequencing, DNA sequencing, or RNA sequencing and refers to sequencing the entire nucleotide pool

isolated from a sample.¹⁵ In contrast to 16S studies, this technique also sequences a small amount of human DNA and also Archaea, fungi, and viruses found in a stool sample.^{15,16} The latter can add valuable information because microbes other than bacteria might also have a role in forming mucosal immune responses,⁵ although less is known about these exposures in the context of NCDs, and they will not be covered in this review. Compared with 16S studies, metagenome studies are more expensive, and analysis of the massive amount of data generated requires additional computational expertise.^{16,17} Metaproteomic and metabolomic analyses are other emerging methods that further characterize the functional capacities of the microbiome.^{15,16}

The site and nature of sample collection are also important. Most published studies have analyzed microbial communities in stool samples rather than using mucosal biopsy specimens or analysis of luminal content, which differ in composition. Although the collection of mucosal biopsy samples pose practical and ethical challenges, it is recognized that the mucosal microbiota might be more pertinent for human disease.¹⁷

GUT MICROBIOTA ARE ESSENTIAL FOR NORMAL DEVELOPMENT AND REGULATION: MULTISYSTEM EFFECTS

The critical role of the gut microbiota in immune development has been well documented in germ-free animal models, demonstrating the failure of normal maturation and, in particular, failure of the systemic immune regulatory networks, which result in both allergic and autoimmune phenomena, as reviewed by Renz et al,⁵ Sommer and Bäckhed,⁶ Garn et al,⁷ and West et al.⁸ Significantly, early experiments clearly demonstrated a critical early developmental window during which microbial colonization could induce appropriate maturation of the T_H2 response and IgE regulation, after which this was no longer possible.²² More recently, it has been shown that diversity of colonization is crucial for development of an immunoregulatory network that protects against IgE induction in the mucosa.²³ It remains unclear whether such a window exists also in human subjects, but this is nonetheless consistent with observations that gut microbial exposures in infancy might affect the maturation of mucosal and systemic immune responses in infants.^{8,24,25}

The proinflammatory and metabolic effects of altering the gut microbiota have also been observed in animal models of obesity.⁶ Notably, more subtle manipulation with low doses of antibiotics in early life (at levels likely to be encountered in human food) increased the risk of adiposity, with significant effects on short-chain fatty acid (SCFA) levels and regulation of the hepatic metabolism of lipids and cholesterol.²⁶ Equally fascinating are results from experimental models reporting changes in behavior and gene expression in the brain after changes in the gut microbiota.^{6,8}

Possibly less well known to immunologists, in another aspect of their work in germ-free animals, Sudo et al²⁷ also demonstrated reduced expression of the neuronal growth factor brain-derived neurotrophic factor in the cortex and hippocampus, which is consistent with patterns seen in depression and anxiety. They also had heightened cortisol stress responses. This shows that without normal bacteria, germ-free animals will experience potentially maladaptive changes in brain function and endocrine regulation of the stress response through the

hypothalamic-pituitary-adrenal axis. Critically, these effects can all be reversed by either administering probiotics or by means of fecal transplant with normal mouse microbiota.²⁷

Perhaps of even greater significance, timing was critical; reversal is only possible at an early stage of development. Once the response patterns become established with age, they cannot be readily changed. This is true of both the stress and immune responses, which remain abnormal once they are committed. It is also consistent with what we know about critical early periods for both immune and neural development and the decreasing plasticity of neural networks with age. Collectively, these findings form a basis to further explore the role of gut microbiota in early programming of host responses also in human subjects.

ESTABLISHMENT OF THE HUMAN GUT MICROBIOTA

The human gut microbiota evolves through ecologic succession, with compositional and functional changes over the first years of life²⁸ eventuating in a relatively stable microbial community.^{4,28} This is influenced by interactions between the environment, diet, and microbe-associated and host-related factors.^{29,30} For the majority of human subjects, the first postnatal microbial exposures originate from the maternal vaginal and perianal microbiota. Shortly after delivery, the gut microbiota of a vaginally delivered infant resembles that of his or her mother's vagina.³¹ In contrast, infants delivered by means of cesarean section (CS) initially acquire microbial communities typically found on maternal skin, followed by the gradual acquisition of a more complex microbiota, although typically more slowly than in vaginally delivered infants. However, there is high individual variability in the patterns of bacterial species and the timing of acquisition.^{4,28} Even so, across individual subjects and populations, infant microbiomes generally have a higher proportion of bifidobacteria and lower species richness than those of adults²⁸ and share common functional characteristics, such as infants typically showing metagenomes enriched in genes for simple sugar breakdown and folate synthesis.^{4,28}

