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Proceedings of the 2014 A.S.P.E.N. Research Workshop: The Interface Between Nutrition and the Gut Microbiome: Implications and Applications for Human Health

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Abstract

The human and earth microbiomes are among the most important biological agents in understanding and preventing disease. Technology is advancing at a fast pace and allowing for high-resolution analysis of the composition and function of our microbial partners across regions, space, and time. Bioinformaticists and biostatisticians are developing ever more elegant displays to understand the generated megadatasets. A virtual cyberinfrastructure of search engines to cross-reference the rapidly developing data is emerging in line with technologic advances. Nutrition science will reap the benefits of this new field, and its role in preserving the earth and the humans who inhabit it will become evidently clear. In this report we highlight some of the topics of an A.S.P.E.N.-sponsored symposium held during Clinical Nutrition Week in 2013 that address the importance of the human microbiome to human health and disease. (*JPEN J Parenter Enteral Nutr*. 2014;38:167-178)

Keywords

adult; life cycle; pediatrics; genomics; research and diseases

Introduction

The emerging role of the microbiome in human health and disease is being defined across various diseases and disorders that span every aspect of human illness. Diseases, their progression, and even human behaviors not imagined to be influenced by our microbiome are now being defined by subtle changes in the composition and function of microbiota present in various compartments from skin to feces. There is no doubt that nutrition, from as early as in utero, through the neonatal period, and up to adulthood, has a profound effect on the shape and trajectory of our body's microbiome. Technical capabilities in genomics, proteomics and metabolomics, and bioinformatic management are now a reality, and the information generated is nothing short of startling in revealing the immense influence that our microbiome has on our early development, behaviors, susceptibility to disease, and recovery from disease. Although the data display can be enormous and appear complicated at first, advances in bioinformatics and biostatistics are making pattern and signature recognition ever more understandable even to the uninitiated. Interpretation of changes in the composition and function of the microbiome must also be contextualized to the spatial and temporal dynamics that constantly exist in complex microenvironments such as the mouth, gut, vagina, skinfolds, and elsewhere. The virtually limitless capacity to sample and analyze across the spatioregional landscape of these various compartments and provide temporal and clinical contexts to the development of and recovery from disease has the potential to generate an unimaginable number of novel hypotheses to explain conditions that have remained beyond

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the reach of medical science, such as autism, antibiotic resistance, outcome from sepsis, and autoimmune disease, to name a few. Sequence technology and mass spectroscopy are becoming better, faster, and cheaper. The future of medical science will embrace these efforts as systems biology takes the front stage in explaining the human condition, from early development to disease incidence and disease recovery.

Nutrition science will reap enormous benefits in defining the systems biology of human disease since what and how we eat affects every aspect of our integrated physiology. The "first pass" aspect of nutrients as they enter the human intestinal bioreactor is an open line of inquiry. When this first pass effect is eliminated, such as occurs with parenteral nutrition, much of human physiology is changed. When antibiotics alter the human intestinal bioreactor, nutrients, drugs, and overall metabolism are changed. Finally, when foreign invaders take hold, such as occurs in *Clostridium difficile* colitis, reestablishing the core microbiome may be the patient's only chance for recovery. Last, the etiopathogenesis of complex autoimmune diseases such as inflammatory bowel disease may only be disentangled by understanding and defining how the microbiome interacts with the immune system to trigger and sustain mucosal inflammation.

In this report, leaders in the field of microbiome research highlight a few of the above concepts. The symposium took place as a workshop during Clinical Nutrition Week in February 2013. The workshop was organized to introduce the idea that nutrients play a major role in shaping a core microbiome that directly interacts with every aspect of human physiology, immune function, and health. As such, nutrition science and its clinical application will need to align with efforts in microbiome research and incorporate many of its finding into research and clinical care in the field.

A major aspect of incorporating microbiome research into nutrition sciences is to recognize the importance of diversity as a key determinant of microbial community health and function. This is reviewed by Dr. Jack Gilbert, associate professor of ecology and evolution. Recognizing the effect of nutrition management on the microbiome is also important, and this is addressed by Dr. Daniel H. Teitelbaum, professor of pediatric surgery. Precisely how the microbiome influences the incidence and progression of complex diseases such as necrotizing enterocolitis and inflammatory bowel disease is addressed by Dr. Michael J. Morowitz, assistant professor of pediatric surgery and Dr. B. Eugene Chang, professor of medicine. Last, Dr. Michael J. Sadowsky, professor of microbiology, and others discuss how disease states such as recalcitrant and relapsing *C difficile* diarrhea may progress beyond antibiotics and nutrition management and may require refaunation of the core microbiome through fecal transplantation.

Closing remarks by Dr. John Alverdy, professor of surgery and director of the workshop, explain how the incidence and outcome of sepsis may be influenced by loss of the core microbiome and how future strategies might include methods to preserve the core microbiome.

What is learned is that microbial communities are diverse, complex, and important components of the biology of all higher animals, including humans. Research is just beginning to elucidate these complexities and is both exciting and daunting. As our understanding expands, it is becoming clear that all facets of medical science will need to incorporate microbiome effects into current paradigms of disease pathophysiology.

Modeling the Microbiome: Creating Predictive Models From Deterministic Patterns (Jack Gilbert)

Most microbial communities exhibit exquisitely complex structure. Many aspects of this complexity, from the number of species to the total number of interactions, are currently very difficult to examine directly. However, extraordinary efforts are being made to make these systems accessible to scientific investigation. While recent advances in high-throughput sequencing technologies have improved accessibility to the taxonomic and functional diversity of complex communities, monitoring the dynamics of these systems over time and space—using appropriate experimental design—is still expensive and not commonplace. Fortunately, modeling can be used as a lens to focus low-resolution observations of microbial communities to enable mathematical abstractions of functional and taxonomic dynamics. Here we review the approaches for modeling bacterial diversity at both the very large and the very small scales at which microbial systems interact with their environments.

