REVIEW SUMMARY

MICROBIOTA

Childhood undernutrition, the gut microbiota, and microbiota-directed therapeutics

Laura V. Blanton, Michael J. Barratt, Mark R. Charbonneau, Tahmeed Ahmed, Jeffrey I. Gordon*

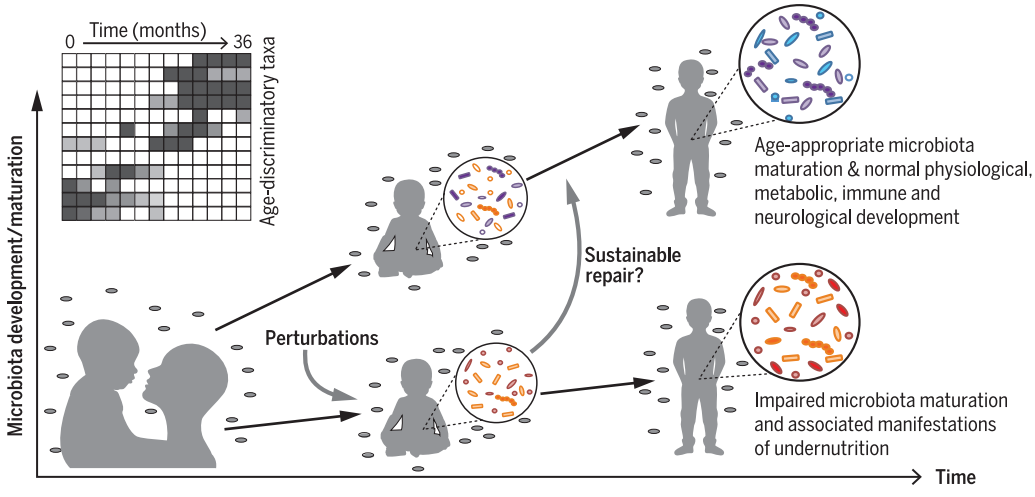
BACKGROUND: Childhood undernutrition is a global health challenge. Undernutrition in early life is associated with a number of adverse outcomes, including persistent stunting, immune dysfunction, and neurocognitive deficits. Current approaches to treatment have only modest effects in correcting these long-term sequelae, suggesting that certain features of host biology are not being adequately repaired. This has led to the hypothesis that healthy growth is dependent, in part, on normal postnatal development of the gut microbiota and that perturbations in its development are causally related to undernutrition. Testing this hypothesis illustrates a number of the challenges that human microbial ecology research faces: (i) defining "normal," both in terms of community structure and expressed functions; (ii) determining whether normal in one population generalizes to other populations; (iii) ascertaining whether deviations from normal correlate with disease and are a cause rather than an effect of pathology; (iv) determining whether abnormal microbial community configurations can be repaired in a sustained fashion, and what route and time course are optimal for such repair; (v) deciphering the short- and long-term effects and safety of repair; and (vi) proactively addressing the ethical, regulatory, and other societal implications of microbiota-directed food and/or microbial interventions designed to deliberately manipulate this facet of postnatal human development.

ON OUR WEBSITE

Read the full article at http://dx.doi.org/10.1126/science.aad9359

ADVANCES: Culture-independent studies of the gut microbiota of members of birth cohorts with healthy growth phenotypes have identified a program of community assembly ("maturation") defined by the changing representation of a group of age-discriminatory bacterial taxa. Features of this program are shared across individuals living in several low-income countries. Applying metrics for defining deviations from this program (microbiota-for-age Z score) has disclosed that children with severe acute malnutrition have gut microbial communities that appear younger than would be expected on the basis of their chronological age. The resulting microbiota "immaturity" is not repaired by current therapeutic food interventions. Compared with healthy children, microbiota from undernourished children transmit impaired growth phenotypes to recipient gnotobiotic mice fed diets representative of those of the human donors; moreover, some of the transplanted age-discriminatory strains are growth-discriminatory. These findings provide early preclinical proof-of-concept that gut microbiota immaturity is causally related to a number of the manifestations of childhood undernutrition.