Microorganisms colonizing the gastrointestinal tract are involved in a number of vital processes.^{5,6,8,29} With about 70% of the cellular component of the immune system present as gut-associated lymphoid tissue, the potential for crosstalk between the microbiota and the immune system is substantial and likely to play a central role in enabling microbial imprinting and immune programming of the newborn microbiota.⁸ Recent discoveries that bacterial DNA is present in the newborn's first stool (meconium)^{32,33} and in the fetoplacental unit,^{33,34} with the placental microbiome profiles being most similar to those of the oral microbiome,³⁴ challenges the "sterile womb" paradigm. This suggests that imprinting of the gut microbiota might commence already *in utero* and is then further shaped by postnatal exposures.^{8,30,33}

ENVIRONMENTAL FACTORS AFFECT GUT MICROBIOTA ESTABLISHMENT IN INFANTS

In addition to CS,^{31,35-37} antibiotics given to the mother or infant,²⁹ breast-feeding,^{35,37} and introduction of solid foods²⁸ influence establishment of the gut microbiota. Infants born by means of CS have a higher incidence of respiratory distress³⁸ and appear to be at higher risk for asthma and atopy,³⁹ obesity,⁴⁰

and type 1 diabetes.⁴¹ It has been hypothesized that delayed and aberrant intestinal colonization might contribute to this risk conferred by CS delivery. Recent studies that have applied DNA-based approaches have demonstrated perturbations in infant gut microbiomes, with lower diversity of Bacteroidetes³⁶ and abundance of the genus *Bacteroides* in CS-delivered infants,³⁵⁻³⁷ suggesting that dysbiosis might contribute at least partially. Beyond its direct effect on microbial exposure, CS requires antibiotic treatment and can delay the onset of breast-feeding, which can lead to further ecologic perturbations.³⁵⁻³⁷ Retrospective studies have reported antibiotic treatment in early life to be associated with increased risk of asthma, although the results might still be influenced by reverse causation or confounding by indication.⁴²

The gut microbiota evolves over the preschool years, with an adult-like profile emerging by 3 to 4 years of age in human subjects.^{4,28} Established microbiota of adults can be divided into different enterotypes based on clusters of predominant bacterial genera in the gut microbiome.⁴³ As yet, it is not clear how these enterotypes are formed, but this process is likely to be influenced by diet and other environmental factors.^{4,43,44} Recently, it has also been argued that there are gradients of key genera rather than distinct clusters.^{4,45,46}

AN ABERRANT GUT MICROBIOME IN PATIENTS WITH EARLY- AND LATE-ONSET NCDs

Changes in the gut microbiome are the inevitable result of complex changes in both our nutritional patterns and our built environment. The consequences of early environmental change are evident in the dramatic increase in early-onset inflammatory NCDs (eg, allergic disease). Effects on the developing immune system are consistent with the hygiene hypothesis, which initially focused on decreasing exposures to "external" infectious microorganisms.⁹ A more recent focus on the gut microbial deprivation hypothesis has shifted the emphasis to the importance of gut microbial exposures for normal immune development and regulation.¹¹ This concept is supported by culture-independent DNA-based studies demonstrating associations between reduced gut microbiota diversity and early-onset NCDs, including atopy,⁴⁷ eczema,^{37,48-50} and asthma.⁵¹ However, this has not been seen in all studies.⁵²