To understand microbial systems, it is necessary to consider the scales at which they interact with their environment. These scales range spatially from microns to kilometers and temporally from eons to hours. Accounting for 350–550 billion tons of extant biomass, microbes are the principal form of life on earth, and they have dominated earth's evolutionary history.[1] While their effects might be global, microbial systems interact with their environments at microscopic scales. A single gram of soil might contain around 109 microbial units,[2] and an average milliliter of seawater will contain approximately a million bacterial cells. The wide taxonomic diversity of the populations[3] is fostered, at least in part, by myriad micro-environments accessible to the bacteria. In soil and marine systems, the majority of microbial diversity is represented in the minority of biomass.[3–6] Generally, in highly diverse microbial communities a few highly abundant taxa predominate in a community with a long tail of low abundance taxa.[4] These low-abundance taxa in particular are crucial to our understanding of microbial ecosystems, as they represent the vast functional diversity that can rapidly blossom to high abundance under the appropriate environmental conditions.[7,8]

Microbial systems can be described using environmental DNA sequence information and contextual metadata, which reveal dynamic taxonomic and functional diversity across gradients of natural or experimental variation.[9–13] Taxonomic diversity is a measure of the community species composition and relative abundance of its members, which is maintained or altered via interactions and adaptations between each species and with environment. Functional diversity is a measure of the frequency and the type of predicted enzyme functions encoded in a community's metagenome and represents the potential to express a phenotype that interacts with a particular environmental state. The continuously increasing depth of observation provided by contemporary sequencing technologies has now enabled whole genomes to be reassembled from metagenomic data, which permits appropriate descriptions of the taxonomic and functional potential of individual species imbedded within each community.[14–16]

Each community, whether on the epithelial lining of the gut, in an oral cavity, embedded in a desiccated soil particle, or in a biofilm attached to a hermit crab in a coral sea, presents a potentially unique set of interactions with the ecosystem. Microbial ecology aims to characterize these dynamics in different environments and identify trends and relationships that predict these dynamic patterns. The aim is to generate predictive models that incorporate taxonomic and functional diversity into the biological, chemical, and physical interactome, for microbial communities at the micro (bottom-up) and macro (top-down) scales.

The human microbiome is particularly interesting as it shows both amazing resilience to perturbation and also dynamic variability over time and body site.[17] The stability of the microbiome in humans has been shown to increase with the age of the subject. In fact, during the first year of life microbial communities are extremely unstable, and they start to reach "adult" stability around 1–1.5 years of age.[18] Their diversity and stability have been linked to many aspects of human disease, including mental health and neurological conditions in general.[19,20] Of specific interest is the role that the intestinal microbiota plays in neurological conditions such as autism,[21-25] with strong evidence that the microbiome shows significant correlations with autistic traits.[26] To determine where the microbes that colonize our bodies and affect our health and well-being originate, we have instigated a series of comprehensive studies into the microbiology of homes (www.homemicrobiome.com) and hospitals (www.hospitalmicrobiome.com). These are designed with longitudinal interpretation in mind, so that the diversity and succession of the bacteria in these living environments can be tracked against the resultant microbial colonization and shed from the humans and animals in that ecosystem. The aim is to create predictive models that can be used to predict when certain types of bacteria will become abundant and what that will mean in terms of impacts on human and building health. Comprehensively, this means encapsulating the relationships between bacteria, the environments, and the humans and animals that live in those ecosystems. This will lead to the development new architectural and building design practices.

Parenteral Nutrition and the Intestinal Microbiome (Daniel H. Teitelbaum)

Parenteral nutrition (PN), or enteral nutrient deprivation, is commonly used as treatment for many patients, ranging from short-term use in patients with gastrointestinal dysfunction[27] to long-term use in patients with short bowel syndrome.[28] While it is life-saving for many, PN use is associated with numerous complications ranging from an increase in enteric-derived infections to a loss of immune reactivity.[29,30] Previous studies from our laboratory and others have shown that in a mouse model of PN, a number of significant physical and immunologic changes occur in the intestinal mucosa.[31] Physically, there is atrophy of small intestinal villi, an increase in intestinal epithelial cell apoptosis, and a decrease in intestinal epithelial cell proliferation.[32] Immunologically, a proinflammatory state develops within the gastrointestinal tract, including increased mucosal and intraepithelial lymphocyte-derived tumor necrosis factor-α (TNF-α), interferon-γ, and decreased interleukin-10.[33,34] However, the mechanisms driving these changes are unknown. The implications of this shift toward a proinflammatory state, however, can be a major contributor to the known association of PN with subsequent loss of epithelial barrier function[34–36] and increases in bacterial translocation.[37,38]

Over the past few years, the scientific community has developed a great appreciation for the dependence of the intraluminal microbial population on the supply of enteral nutrients delivered to these microbes. The intestinal microbiota is highly sensitive to local environmental changes, and the composition of the population may be rapidly altered in response to such dramatic changes in the local environment.[39,40] Because of this, our group hypothesized that the acute deprivation of enteral nutrition with the administration of PN would place the intestinal microbiota into an abrupt state of nutrient withdrawal. The consequences of PN administration on the resident microbial community during this environmental change have not been fully addressed but might have far-reaching consequences on the health of the host.