OUTLOOK: Gnotobiotic animal models can be used to test a number of concepts. Gut microbiota immaturity, increased enteropathogen burden, and gut barrier dysfunction are interrelated factors that affect disease risk and pathogenesis. Microbiota development is linked to maturation of the gut mucosal immune system, metabolic function in multiple host tissues, plus musculoskeletal and brain development. Age- and growth-discriminatory bacterial strains identified in the normally developing microbiota represent therapeutic targets in children with undernutrition. The representation of these strains provides a way not only for defining the efficacy of these therapeutic interventions but also for assessing the effects of various parameters postulated to contribute to disease risk and pathogenesis (such as maternal health status, breast milk composition, history and quality of complementary feeding, poor sanitation and enteropathogen burden, and antibiotic exposures). Microbiota-directed strategies for treating and ultimately preventing childhood undernutrition raise intriguing questions about the mechanisms that define human development. They also highlight the need to add a microbial dimension to our conceptualization of human biological immaturity and its associated adaptations and compensations, and to consider whether interventions that promote healthy microbiota development can spawn a form of preventative medicine that has lifelong benefits. ■



The concept that impaired postnatal gut microbiota development (maturation) is causally related to childhood undernutrition. The representation of age-discriminatory bacterial taxa defines a program of normal gut microbiota development. Disrupting the coordinated functional codevelopment of microbiota and host affects multiple biological regulatory systems through largely unknown mechanisms. Developing effective strategies for sustained repair of microbiota immaturity through food or microbial interventions requires preclinical studies of these mechanisms and modeling of the effects of different rates and routes of repair.

The list of author affiliations is available in the full article online.
*Corresponding author. Email: jgordon@wustl.edu
Cite this article as L. V. Blanton *et al.*, *Science* 352, aad9359 (2016). DOI: 10.1126/science.aad9359

REVIEW

MICROBIOTA

Childhood undernutrition, the gut microbiota, and microbiota-directed therapeutics

Laura V. Blanton,[1,2] Michael J. Barratt,[1,2] Mark R. Charbonneau,[1,2] Tahmeed Ahmed,[3] Jeffrey I. Gordon[1,2]*

Childhood undernutrition is a major global health challenge. Although current therapeutic approaches have reduced mortality in individuals with severe disease, they have had limited efficacy in ameliorating long-term sequelae, notably stunting, immune dysfunction, and neurocognitive deficits. Recent work is providing insights about the role of impaired development of the human gut microbiota in disease pathogenesis, leading to new concepts for treatment and prevention. These findings raise intriguing basic questions about the mechanisms that direct normal gut microbial community assembly and functional maturation. Designing and implementing new microbiota-directed therapeutics for undernutrition highlights the need to simultaneously consider a variety of features of human biology as well as broader societal issues.

Childhood undernutrition is a vexing, pressing, and in many respects overwhelming global health issue. Undernutrition contributes to more than 40% of deaths worldwide among children under 5 years old (*1*). Acute undernutrition affects more than 50 million children and is defined by a low weight-for-height Z (WHZ) score [the number of standard deviations from the median value for a reference, multinational World Health Organization (WHO) cohort of children with healthy growth phenotypes (*2*)]. Preschool children with severe wasting (WHZ < −3) have a 10-fold higher mortality rate than that of their well-nourished counterparts. In 2014, chronic undernutrition, which manifests as stunting [low height-for-age Z score (HAZ)], affected 159 million children, with almost all living in low-income countries (*3*). Despite these categorical distinctions, deficits in ponderal and linear growth frequently coexist and increase the risk that children will experience persistent stunting, defective immune responses, and impaired neurocognitive function into adulthood (*4*).

Epidemiologic studies have emphasized that undernutrition cannot be ascribed to food insecurity alone and reflects the intersection of multiple factors that operate within and across generations (*4*, *5*). Genetic analyses of risk factors have produced few leads. Longitudinal studies of a cohort of 317 Malawian twin pairs, aged 0 to 3 years and living in rural villages, revealed a high incidence of discordance (>40%) for either severe acute malnutrition (SAM) or moderate acute malnutrition (MAM). The rate of discordance (in which one twin was healthy by anthropometry and the other was diagnosed with SAM or MAM) was not significantly different between mono- versus dizygotic pairs, suggesting that early life environmental exposures play a key role in disease pathogenesis (*6*).