Also, in patients with intestinal inflammatory disease, several studies have now reported dysbiosis to be associated with inflammatory bowel disease (IBD)⁵³ and celiac disease.⁵⁴ Increased gut permeability and intestinal inflammation with impaired immune-regulatory mechanisms have been shown in children with allergic disease⁵⁵ and also in children with type 1 diabetes.⁵⁶ Consistent with reports of dysbiosis in children with allergic manifestations, an aberrant gut microbiome has been associated with type 1 diabetes.^{57,58} 16S sequencing revealed differences in the gut microbiome in children with signs of β -cell autoimmunity compared with that in healthy children of the same age and sex with a similar feeding history and genetic risk.⁵⁷ In particular, children with β -cell autoimmunity had lower levels of bifidobacteria, which normally enhance intestinal epithelial barrier function and suppress inflammation. For comparison, by using denaturing gradient gel electrophoresis PCR and q-PCR, dysbiosis was observed in diabetic children, with numbers of bifidobacteria and lactobacilli and the Firmicutes/Bacteroidetes ratio correlating negatively with plasma

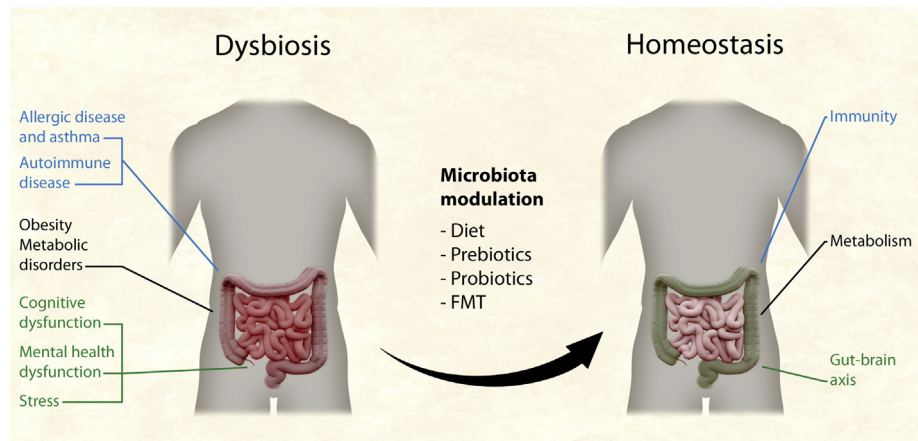


FIG 1. Dysbiosis, an “imbalance in the structure and/or function of the microbiota that leads to disruption of host-microorganism homeostasis,”⁶ has been implicated in a broad range of inflammatory disease states. There is also suggestive evidence that changes in gut microbiota have implications for cognitive and mental health dysfunction and stress responses. These diverse multisystem influences have sparked interest in strategies to favorably modulate the gut microbiota to attain homeostasis.

TABLE II. Treatment strategies for gut microbiota modulation in patients with NCDs

Treatment	Definition	References
Probiotics	“Live microorganisms which when administered in adequate amounts confer a health benefit on the host” ⁶⁹	Bertelsen et al, ⁷² 2014 Kalliomäki et al, ⁷⁴ 2007 Jensen et al, ⁷⁵ 2012 West et al, ⁷⁶ 2013 Abrahamsson et al, ⁷⁷ 2013 Wickens et al, ⁷⁸ 2013 Loo et al, ⁷⁹ 2014 Azad et al, ⁸⁰ 2013 Kuitunen et al, ⁸² 2009 Wang et al, ⁸³ 2014 Chorell et al, ⁸⁴ 2013 Kadooka et al, ⁸⁵ 2010 Luoto et al, ⁸⁷ 2010
Prebiotics	Nondigestible fermentable oligosaccharides	Thorburn et al, ⁹⁰ 2014 Osborn and Sinn, ⁹¹ 2013 Vulevic et al, ⁹² 2013
Dietary fiber	Nondigestible carbohydrates of plant origin	Thorburn et al, ⁹⁰ 2014
FMT	Transplantation of fecal bacteria from a healthy donor to a recipient	Smits et al, ⁹⁴ 2013 Kassam et al, ⁹⁵ 2013 van Nood et al, ⁹⁶ 2013 Postigo et al, ⁹⁷ 2012 Hamilton et al, ⁹⁸ 2013

glucose levels, whereas numbers of *Clostridia* displayed a positive correlation.⁵⁸