Figure 1 shows a dramatic shift in the composition of the microbial population with the administration of PN from *Firmicutes*-rich to a population dominated by *Proteobacteria* and *Bacteroides*.[41] The major way that these microbes signal host enterocytes and immunocytes is via the Toll-like receptor pathway and through the myeloid differentiation primary response gene 88 (MyD88), with subsequent activation of nuclear factor-κ (NFκB) signaling. NFκB activation is known to mediate the expression of several proinflammatory cytokines including TNF-α.[42] We have demonstrated that blockade of the MyD88 pathway using MyD88 knockout mice resulted in prevention of the proinflammatory state within the epithelium of the small bowel, with subsequent preservation of T-regulatory

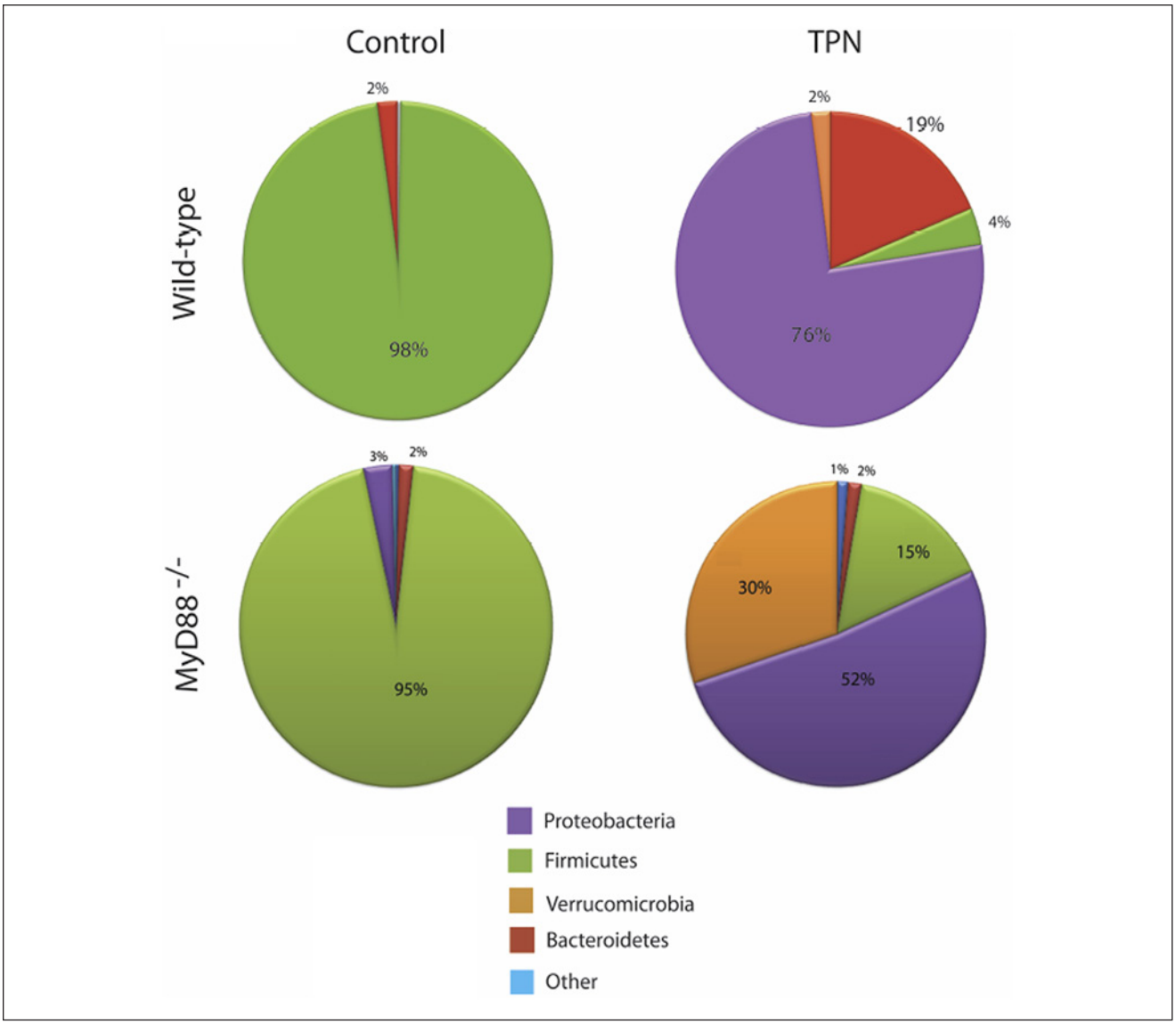

Figure 1. Summary of 454 pyrosequencing results from mucosally derived microbial populations in enterally fed and enterally deprived (parenteral nutrition) populations of mice. Note a dramatic shift in the composition of the microbial population with the administration of PN, from *Firmicutes*-rich to a population dominated by *Proteobacteria* and *Bacteroides*. Note also an expansion of the *Verrucomicrobia* population in MyD88 (a common signaling protein for the immune signaling of bacterial products) knockout mice. Results are derived from Miyasaka et al.[41]

cells and maintenance of epithelial barrier function. This suggests that the shift toward a *Proteobacteria*-rich intraluminal environment may be a key trigger for many of the adverse events seen with PN administration. Future work will examine whether prevention of these microbial shifts can also prevent the PN-related proinflammatory state. Another fascinating area is the marked expansion in the *Verruco* microbial population with PN and a further expansion in this population in MyD88 KO mice (Figure 1). This group of organisms, particularly the dominant species *Akkermansia muciniphila*, thrives on mucous and has been associated with a number of pathologic processes, including obesity.[43] It also intriguing that administration of PN is associated with a marked increase in intestinal goblet cells and altered mucous production,[44] suggesting that these microbes may be expanding to take advantage of a new nutrient source—the patient's own mucous.