Breast milk

For many infants, breast milk represents one of the earliest postnatal environmental exposures. WHO/United Nations Children's Emergency Fund (UNICEF) recommends exclusive breastfeeding for the first 6 months of postnatal life and non-exclusive breastfeeding up to 24 months (*7*). Breast milk reduces infant mortality from common infections, including those that cause diarrhea and pneumonia (*8*). Despite the biological importance of breast milk, large gaps in knowledge remain, including how breast milk composition varies as a function of maternal nutritional status and whether and how variation in its composition affects risk for undernutrition in offspring. Fortunately, recent advances in mass spectrometric (MS) methods have set the stage for addressing how breast milk components vary as a function of age, genotype, parity, length of pregnancy, time after parturition, and health status in geographically and culturally diverse populations of women (*9*). Human milk oligosaccharides (HMOs) represent attractive targets for MS-based analyses, particularly if they are incorporated into birth cohort studies that couple serial breast milk sampling with comprehensive assessments of maternal and infant health status (*9*). HMOs contain a lactose core and linked glucose, galactose, N-acetyl galactosamine, fucose, and/or sialic acid residues [3–20 monosaccharides per HMO structure; ≥100 structures present in a given mother's breast milk (*9*)]. HMOs do not directly provide nutrition to the host. HMOs act as prebiotics that promote colonization of the infant gut with bifidobacterial taxa associated with multiple benefits to the host, including improved vaccine responses (*10*), enhanced gut barrier function (*10*, *11*), and protection from enteropathogen infection (*12*).

A recent study documented reduced levels of total, fucosylated, and sialylated HMOs in the breast milk of Malawian mothers with severely stunted compared with healthy 6-month-old infants (*13*). In follow-up preclinical experiments, young gnotobiotic mice were colonized with a consortium of bacterial strains cultured from the fecal microbiota of a severely stunted 6-month-old Malawian infant and fed a prototypic micro- and macronutrient-deficient Malawian diet, with or without added purified sialylated bovine milk oligosaccharides (S-BMOs) that are structurally similar to sialylated HMOs (*13*). A separate group of animals was fed the same Malawian diet supplemented with fructo-oligosaccharides, which are present in a number of current infant formulas. Even though all diets were isocaloric and animals in each of the groups consumed the same amount of diet, S-BMO but not fructo-oligosaccharide supplementation significantly augmented the rate of lean body mass gain. Growth augmentation was microbiota-dependent. S-BMO supplementation also produced changes in liver, muscle, and brain metabolic profiles indicative of increased capacity to use nutrients for anabolism in the fed condition and break down lipids during fasting [a state of increased metabolic flexibility (*14*)]. S-BMO–treated animals also manifested alterations in bone morphometry (*13*). The effects of S-BMOs were also observed in gnotobiotic piglets fed the same prototypic Malawian diet and colonized with cultured bacterial components of the same donor's microbiota (*13*). These findings underscore the need to understand genetic, physiologic, metabolic, and environmental factors that influence the representation of different HMO structures in lactating mothers' milk, how these structures are processed by the gut microbiota of healthy versus undernourished infants, and whether HMOs and the products of their biotransformation by the microbiota correlate with infant growth outcomes. In addition, these preclinical results illustrate how S-BMOs can be used as a tool to explore the mechanisms by which at least one class of HMOs operate, via the microbiota, to influence postnatal growth and metabolism.

Enteropathogen burden

Enteropathogens represent another common early life environmental exposure. The breadth of exposure is enormous: ~2.5 billion people currently live under unsanitary conditions, although efforts to sponsor innovations that yield affordable and sustainable solutions are under way (for example, www.gatesfoundation.org/What-We-Do/Global-Development/Reinvent-the-Toilet-Challenge).

[1]Center for Genome Sciences and Systems Biology, Washington University School of Medicine, St. Louis, MO 63110, USA. [2]Center for Gut Microbiome and Nutrition Research, Washington University School of Medicine, St. Louis, MO 63110, USA. [3]Nutrition and Clinical Services Division, International Centre for Diarrhoeal Disease Research, Dhaka 1212, Bangladesh.
*Corresponding author. Email: jgordon@wustl.edu

The development and implementation of robust, high-throughput methods for simultaneous quantitative polymerase chain reaction assays of multiple enteropathogens in a single fecal sample (*15*) is critical, enabling large-scale surveys of the relationship between enteropathogen burden and undernutrition. Birth cohort studies designed to address this question are now under way that also include serial anthropometric assessments as well as tests of immune and neurocognitive function (*16*).

Environmental enteric dysfunction (EED)

EED is an enigmatic disorder prevalent in low-income countries where sanitation is poor and enteropathogen burden is high. EED is often invoked as a key factor underlying the pathogenesis of undernutrition. However, accurate diagnosis remains a challenge. The "gold standard" for diagnosis is small intestinal biopsy, in which histopathologic manifestations of disease include infiltration of the lamina propria with $CD8^+$ T cells and villus atrophy (*17*). These changes are accompanied by marked reductions in the surface area available for active nutrient uptake and disruption of gut barrier function (*17*, *18*).