It has also been suggested that the early microbial environment drives more sustained predisposition to low-grade inflammation into adulthood and the propensity for later-onset NCDs.⁵⁹ In high-income countries baseline C-reactive protein levels, as a measure of the inflammatory state, are higher than in traditional environments, are a risk factor for cardiovascular disease⁶⁰ and type 2 diabetes,⁶¹ and are associated with higher all-cause mortality.⁶² A significant component of the risk of these disorders is programmed during early development,⁶³ and many children already exhibit the inflammatory antecedents of cardiometabolic risk.⁶⁴ Therefore it has been hypothesized that the early

environment and early patterns of microbial exposure might influence the dynamics of inflammation in adulthood.⁵⁹ Although there has been a focus on infectious exposures, the commensal gut microbiota, which is also heavily influenced by early nutrition, is likely to play an even more important role in developing immune and metabolic homeostasis. Some support for this was recently reported in mice, in which low-dose penicillin in early life transiently affected the gut microbiota, affected ileal expression of genes involved in immunity, and induced long-term metabolic perturbations.⁶⁵ Notably, the combination of low-dose penicillin and dietary excess further increased fat mass.

Although diet and a sedentary lifestyle are important contributors to the obesity epidemic, there is growing recognition that gut microbiota regulate metabolic function and energy balance.⁶ Studies in obese adults reported reduced diversity, an increase in Firmicutes, and a corresponding decrease in Bacteroidetes, although this was not seen in all studies.^{4,6,43} Recently, a tendency toward decreased gut microbiota diversity in overweight/obese children assessed by using q-PCR and terminal RFLP was reported.⁶⁶ By using fluorescence *in situ* hybridization, reduced numbers of bifidobacteria in infancy have also been shown to precede the development of obesity.⁶⁷ Aberrations in the gut microbiota might also have implications for obesity-associated NCDs. In a recent report adults with reduced gut microbial richness had higher overall adiposity, insulin resistance, and dyslipidemia and a more pronounced inflammatory phenotype than adults with higher gut microbial richness.⁶⁸ Dysbiosis has even been associated with neurodevelopment and mental health in a series of experimental models.⁶ Whether microbial variation is the cause or effect of these diseases is still the subject of conjecture, but this highlights the multisystem effects of the microbiome.

MODULATION OF THE GUT MICROBIOTA FOR TREATMENT AND PREVENTION OF NCDs

Collectively, the above has led to intense interest in strategies to correct dysbiosis for treatment purposes and to favorably affect intestinal colonization to prevent NCDs (Fig 1).

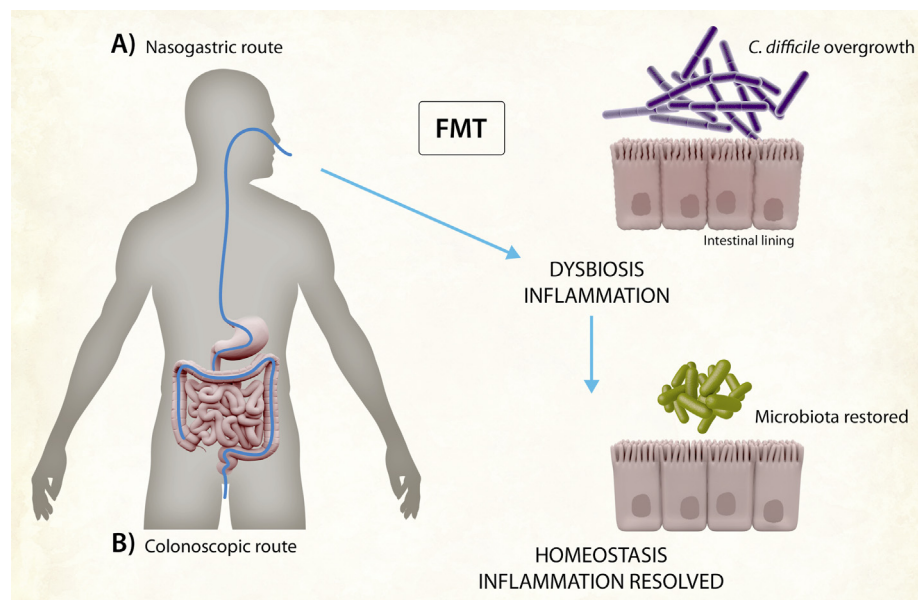


FIG 2. Overuse or repeated courses of broad-spectrum antibiotics disrupt the normal ecology, allowing colonization of *C difficile* and leading to dysbiosis. FMT with feces from a healthy donor administered through the nasogastric (A) or colonoscopic (B) routes restores microbial ecology and resolves inflammation.