Whether such changes in the intestinal microbial population occur in humans receiving PN has only recently been examined. Our laboratory had the opportunity to examine a series of human small bowel specimens harvested at the time of surgery

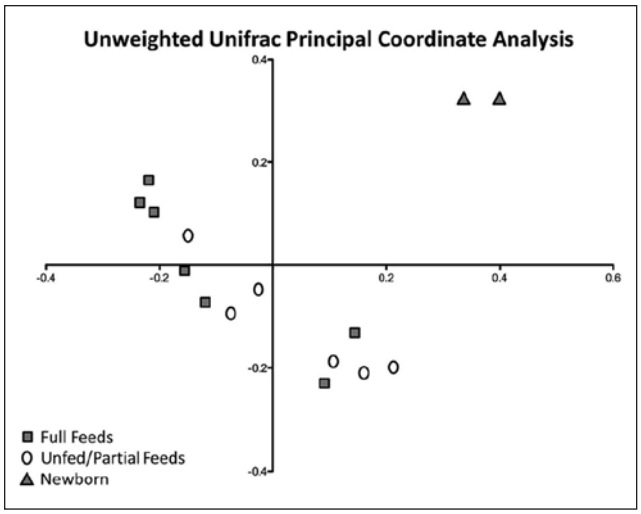

Figure 2. Summary of 454 pyrosequencing results from human distal small bowel specimens from patient specimens where enteral nutrients were delivered or deprived for at least 2 weeks. Note a shift in microbial species with differences in degree of nutrient deprivation. Data derived from Ralls et al.[45]

from patients who had segments of bowel that lacked exposure to enteral nutrition for a prolonged period of time (>2 weeks). Interestingly, 454 pyrosequencing of these specimens demonstrated a shift in the microbial population between segments that were or were not exposed to enteral nutrients[45] (Figure 2). While the significance of these microbial population shifts has not been fully examined, they suggest that patients receiving PN may undergo changes similar to those seen in mice.

Thus, nutrient deprivation appears to have profound changes in the microbial populations of both mouse models and humans. Future challenges will include our ability either to block virulent pathogens in the gut lumen from effectively signaling a patient's immunocytes and forming a proinflammatory state or to provide a healthier luminal environment, during PN administration, that will sustain a more favorable microbial population.

Host-Microbe Interactions in Babies at Risk for Necrotizing Enterocolitis (Michael J. Morowitz)

Necrotizing enterocolitis (NEC), a disease characterized by severe intestinal inflammation that can progress to multiorgan failure and death, remains an important source of morbidity and mortality among premature infants.[46] The disease provides one of the starkest examples of the intersection between nutrition and human pathology, since epidemiologic studies have consistently shown the incidence of the disease to be significantly higher in babies receiving artificial formula than babies receiving maternal milk.[47] Although it has long been suspected that abnormal patterns of gut bacterial colonization contribute to NEC pathogenesis,[39,47] we lack an understanding of specific mechanisms by which bacteria might trigger the excessive gut inflammation and epithelial cell death seen in the disease. However, recent advances in high-throughput platforms have made it possible to revisit the relationship between babies and gut microbes at high resolution.

The complex interactions between the gut epithelium and intestinal microbes can be mutually beneficial; for example, bacteria can serve specific functions such as vitamin production and in return they benefit from the nutrient-rich microenvironment of the human gut.[48] Current understanding of such symbioses in the human gut is relatively limited, but there is much to be learned from studies of the host-microbe relationship in well-studied model organisms such as the Hawaiian Bobtail Squid. Within hours after newborn squid have hatched, a mucus layer within specialized epithelial cells of the squid light organ becomes colonized by a *Vibrio* species of marine bacteria with luminescent properties. This specific bacterial population ultimately contributes to the health and fitness of the squid throughout its lifespan.[49] Elegant work in this field has demonstrated how the squid and the bacteria "find each other" using a molecular dialogue. Remarkably, despite the high microbial diversity in ocean waters, it seems that only *Vibrio fischeri* is capable of successfully colonizing the squid light organ.

Lessons learned from model symbiotic relationships such as this have generated interest in the exploration of similarly sophisticated relationships established and maintained over time in humans. Recent research, for example, has demonstrated how colonic bacteria can affect brain development in the newborn period and also coordinate the education of regulatory T cells.[51,52] Importantly, there is increasing recognition that complex intestinal disorders such as NEC and inflammatory bowel disease probably represent a global disruption of such host-microbe interactions.[52,53] This is a paradigm shift from earlier approaches to "pathogen hunting," in which clinicians and researchers have sought to find individual causative pathogens that could be "blamed" for microbe-related clinical problems. In the case of NEC, prior attempts to identify causative pathogens have been inconclusive[39]; babies with NEC have repeatedly been found to have a microbiota distinct from that of healthy controls, but the observed changes have varied across studies. In other words, it has not been possible to satisfy Koch's postulates regarding bacteria, causality, and NEC pathogenesis.