The fact that small intestinal biopsies carry substantial risk for children with undernutrition has limited the ability to assess the specificity or sensitivity of current fecal-, serum-, and urine-based biomarkers (*19*). Nonetheless, reports indicate that biomarkers of gut inflammation, intestinal permeability, and systemic immune activation are inversely correlated with linear growth and the efficacy of oral vaccines (*20*, *21*). Preclinical models with features of EED (*22*) provide an opportunity to help identify disease mechanism–based biomarkers and to dissect interactions between dietary components, enteropathogens, community members, gut barrier function, and inflammatory responses. For example, some gut microbes appear to elicit and benefit from inflammation (*23*), whereas others appear to blunt the immune response (*24*, *25*).

The limitations of current therapies and our knowledge of their long-term effectiveness

There is ongoing debate about the role of antibiotics in treating children at risk for or with already manifest acute or chronic undernutrition (*26*, *27*). A recent large, double-blind, placebo-controlled trial involving children with SAM living in Niger found no benefit of routine antibiotic use on nutritional recovery (*26*). Using random effects models, a meta-analysis of 10 randomized control trials (RCTs) involving children living in low- and middle-income countries given a variety of oral antibiotics for different indications and durations concluded that treatment was associated with modest improvement in ponderal growth and smaller improvements in linear growth (*28*). Extrapolating, these effects would correspond to a 0.2 to 0.3 increase in WAZ and a 0.1 increase in HAZ over 6 months in the most vulnerable populations (*28*).

Current nutritional interventions also have limited efficacy. A meta-analysis of RCTs, conducted in low- and higher-income countries, involving various forms of antenatal diet supplementation indicated that these interventions contributed to improved birth outcomes, including gestational age and birth weight (*1*). However, because primary end points were assessed at birth, the long-term effects on child growth and development remain unclear (*1*, *29*). The levels of certain micronutrients in breast milk that are essential for growth (such as vitamins A, B1, B2, B6, B12, and D and choline, selenium, and iodine) are influenced by maternal nutritional status (*30*). A meta-analysis of 16 controlled clinical studies of "multiple micronutrient powders" (MNPs) found no evidence of substantial effects on linear growth, despite improvements in micronutrient status (*31*). A separate analysis of seven clinical trials conducted in 6- to 24-month-old children living in food-insecure populations indicated that provision of complementary foods with or without nutritional education did not significantly reduce the risk of stunting (*1*). As with antenatal supplementation, studies have generally not examined the long-term effects of postnatal interventions on immune function or neurodevelopmental outcomes (*1*).

Energy-dense, micronutrient-fortified, ready-to-use therapeutic foods (RUTFs) are effective in accelerating short-term weight gain in individuals with SAM (*1*). Nutritional recovery (defined as attaining WHZ > −2) is believed to be facilitated by the provision of high-quality dietary protein in RUTFs, resulting in a switch from fatty acid to amino acid oxidation, with accompanying promotion of fat deposition and weight gain. Concomitant increases in insulin and IGF-1 and decreases in cortisol levels limit protein catabolism and permit accretion of muscle mass (*32*). However, in SAM as well as MAM there is a general lack of information on the appropriate timing and duration of current nutritional interventions or their overall effectiveness in overcoming the long-term morbidities that ensue from childhood undernutrition (*33*, *34*).

What are we missing in our understanding of the pathogenesis of undernutrition, and how can new therapeutic strategies be identified that more durably repair its short and long-term effects? Recent work points to a persistent developmental abnormality in children with undernutrition that affects their gut microbiota.

Box 1. A hypothesis about gut microbiota development and the pathogenesis of undernutrition.

- Initial gut community assembly is disrupted by one or more factors (for example, failure to acquire organisms with key metabolic capabilities, whether due to insufficient exposure, deficiency of required nutrients, antibiotic administration, undefined host biological features, and/or enteropathogen competition).
- Disrupted microbiota development provides opportunities for enteropathogens to establish themselves and/or the virulence potential of pathobionts to be expressed, further disrupting already impaired microbiota maturation.
- Impaired microbiota development impairs codevelopment of the mucosal immune system, compromising barrier function, defense against enteropathogens, and vaccine responses and impeding further maturation.
- Disrupting the coordinated, mutually beneficial, functional codevelopment of the microbiota and host affects multiple biological regulatory systems through mechanisms that are largely unknown but manifested in part by impaired muscle and bone growth, impaired metabolism that jeopardizes the supply of nutrients and energy required by the developing brain, and impaired immune/gut barrier function.