Probiotics

The most widely used approach has been to administer probiotics (Table II).⁶⁹ In most studies single or several strains of lactobacilli and bifidobacteria have been used for the treatment and prevention of allergic disease; the demonstrated effect of one probiotic strain cannot be extrapolated to another strain. Some probiotics exert immunomodulatory effects, mostly shown in experimental models but also in human intervention studies.^{8,70} Mechanistic effects are thought to be mediated by Toll-like receptors to promote T_H1 cell differentiation through enhanced production of IL-10, TGF- β , and IgA; inhibition of antigen-induced T-cell activation; and suppression of TNF.^{8,70} The timing of probiotics for promoting immune tolerance appears critical,⁸ and a combination of prenatal and postnatal probiotic supplementation for allergy prevention (namely eczema) has shown the most consistent benefit, although their routine use cannot be recommended.^{8,30,70} To date, reports on later-onset allergic manifestations, such as respiratory allergic disease, from initiated cohorts are limited and need to be prospectively collected and reported.^{8,30,70} If prenatal microbial exposure enhances the preventive effect, as also suggested by epidemiologic^{71,72} and experimental⁷³ studies, starting supplementation in the second trimester of pregnancy, when circulating fetal T cells have developed, might be more effective.⁸ This might include effects on asthma development, which thus far have not been prevented by probiotic interventions.⁷⁴⁻⁸⁰ Using nonconventional probiotics, such as butyrate and propionate producers, immunomodulatory strains of *Bacteroides* species^{8,30} or *Clostridia*⁸¹ might also induce stronger effects. Another area to explore is that of probiotic treatment to CS-delivered infants because data suggest that they might benefit the most.⁸²

The preventive effects of probiotics on other NCDs would be highly interesting to evaluate, but their low incidence, delayed onset, or both compared with allergic diseases make

such studies challenging to perform. Some probiotics have been demonstrated to exert beneficial metabolic effects in experimental models⁸³ and human intervention studies.⁸⁴⁻⁸⁷ There is also growing appreciation that probiotics might have a role in gut-brain function and central nervous system-related conditions, as reviewed by Borre et al.⁸⁸ However, more research is needed for the mechanistic understanding of probiotics, and as yet, probiotics do not have an established role in the treatment or prevention of any NCD. Many meta-analyses of clinical trials using probiotics have been carried out; however, there has been substantial heterogeneity in several aspects of these studies.¹ If we are to translate findings from conducted studies into recommendations in clinical practice, there is need for a more standardized approach using harmonized protocols and outcome measures in clinical trials.^{8,70}

Dietary fiber and prebiotics

Observational studies have reported an association between the high-fat/low-fiber westernized diet and the prevalence of many NCDs, including allergic disease.⁸⁹ With these data in mind, modulation of the dietary substrate of gut microbiota (Table II) might be part of strategies for the prevention and treatment of NCDs. Dietary fibers pass through the upper intestine and are fermented by large-bowel anaerobic microbiota to produce SCFAs.⁹⁰ SCFAs promote gut epithelial integrity and exert immune effects, including stimulation of G protein-coupled receptors, promotion of innate (Toll-like receptor 2) immune responses, and induction of regulatory T cells.⁹⁰

In experimental models switching from a high-fiber to a low-fiber diet induces dramatic changes in gut microbiota composition, such as a decrease in gut bacteria of the Bacteroidetes phylum.⁹⁰ Concordantly, infants in rural Africa, where mothers and infants have a traditional diet very

TABLE III. Current knowledge gaps of the role of the gut microbiota in patients with NCDs and suggestions for further work