Advances in high-throughput platforms such as next-generation DNA sequencing have made it possible to characterize the dynamic process of early gut bacteria colonization in newborn infants at higher resolution. The utility of studying time series of samples rather than a limited number of samples per infant has been illustrated by landmark papers in the field demonstrating the temporal instability, low diversity, and high interindividual variability of gut bacterial communities in newborn infants.[54,55]

It appears that these features of gut microbial communities are even more pronounced in premature infants.[56,57] A unique feature of gut bacterial colonization of newborn premature infants is that it almost always occurs in the context of the administration of broad-spectrum antibiotics. This exposure to antibiotics could theoretically derail normal "programs" of bacterial colonization, and in fact 2 important retrospective studies have linked early antibiotic exposure to an increased incidence of NEC.[58,59]

As noted, molecular studies of the intestinal microbiota in premature newborns have not identified reproducible differences between babies with and without NEC. It is becoming increasingly likely that taxonomic identifications alone using marker genes will be inadequate to resolve the pathogenesis of the disease. Whole genome sequencing of entire bacterial communities (commonly referred to as metagenomic sequencing) offers the possibility to more fully understand the metabolic potential of mixed bacterial population and the genetic differences between closely related but nonidentical strains of bacteria. Metagenomics, however, does not offer functional data about which metabolic and virulence pathways are activated at a specific time and place. For this reason, there is intense interest in novel "omics" platforms that can provide functional information about the gut microbiota. An ideal approach might be to pair this functional information in real-time with tests that assess the health of the gastrointestinal tract.

Metaproteomics is a developing field that allows for the recovery and characterization of proteins directly from biologic samples containing mixed populations of microbial species. This approach stands in contrast to older proteomics technologies developed for individual microbial isolates grown in culture. A common tandem metaproteomics strategy uses liquid chromatography to separate complex mixtures of proteins and then mass spectrometry to characterize and identify the proteins.[60] Whereas older attempts to characterize protein expression in mixed microbial communities were limited by technical considerations, recent advances have made it possible to obtain relatively deep shotgun datasets of expressed proteins. This approach has been used successfully with human fecal samples to determine precisely which bacterial proteins are expressed within the distal human gut.[61,62] Such an approach allows for identification of actively expressed bacterial proteins while simultaneously profiling human proteins that have been secreted into the gut lumen and/or expressed on exfoliated epithelial cells. Importantly, many proteins found in high abundance in human stool samples—for example, mucin 2—are known to participate in the host-microbe dialogue.[63,64] Monitoring fecal concentrations of microbial and human proteins may prove useful in improving our ability to diagnose and treat gastrointestinal disorders such as NEC.

The increased risk of NEC in formula-fed babies could result from the growth of particularly virulent strains of gut bacteria or alternatively from the induction of virulence genes and proteins due to the specific nutrition components present in artificial formula. Efforts to untangle these considerations, and to decipher the contributions of gut microbes to NEC pathogenesis, have been reinvigorated in recent years by the development of high-throughput culture-independent experimental platforms. At present, we still have a limited understanding of genomic and functional differences in the gut microbial communities of babies with and without NEC. However, in coming years, it should be possible to more clearly define these differences and to place microbiome data squarely in the context of clinical information about intestinal health. Ideally, an enhanced understanding of the disturbed host-microbe relationship in NEC will be translated into improved diagnostics for early detection of disease and/or therapeutics to "rescue" unstable gut microbial communities. Outcomes for babies with NEC have not changed significantly over the past few decades, but this may soon change as we learn more about the host-microbe relationship during the newborn period.

The Intestinal Microbiome and Inflammatory Bowel Diseases (Eugene B. Chang)

Inflammatory bowel diseases (IBDs), as well as other immune-related human disorders, are "Western" disorders that have significantly increased in incidence and prevalence over the last half century, particularly in highly developed urban populations.[65,66] While it is very clear that these diseases have a genetic basis, their emergence over such a short period of evolutionary time is not likely caused by genetic drift[67] but rather by exposure to nongenetic factors introduced through changes in the environment and lifestyle of genetically susceptible individuals, triggering aberrant host responses that lead to IBD. The nature of these triggering factors remains unclear, although important leads have been established through experimental models and human genome-wide studies that have increased our understanding of etiopathogenesis. For instance, expression qualitative trait loci and genes associated with risk polymorphisms implicate gut microbiota in the pathogenesis of IBD—that is, IBD caused by an unfortunate combination of aberrant gut microbiota (dysbiosis) against a background of genetic susceptibility. However, to explain the recent epidemiological trends in IBD, the supposition must be made that the collective human microbiome of Western populations has shifted as a result of societal and cultural changes. In support of this, several studies have convincingly shown substantial differences in the gut microbiota of individuals from developing populations and those from Western populations. Children from Burkina Faso, for instance, have a substantially different gut microbial profile that their counterparts in the European Union.[67] Difference in diet appeared to be the strongest correlation with these findings, dominating over other possible variables such as ethnicity, sanitation, hygiene, geography, and climate, in shaping the gut microbiota.