(Left) Young girl in Dhaka, Bangladesh, consuming a meal with a diversity of dietary ingredients. Identifying those ingredients that promote healthy development of the gut microbiota may yield more effective treatments for undernutrition. [Photo courtesy of Mustafa Mahfuz] (Right) Measurement of mid-upper-arm circumference (MUAC) to assess for acute wasting in a child in Mitondo, Malawi. [Photo courtesy of Indi Trehan]

Postnatal development of the gut microbiota and the pathogenesis of undernutrition

Defining "normal" gut microbiota development

The tens of trillions of microbes that inhabit the human gut are tasked in part with the biotransformation of dietary ingredients into products that affect various aspects of our physiology, metabolism, and immune function. Asking whether and how postnatal assembly of the microbial community is related to the risk for and pathogenesis of undernutrition requires a definition of normal microbiota development. One approach used machine learning (Random Forests) to mine bacterial 16*S* ribosomal RNA (rRNA) data sets generated from fecal samples, collected monthly from birth through the first 2 to 3 years of postnatal life from infants and children living in an urban slum in Bangladesh who had healthy growth phenotypes (as judged with serial anthropometry). The results yielded a group of the most age-discriminatory

bacterial taxa; changes in their relative abundance provided a microbial signature that described a program of normal gut microbiota development shared by biologically unrelated healthy Bangladeshi infants/children (*35*).

This approach is being applied to additional birth cohorts living in different regions of the world, including low-income countries where the burden of undernutrition is great as well as high-income countries where the level of sanitation and consumption of processed foods is considerably higher. The results are beginning to provide information about the degree to which this program of gut community development is shared (*36*, *37*). Applying advances in genome assembly by using shotgun sequencing data sets generated from fecal DNA, as well as advances in culturing phylogenetically diverse members of the gut microbiota, should reveal the extent of strain-level variation and insights into the functional potential of these age-discriminatory bacterial taxa, both within and between different human populations. The representation of archaeal, eukaryotic, and viral components [including phage (*38*)] in the developing microbiota of healthy infants and children also needs to be delineated. One of the many motivations for obtaining this expanded view comes from recent work emphasizing how enteric viruses affect, and are affected by, members of these other domains of life ["transkingdom interactions" (*39*)].

Gut microbiota "immaturity" in undernourished children

Bacterial 16*S* rRNA–based Random Forests–generated models composed of age-discriminatory bacterial taxa allow a microbiota-for-age Z (MAZ) score to be computed that defines the degree of deviation of an unhealthy individual's microbiota age (state of development) from a reference cohort of chronologically age-matched children with normal growth. Applying this metric disclosed that community assembly is perturbed in Bangladeshi and Malawian children with undernutrition, resulting in persistent gut microbiota "immaturity"; their microbial community configuration looks "younger" than in chronologically age-matched healthy individuals (*35*, *36*). Children with MAM also have microbiota immaturity, albeit less severe than those with SAM (*35*, *36*). MAZ scores documented at 12 months of age in members of a Malawian birth cohort with minimal antibiotic exposure were predictive of anthropometric scores at 18 months [WHZ, WAZ, and to a lesser degree HAZ (*36*)]. Although additional studies of birth cohorts are needed to define the degree of correlation between MAZ and growth phenotypes, applying this metric in a randomized clinical study that assessed the effects of two different RUTFs given to Bangladeshi children with SAM revealed that neither RUTF rescued microbiota immaturity; improvement was partial and transitory (*35*).

Cause versus effect

What is the biological consequence of persistent gut microbiota immaturity? Preclinical proof-of-concept (POC) that it is a contributing cause rather than simply an effect of undernutrition has come from transplanting immature gut microbiota from undernourished Malawian infants and children, or normally maturing microbiota from those with healthy growth phenotypes, into young recently weaned germ-free mice fed a macro- and micronutrient-deficient diet representative of those consumed by the human donors (*36*). Immature microbiota from undernourished infants and children transmitted significantly reduced lean body mass gain phenotypes, alterations in bone morphometric phenotypes, and metabolic abnormalities evident in muscle, liver, and brain. Remarkably, the gain in lean body mass was significantly greater when the microbiota were from 6-month-old as compared with 18-month-old donors, although in each chronological age bin, growth promotion in recipient mice was greater when the microbial community originated from a healthy individual (*36*). These latter findings provide evidence supporting the hypothesis that microbiota maturation is functionally linked to the growth rate of the host.