Current situation	References	Future prospects
The healthy gut microbiome is still not clearly defined.	Lozupone et al, ⁴ 2012 West et al, ⁸ 2014 Gevers et al, ¹³ 2012 Aagard et al, ¹⁴ 2013	There is need for further characterization of both gut microbial ecology and the functional activities of the gut microbiota.
Dysbiosis and/or reduced gut microbial diversity have been reported in human studies in various NCDs: cause or effect?	Gosalbes et al, ³² 2013 Penders et al, ³⁷ 2013 Bisgaard et al, ⁴⁷ 2011 Abrahamsson et al, ^{48,51} 2012 and 2014 Ismail et al, ⁴⁹ 2012 van Nimwegen et al, ⁵⁰ 2011 Nylund et al, ⁵² 2013 Frank et al, ⁵³ 2007 Cheng et al, ⁵⁴ 2013 de Goffau et al, ⁵⁷ 2013 Murri et al, ⁵⁸ 2013 Karlsson et al, ⁶⁶ 2012 Kalliomäki et al, ⁶⁷ 2008 Le Chatelier et al, ⁶⁸ 2013 Korpela et al, ⁹³ 2014	Multidisciplinary integrative approaches and careful monitoring of gut microbiota, other environmental exposures, and host-specific factors before disease onset will further our understanding.
There is potential confounding in observational studies because of lack of information on all exposures that might have affected the gut microbiota and/or short period of observation.	Lozupone et al, ⁴ 2012 Gevers et al, ¹³ 2012 Aagard et al, ¹⁴ 2013	Longitudinal studies in multiple settings using the same protocols and methodological platforms might enable direct comparison of studies.
Current gut microbiota modulation strategies for treatment and prevention are preliminary, variable, or limited in magnitude.	West et al, ⁸ 2014 Fiocchi et al, ⁷⁰ 2012 Ellwood et al, ⁸⁹ 2012 Osborn and Sinn, ⁹¹ 2013 Smits et al, ⁹⁴ 2013	Monitoring of long-term effects and safety, especially for FMT, is needed. Development and evaluation of optimized dietary interventions, prebiotics, nonconventional indigenous gut bacteria as next-generation probiotics, and FMT are needed.

high in fermentable carbohydrates, have substantially higher Bacteroidetes and SCFA levels in stool compared with infants from Europe, where mothers and infants have a Western diet.⁴⁴ In relation to early-onset NCDs, there is preliminary evidence that feeding prebiotics (Table II) from early infancy might prevent eczema, but these findings are yet to be confirmed.^{8,30,91} In the most recent Cochrane review,⁹¹ which included 1428 infants at high risk of allergic disease, prebiotics added to infant feeding reduced the risk of eczema (risk ratio, 0.68; 95% CI, 0.48-0.97), with no effect on any other allergic outcomes.

There are also clinical trials that have assessed prebiotics for metabolic outcomes. In overweight adults treatment with galactooligosaccharides induced “favorable” changes in gut microbial composition, increased secretory IgA levels, and reduced inflammation and measures of the metabolic syndrome.⁹² Notably, the combination of a healthy dietary intervention (increased fiber/reduced fat) and probiotics in pregnancy decreased the risk of gestational diabetes compared with the healthy dietary intervention alone or no intervention.⁸⁷ Either alone or in combination, dietary approaches might be a useful tool for gut microbiota modulation in both the treatment and prevention of NCDs. However, the effects of dietary modulation on the gut microbiota and host response appear highly individual and difficult to foresee. Interestingly, a proof-of-concept study combining microbiome data sets from different dietary intervention studies in obese adults, clostridial species in particular, indicated the amenability of the gut microbiota to dietary modulation, which in turn was associated with the host’s lipid metabolism.⁹³

Fecal microbiota transplantation

There has been emerging interest in fecal microbiota transplantation (FMT; Table II) in both the treatment and prevention of NCDs.⁹⁴ It was first used in the treatment of *C difficile*-induced diarrhea, with a high rate of clinical resolution and no observed adverse events, although these initial trials were not controlled.⁹⁵ The principal of FMT for this indication is predicated on the concept that antibiotic therapy (repeated courses or overuse of broad spectrum antibiotics) disrupts the normal ecology, allowing colonization of *C difficile* (Fig 2). FMT offers both an investigational tool in rodent models to study the role of microbes in disease development and treatment response,⁶ as well as a new therapeutic intervention that has gained credibility in the clinical world after publication of the first RCT demonstrating the effectiveness of FMT for treatment of antibiotic-resistant *C difficile*-induced diarrhea.⁹⁶ The authors also demonstrated a significant increase in Bacteroidetes species and *Clostridium* species clusters IV and XIVa and a decrease in Proteobacteria in line with healthy donor profiles.