In this regard, diet is thought to play an important role in the increase of complex autoimmune and inflammatory disorders in Western countries.[68] In our study, we examined whether certain dietary fats that are well represented in Western diets are capable of either precipitating or preventing/ameliorating colonic inflammation through their actions on the enteric microbiome.[69] On a background of genetic susceptibility, we hypothesized that these microbial changes affect host immune homeostasis, shifting the state to one favoring increased risk for disease. The effects of the 3 different high-fat, "Western-type" diets on the enteric microbiota of specific pathogen-free wild-type C57Bl/6 mice were tested. With the exception of the low-fat purified mouse diet, the high-fat diets were isocaloric and differed only in the type of dietary fat used, which was held constant at 37% of total calories and closely paralleled Western consumption.[70] As an aside, these fat sources are commonly used in numerous processed and confectionary foods or are consumed as animal food products. Twenty-one-day exposure to the 3 study diets resulted in significant differences in the structure of the enteric microbiota as assessed by both Sanger-based and 454-based DNA sequencing of 16S rRNA libraries from cecal contents and stool, and the findings were evident even within the first week of dietary intake. All high-fat diets reduced the richness of the microbiota compared with the low-fat diet. Furthermore, the low-fat diet promoted *Firmicutes* but decreased most other phyla, whereas polyunsaturated fatty acids and the diet high in anhydrous milk fat promoted *Bacteroidetes* and decreased *Firmicutes*. Interestingly, these changes differed from those induced by lard-based, saturated fats,[71,72] where *Firmicutes* predominate. While diets with milk fat and polyunsaturated fatty acids had similar effects on *Bacteroidetes* and *Firmicutes*, a significant increase in the membership of the *Deltaproteobacteria, Bilophila wadsworthia*, was consistently observed only with the milk-fat diet. *B wadsworthia* has been reported under pathological conditions in humans such as appendicitis and other intestinal inflammatory disorders, where it has been shown to be genotoxic as well as proinflammatory, in part related to its sulfite-reducing properties and production of H2S.[73–75] The bloom of this rare microorganism without major restructuring of other commensal microbiota most likely reflected changes in conditional factors that promote its growth. One potential and major source for sulfur is taurine-conjugated (TC) bile acids, which are preferentially formed when exposed to milk-fat diets, due to TC bile acids' ability to increase micelle formation and emulsification of milk fats.[76–78] In this regard, the hepatic conjugation of bile acids in mice fed milk-fat diets did indeed shift, to favor the formation of taurocholic acid. This effect mostly likely was the cause of the *B wadsworthia* bloom because gavage with TC bile acids in mice fed low-fat diets recapitulated the bloom of this microorganism. None of the test diets caused the development of colitis or other obvious adverse effects in wild-type C57Bl/6 mice. However, in genetically susceptible interleukin-10-deficient mice of the same background, the rate of spontaneous colitis increased from ~30% to ~70%, with earlier onset of disease. Similarly, mice gavaged with TC bile acids on low-fat diets exhibited elevated mucosal and mesenteric lymph nodes cytokine levels that, like the milk-fat diet treatment, was associated with a TH1 immune response.

Altogether, these findings support the notion that diets are complex and that not all dietary fats have the same effects on the enteric microbiota. The emergence of pathobionts such as sulfite-reducing bacteria can occur through changes in diet-induced taurine conjugation of host hepatic bile acids, which, through repeated cycles of enterohepatic circulation, increases sulfur bioavailability that provides substrate for the growth of colonic microbiota capable of sulfur reduction. These types of bacteria are often recovered from biopsies and stool of patients with IBD[79–83] and possibly represent how certain gut microbes use bile to their advantage. Bile formation is unique to vertebrates and omnivores, providing them with the ability to digest and use a far greater variety of dietary substrates. Bile also provides potent antimicrobial properties that contribute to the selection of commensal microorganisms as well as exclusion of many potential gut microbiota. In some cases, such as with sulfite-reducing bacteria, TC bile acids are used as metabolic substrates or for anaerobic respiration, whereas in other cases, bile may be essential to suppress symbiotic, commensal microorganisms, allowing pathobionts and pathogens opportunity to establish a niche in the intestine. In genetically susceptible hosts, this development has the capacity to tip a compensated state of immune balance to one of chronic inflammation.

Dietary fats differ in their effects on the host microbiota and immune system. The findings of our study provide a plausible mechanistic basis by which Western-type diets high in saturated fats may increase the prevalence of complex immune-mediated diseases like IBDs in genetically susceptible hosts. Interventional strategies to reshape the gut microbiome in susceptible individuals should be explored to lower risk and prevent disease.

Use of Fecal Microbial Transplantation to Understand and Cure Multiply Recurrent *Clostridium Difficile* Infection (Michael J. Sadowsky, Alexander Khoruts, Matthew Hamilton, and Alexa Weingarden)

C difficile infection (CDI) is currently among the most common antibiotic-related causes of diarrhea in the developed world and has rapidly become one of the most commonly acquired hospital- and healthcare-acquired infections.[84] The increase in incidence of this infection during the past decade is in large part due to increased use of antibiotics and the global emergence of more virulent strains of *C difficile*. These strains are characterized by higher levels of toxin production, sporulation ability, and persistence in the gut.[85] These properties have contributed to increased incidence and mortality associated with CDI. In the U.S. alone, it has been estimated

that 20,000–100,000 cases occur each year, but this may be underreported.[85,86] Moreover, there is an increasing occurrence of the multiply recurrent form of CDI (MR-CDI)[87,88] leading to morbidity and in some cases mortality. While antibiotics have been routinely used to treat this disease and can generally suppress acute CDI, infection by this bacterium often returns within a few weeks of antibiotic stoppage. Interestingly, this disease generally follows the rule of doubling. That is, while an initial bout of CDI is associated with a 20%−30% chance of recurrence, the chance rises by another 20% with each consecutive recurrence.[89] After 3 or more recurrences, the cycle often becomes indefinite, frequently leading to hospitalization. Furthermore, it is common for each consecutive recurrence to be clinically more severe, leading to multiple hospitalizations, morbidity, and loss of productivity. In the U.S. alone, this has led to escalating costs of CDI, which have been conservatively estimated to be several billion dollars per year.[89]

The prevalence of MR-CDI can be explained, in large part, by the fact that *C difficile* exists as both a free-living bacterium and a spore.[90] The vegetative cells of *C difficile* excrete exotoxins that are destructive to colonic epithelium. In addition, *C difficile* resting spore are highly persistent in the intestinal tract (and hospital and home environments) and are immune to the action of antibiotics. Current hypotheses suggest that the normal distal gut microbiota provides colonization resistance against *C difficile* and that antibiotic-induced dysbiosis leads to disruption of the microbial community structure of the gut—leading to colonization by *C difficile*. Thus, antibiotics are themselves one of the major causes of MR-CDI: They suppress the vegetative forms of *C difficile* but also lead to recurrence of the infection as they destroy normal gut bacteria and are ineffective against spores that germinate once the antibiotic is removed (Figure 3).[91]