Random Forests–based analysis of bacterial 16*S* rRNA data sets generated from the fecal microbiota of these recipient mice identified groups of bacterial taxa whose representation was discriminatory for several host phenotypes, including rate of lean body mass gain and bone morphologic features (*36*). These "growth-discriminatory" taxa included a subset of the age-discriminatory taxa, suggesting that the latter include taxa that are not only biomarkers but also mediators of host development. The overlapping membership of taxa in the different Random Forests models generated from different features of host biology leads to a testable hypothesis: Different configurations of microbiota from undernourished children can transmit different manifestations of disease.

Taking advantage of the coprophagy of mice, cohousing gnotobiotic animals shortly after they received microbiota transplants from 6-month-old infants with either normal linear and ponderal growth or severe stunting and underweight phenotypes resulted in invasion of age- and growth-discriminatory strains from cagemates with the transplanted healthy donor microbiota into the microbiota of cagemates harboring the undernourished donor's microbiota. Invasion was associated with prevention of growth faltering. Addition of a number of cultured invasive taxa to an undernourished donor's microbiota ameliorated growth-faltering and metabolic abnormalities present in control mice that received the undernourished donor's microbiota alone (*36*).

This approach of tracking microbial exchange during cohousing, followed by culturing invasive taxa, can be applied to numerous combinations of healthy and undernourished microbiota to determine the extent to which acquisition of members of a healthy donor's microbiota (or extirpation of members of an undernourished donor's microbiota) can prevent or treat transmissible disease–associated phenotypes. The results could yield probiotic candidates and provide an opportunity to define mechanisms underlying microbial promotion of healthy growth. Relevant to this issue is a report showing that the gut microbiota enhances sensitivity to growth hormone (*40*). In this study, young germ-free mice exhibited significantly reduced rates of weight gain and bone growth as compared with their conventionally raised counterparts. Moreover, the difference between groups was greater when animals consumed a nutrient-deficient compared to -sufficient diet. Whereas levels of growth hormone were comparable, insulin-like growth factor 1 (IGF-1) and IGF-1–binding protein 3 (IGFBP-3) concentrations were significantly lower in animals lacking a microbiota. Remarkably, this growth hormone–resistance was overcome if germ-free animals were monocolonized at birth with one but not another strain of *Lactobacillus plantarum*.

Characterizing codevelopment of the microbiota and gut mucosal immune system

Another important aspect of postnatal development involves functional maturation of the gut mucosal immune system. Quantifying immunoglobulin A (IgA) responses to members of the developing gut microbiota provides one way to characterize this process. IgA is the major class of antibody secreted by the gut mucosa. The intestinal IgA repertoire of breastfeeding infants initially consists of a mixture of antibodies produced locally within the mucosa and antibodies present in breast milk but becomes entirely host-derived as IgA-secreting plasma cells populate the intestinal lamina propria through both T cell–dependent and –independent pathways. Fecal samples can be serially collected from members of birth cohorts and subjected to fluorescence-activated cell sorting (FACS) so as to distinguish IgA-targeted from nontargeted taxa [defined by means of 16*S* rRNA analysis of the IgA^+ and IgA^- populations and expressed as an IgA index for each taxon (*37*, *41*)]. Applying this method to samples collected from 40 pairs of healthy U.S.-born mono- and dizygotic twins revealed IgA responses to 30 bacterial taxa that converge on a pattern shared across pairs by the second postnatal year. This convergence is not simply due to changes in the abundance of the targeted bacterial taxa. Several of the targeted bacteria are age-discriminatory taxa defined by Random Forests–based analysis of microbiota development in this healthy U.S. birth cohort. Zygosity, mode of delivery, and breast versus formula feeding all had small but statistically significant effects on targeting (*37*).