Despite the lack of clear evidence of whether microbial variation is the cause or effect of IBD, there are now several trials of FMT for the treatment of IBD with modest results, although no controlled trials have yet been published.⁹⁴ FMT might be a future treatment option for many conditions; however, a truly controlled trial design might consider autologous versus allogeneic fecal transplant to ensure there is not a colonoscopy or placebo effect.

There are also many recipient and donor factors to consider (Table III). Age is an important factor for both the donor and the

recipient because there is a significant increase in the diversity and stability of gut microbiota over the first years of life.^{4,28} Compositional differences might affect susceptibility to disease but also response to treatment. Factors that might influence the composition of the donor sample include diet, antibiotics/probiotics, and a range of environmental exposures, as well as underlying disease status.⁹⁴ From a practical clinical perspective, the route of administration is important, and at least for the treatment of *C difficile*, colonoscopic and nasogastric administration appear similarly effective,⁹⁷ although a higher stool volume was used for FMT in the colonoscopy group. Notably, standardized partially purified and frozen fecal microbiota might be just as effective as the current fresh-harvest approach,⁹⁸ which might facilitate its clinical use.

FUTURE DIRECTIONS

Although NCDs represent a wide range of diseases, we can see common threads that trace back to the increased tendency of inflammation driven by virtually inseparable changes in nutrition and the gut microbiota. The end result of the inflammatory and metabolic changes might vary with genetics,² epigenetics,⁶³ overall biodiversity,^{10,99} and other risk factors; this provides an attractive shared explanation for why so many of these inflammatory conditions have increased during the same time period under the influence of the same environmental changes. If we are to stand a better chance of solving the complex pathogenesis of NCDs and understanding the role of the gut microbiota in this process, we must call for interdisciplinary approaches working collaboratively in networks.^{1,8} With standardized outcomes and methodologies, we will be better apt to address current knowledge gaps (Table III) as follows:

1. Research should be focused on the ecologic and functional properties of a healthy gut microbiome. Results from the Human Microbiome Project and MetaHit are anticipated to provide more insight.
2. Strategies to improve gut colonization patterns need to be developed because they might have lifelong influences on health.
3. Although dysbiosis and reduced diversity are centrally implicated in many NCDs, it still remains to be determined whether microbial variation is the cause or effect.
4. Longitudinal studies with the same protocols and methodological platforms are needed to overcome potential confounding.
5. Looking at the bacterial components of the microbiome might be overly simplistic and should ultimately include other aspects of the microbiome.
6. A research priority should be the development of microbiota signatures in specific disease states.
7. Ultimately, these can then be used in clinical practice for improved diagnostic, treatment, and prevention strategies that might include optimized dietary interventions, prebiotics, nonconventional indigenous gut bacteria as next-generation probiotics, and FMT. Such multidisciplinary and integrative approaches might ultimately lead to improved strategies to overcome the disease epidemics of modern civilizations.

What do we know?

- Experimental models demonstrate that gut microbiota exert physiologic, metabolic, and nutritional functions and are vital for normal immune development and regulation.
- Observational studies show variations of gut microbiota and reduced microbial diversity to be associated with various NCDs, such as eczema, allergic rhinitis, asthma, autoimmune disease, and obesity.
- Meta-analyses of clinical trials using probiotics for allergy prevention have demonstrated a reduced incidence of eczema but not any other allergic outcomes.
- Meta-analyses of a limited number of clinical trials using prebiotics for allergy prevention suggest a reduction of eczema, although only in high-risk populations.
- FMT regulates inflammation in experimental models.

What is still unknown?

- Whether the dysbiosis and reduced microbial diversity seen in many NCDs is causal
- How probiotics mediate their clinical effects in NCDs
- Whether prebiotics have a role in the prevention of allergic disease in high- and low-risk populations
- What the long-term effects of FMT are in human subjects, both clinical benefit and safety aspects

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