Several lines of evidence indicate that patients who receive concurrent antibiotics for other indications are at greater risk of recurrence.[92] Moreover, recent studies have shown that patients with MR-CDI have significantly reduced diversity in their intestinal bacteria relative to patients having an initial CDI infection or a first recurrence.[93]

Fecal Microbiota Transplantation for CDI

One highly effective solution to this problem is reconstitution of normal functioning gut microbial ecology by way of fecal microbiota transplantation (FMT).[92,94] FMT, previously known as fecal bacteriotherapy, is the therapeutic protocol that allows for the fastest reconstitution of a normal composition of colon microbial communities. We previously showed that FMT resulted in prompt and sustained engraftment of donor fecal bacteria in a patient with recurrent CDI.[95] FMT, administered by infusion during a colonoscopy, resulted in completely normalized bowel functioning within 2 days of treatment. Since its first proposed use in 1958 by Eiseman et al,[96] many hundreds of cases have been reported as individual case reports, or small case series, with an >90% cumulative success rate in clearing recurrent CDI, without any noted adverse events. The history and general method used for FMT have been described in several recent reviews.[97–99]

However, despite the long and successful track record, as well as great clinical need, the availability of the procedure for many patients remains very limited. The lack of wider practice of FMT is in great part due to several practical barriers and is not due to lack of efficacy. These include lack of reimbursement for donor screening, difficulty in material preparation and administration, and aesthetic concerns about conducting the procedure. In 2009, we established the FMT program at the University of Minnesota that has since overcome many of challenges associated with FMT. This has resulted in limited use of patient-identified individual donors and greater use of rigorously screened "universal" volunteer donors. More important, we now use well-prepared standardized frozen fecal extracts instead of fresh crudely prepared fecal slurries.

FMT is also very interesting from a basic science perspective, and this understanding has been aided by use of Illumina-based high-throughput sequencing of 16S rDNA. Therefore, studying the FMT patient population represents an opportunity to gain insights into various roles that the distal gut microbiota plays in host metabolism and physiology. Our studies have shown that patients with MR-CDI have markedly reduced microbial species diversity in their distal gut caused by multiple rounds of antibiotics, leading to severe dysbiosis.[94,95,100,101] In these patients, the intestinal tract is dominated by *Proteobacteria* and few or no members of the *Bacteroidetes* and *Firmicutes.* In contrast, 16S rRNA gene sequence analyses showed that FMT, using frozen fecal bacteria from a healthy donor, resulted in the stable engraftment of gut microbiota.[102] Post-FMT samples from FMT patients showed an increase in the abundance of *Firmicutes* and *Bacteroidetes* that represented ~75%−80% of the total sequence reads.[101] It was also shown in post-FMT patients that the *Proteobacteria* and *Actinobacteria* comprised <5% of reads found in patients prior to FMT. Members of the *Bacteroidetes* phylum were most represented by members of the families *Bacteroidaceae, Rikenellaceae*, and *Porphyromonadaceae* and were mostly comprised of members of the genera *Bacteroides, Alistipes*, and *Parabacteroides*. Members of the phylum *Firmicutes* were represented by members of the families *Ruminococcaceae, Lachnospiraceae, Verrucomicrobiaceae*, and unclassified *Clostridiales* and members of the *Firmicutes*.

Taken together, our results demonstrate that frozen, standardized, fecal microbiota from healthy donors can be used to effectively treat recurrent CDI. This procedure restores the structure of gut microbiota and clears *C difficile* in the vast majority (>90%) of patients.

How Critical Illness Shifts and Reshapes the Microbiome Into a Pathobiome (John Alverdy)

No greater stress is imposed on the intestinal microbiome than that which develops during human critical illness. Although the human microbiome is composed of microorganisms that