These approaches for quantifying development of gut mucosal IgA responses and development of the gut microbiota can be applied to healthy and undernourished members of birth cohorts representing different geographic areas, distinctive cultural and dietary traditions, and varying degrees of sanitation. The results should reveal the extent to which features of the maturation of gut mucosal IgA responses generalize across populations and whether they correlate with MAZ scores, anthropometry, enteropathogen burden, and vaccine responses. (If the latter is true, this may help inform vaccination strategies

in populations with poor responses.) Intriguingly, analysis of feces obtained from gnotobiotic mice colonized with fecal microbiota obtained from twin pairs discordant for SAM revealed a group of IgA-targeted bacterial taxa in the SAM donor microbiota. The FACS protocol allowed these strains to be recovered in a viable form. Germ-free mice receiving the purified consortium of SAM-associated IgA-targeted taxa and fed a Malawian diet developed an enteropathy characterized by disruption of small intestinal and colonic barrier function. The effects were diet-dependent (they did not occur if animals were fed a macro- and micronutrient-sufficient diet) and were prevented when a consortium of purified IgA-targeted bacterial strains from the healthy co-twin's microbiota was added just before transfer of the SAM-derived strains (*41*). These findings indicate that the IgA response can be used to identify and characterize bacterial effectors of enteropathy, strains that are potential therapeutic candidates, as well as the impact of specified dietary ingredients on pathogenesis (*42*).

A recent mouse study demonstrated a role for maternal microbial exposure in programming the immune responses of their pups (*43*). Pregnant germ-free mice were colonized with a genetically engineered *E. coli* strain that transiently colonizes the host, allowing dams to return to a germ-free state and deliver germ-free offspring. Transient maternal colonization was associated with changes in expression of pup intestinal genes involved in various facets of barrier function. This transmissible phenotype appears to be mediated in part by components of maternal microbes transferred, via antibodies, during pregnancy and in breast milk.

Mechanisms and future directions

Identifying age- and growth-discriminatory bacterial taxa in preclinical models provides an opportunity to use serially collected biospecimens and clinical metadata from completed, ongoing, or future birth cohort studies to examine, in a focused and hypothesis-driven manner, the effects of a variety of factors implicated in the pathogenesis of childhood undernutrition on the gut microbiota. One conceptualization of pathogenesis is presented in Box 1. Birth cohort studies also offer an opportunity to examine the extent to which microbiota immaturity arises through a failure to progress through a normal developmental program and/or through a regression owing to harmful perturbations (with an attendant failure to recover). Given the high rates of discordance for undernutrition documented in twins (*6*), incorporating them into these birth cohorts studies should be very informative.

Deciphering mechanisms that direct microbial community assembly

Although progression from milk feeding to complementary feeding to a fully weaned state has a large effect on gut microbial community configuration, the mechanisms that shape postnatal assembly of the human gut microbiota remain poorly understood. Preclinical gnotobiotic models should help address this challenge. The ability to generate clonally arrayed and genome-sequenced collections of cultured bacterial strains that represent the diversity present in a given individual's gut community makes it possible to introduce subsets of these organisms that represent different stages of community assembly into young germ-free animals. This will permit assessments of how each subset affects host biology, how varying the order of presentation of these community subsets affects community "fate," and what the effects of manipulating host genes have on community assembly. High-resolution time series studies need to be applied to these models that look beyond defining community structure, to expressed functions. If cultured strains are amenable to whole-genome transposon mutagenesis, then gene-level definitions of the origin of their fitness in the evolving community can be ascertained (*44*). These approaches can also be used to model the effects of enteropathogen invasion and/or antibiotic administration at various stages of community assembly, including analyses of how the extent and route of recovery from these perturbations (resiliency) relate to stages of community development and composition. Intriguingly, recovery from cholera infection in adults living in Bangladesh recapitulates many of the features of normal gut microbial community assembly seen in healthy infants and children, albeit over a much shorter time scale [weeks as opposed to years (*45*)]. Yet another opportunity provided by these types of defined gnotobiotic models is to characterize the effects of these manipulations along the length of the gut (at least at the time of euthanasia); this includes the proximal small intestine, where substantial host nutrient absorption occurs, and the ileum, where there is considerable cross-talk between microbes and the immune system.

At a minimum, these gnotobiotic experiments require that representative human diets be developed, including those that support early colonizers of the human gut (including milk-based components if the recipient germ-free animals have already been weaned). More detailed biochemical knowledge of the composition of commonly consumed dietary components is needed if their effects on the structural and functional maturation of the microbiota are to be deciphered. Developing

Box 2. Societal considerations for microbiota-directed interventions for childhood undernutrition.