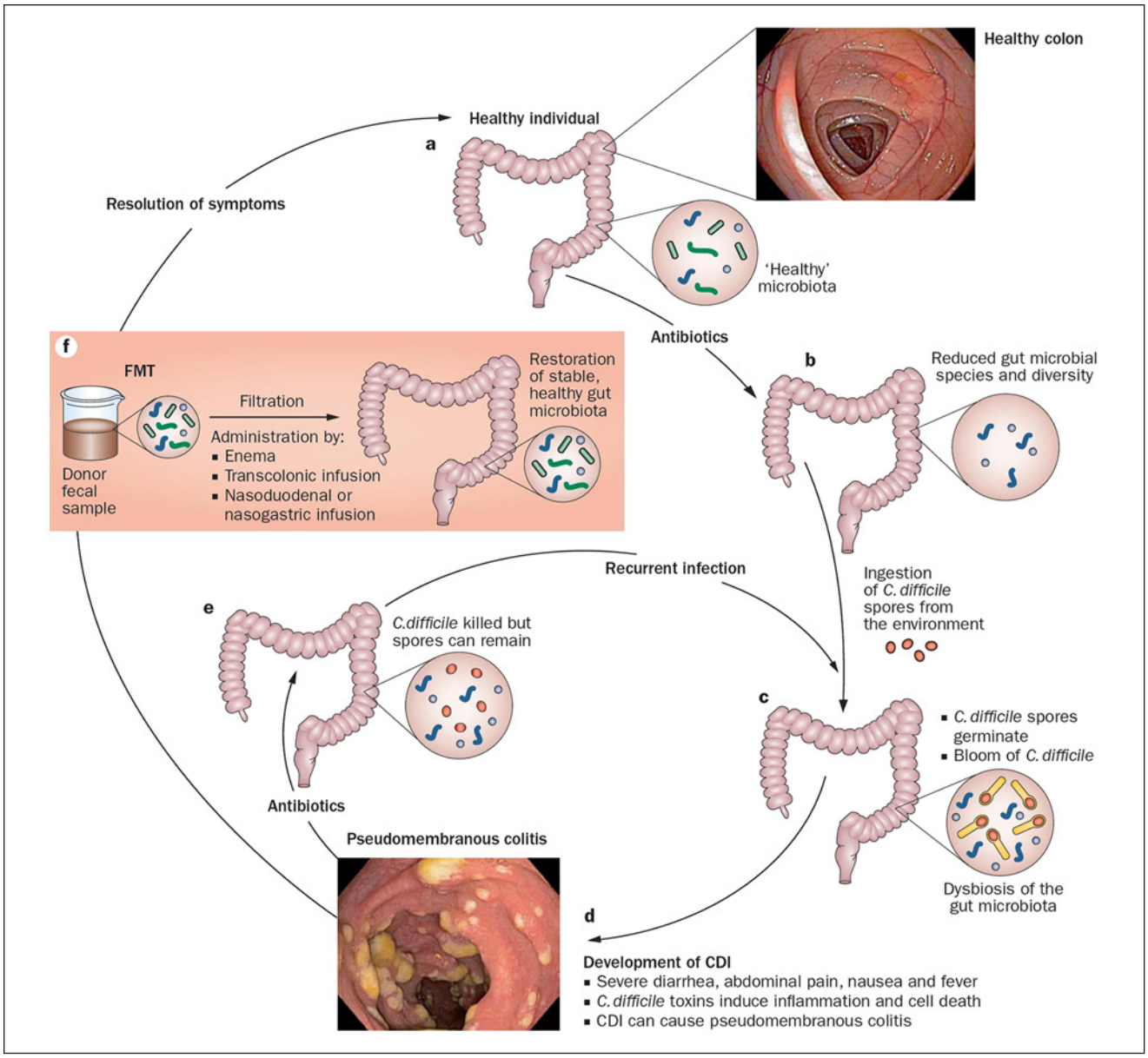

Figure 3. Concept of an abnormal microbial population during an episode of *C difficile*, and an approach for the treatment of this with fecal transplantation. CDI = *C difficile* infection; FMT = fecal microbiota transplantation. Reprinted by permission from Macmillan Publishers Ltd: Nature Reviews Gastroenterology & Hepatology,[91] copyright 2011. (http://www.nature.com/nrgastro/index.html)

have coevolved with their hosts and share similar life histories of famine, pestilence, plagues, and exposure to xenobiotics, there is little evolutionary precedent for the seismic environmental shift that occurs following a major injury (trauma, burns, and organ transplantation) and its treatment. Under such conditions, the human gut is exposed to alterations in nutrient availability (PN), oxygen deprivation (hypoxia, hypotension, pressors) or oxygen excess, exposure to agents that affect motility (opioids), and multiple antibiotics that erode or eliminate the core microbiome. Evidence is now accumulating that this degree of stress, in concert with loss of the core microbiome, has a major impact on the incidence, course, and outcome of critical illness.[103] Although recovery from critical illness is often framed as a function of immune capacity, new evidence suggests that the intestinal microbiome itself is a potent booster of systemic immune function and, thus, its erosion can adversely affect recovery.[104] A new concept in critical care management could consider that the extent to which we

can understand and maintain core functions of the intestinal microbiome during critical illness may itself be immune-enhancing. Given the degree of diversity and metabolic function that is present in the normal core microbiome, it is likely naïve to think that a few strains of *Lactobacilli* can fully supplant the degree of functionality required of the intestinal microbiome to bolster systemic immune function during critical illness. Approaches to maintain the core microbiome during critical illness need to consider the fact that use of systemic antibiotics during critical illness is virtually unavoidable, as infections in soft tissues and organs (lung, kidney, wounds) will always mandate the use of these agents. A strategy of core microbiome maintenance therapy will be necessary even before we have a full understanding of all the components of the intestinal microbiome that preserve systemic immune function. This will clearly involve a better understanding of how nutrition support during critical illness affects the core microbiome and its downstream effect on immunity and inflammation.

Conclusions

The promise of personalized medicine is becoming a reality as we witness the rapid reduction in cost and time to sequence whole genomes and measure whole proteomes and metabolomes. Harnessing and displaying the power inside these high-throughput megadatasets are becoming realities as computational scientists, mathematicians, physicists, and bioinformatics experts come together to tackle the data. Yet several limitations still exist. As different locations within the gut contain unique microbial communities and there seems to be evidence of "niche specialization," there must be a uniform approach to account for the sample source and the >200 m^2 of gastrointestinal surface area. We must be cautious not to make inferences with data that are purely descriptive, so as to avoid the pitfall of overinterpretation of correlative pattern recognition in lieu of causative mechanisms of disease. We must also recognize that an expelled fecal sample may not be an average representation of the entire spatial biogeography of the gut. Therefore, we should be careful not to display these data and make inferences that lack clinical context, as we witnessed in the early period of DNA microarrays. Rather the hope is that we will witness microbiome-related readouts that correlate with disease states and inform the system's biology. Practitioners and patients will then realize the immense power of controlling the intake and quality of their food and the importance of nourishing their microbial partners to foster mutualism and molecular détente within the complex and diverse host-microbial interactome.

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