- Educating women about the microbiota and health
- Envisioning the impact of this knowledge and empowerment on child-rearing practices
- Assessing the cultural acceptability of microbiota-directed food products (includes labeling and associated public educational/advertising efforts)
- Defining price points for "affordability" and development of strategies that promote consumer compliance
- Identification of sources of ingredients, and designing manufacturing systems and distribution infrastructures that provide local economic benefit in order to ensure sustainable production of these products
- Ensuring partnerships between governmental, nongovernmental organization, and other potential stakeholders to enable broad implementation and mitigate risk for investment by industrial partners
- Understanding and shaping national views and policies related to ownership of foods (cultivars and recipes), their manufacture, branding, and associated intellectual property
- Assessing the downstream consequences of success in demonstrating the interrelationship between food, the gut microbiota, and healthy growth on national policies regarding (i) nutritional recommendations for children (including school age), (ii) the identification of more affordable nutritious sources of foods, and (iii) how best to define and ensure food safety (and support associated costs of implementing surveillance/testing)
- Delineating policies about the preservation, stewardship, and ownership of human-associated microbial resources within and across generations, within national borders, as well as policies related to the distribution of these resources across national borders
- Supporting anthropologic studies of how views about the association between microbes and humans ("self") are shaped by cultural traditions and religious beliefs
- Conducting analyses of the ethical and regulatory implications and long-term consequences of deliberately shaping human development through manipulating the assembly of microbial communities

(Left) Woman cooking food in a slum in Dhaka City, Bangladesh. [Photo courtesy of Mustafa Mahfuz] (Right) Rural village of Makhwira in Chikwawa District, Malawi, an area with some of the highest rates of stunting and wasting in sub-Saharan Africa. [Photo courtesy of Indi Trehan]

improved methods for quantifying spatial relationships between community members on partially digested food particles and at the mucosal-luminal interface would also add value to these analyses.

The massive data sets emanating from these preclinical studies should provide unprecedented opportunities (and requirements for new tools) to model microbe-microbe interactions, including the underpinnings of syntrophic relationships and competition. The results of the modeling can be tested in vivo by using the types of gnotobiotic models described above, or in vitro including in microfluidic systems (*46*). Throughput is a bottleneck for gnotobiotic experiments involving mice, but new caging systems offer the promise of increasing the ability to simultaneously test multiple combinations of defined collections of cultured gut microbes, or intact uncultured microbiota from multiple donors, outside of traditional flexible-film isolators. A variety of nonmammalian, genetically manipulatable vertebrate and invertebrate model organisms (such as *Danio rerio*, *Drosophila melanogaster*, and *Caenorhabditis elegans*) have been, are being, and need to be adopted for gnotobiotic studies. Moreover, in contrast to the hunt for genetic polymorphisms that affect susceptibility to infections in childhood with various pathogens (*47*), the search for polymorphisms that influence host-microbe interactions in undernutrition has produced few leads (*48*). Genetically manipulatable preclinical models will hopefully help fill this gap in knowledge.

Communication between the microbiota and host

Identifying the mechanisms by which normally developing versus immature gut microbiota regulate host physiology and metabolism is a formidable and intimidating yet inspiring challenge because it offers an opportunity to greatly advance our fundamental understanding of biological regulation in holobionts (hosts together with all of their microbial symbionts, both stable and transient). Basic principles can be gleaned by using preclinical models of the type described above, in which microbial community membership, host genotype, and environmental exposures can be systematically manipulated. The therapeutic implications could be great. For example, there is a dearth of metabolomic studies of undernourished children before, during, and/or after treatment and of suitable healthy controls (*32*). Understanding how immature microbiota from undernourished donors transmit a reduced metabolic flexibility phenotype to recipient gnotobiotic animals is important for a number of reasons, including the fact that low-income countries are experiencing the dual burden of under- and overnutrition [obese individuals with metabolic syndrome also manifest reduced metabolic flexibility (*14*)].

Therapeutic considerations

We need clinical POC that "catch-up" maturation of immature microbiota is feasible and sustainable. If the means are identified for sustained repair, then appropriately powered follow-on studies can be conducted to ascertain the effect on clinically meaningful growth outcomes, enteropathogen burden, gut barrier function and EED biomarkers, vaccine responses, and neurologic function.

One approach for shaping microbial community maturation is through development of microbiota-directed therapeutic foods that target age- and growth-discriminatory taxa. Commonly consumed complementary foods represent a source of culturally accessible, locally available, and affordable food ingredients that have "co-evolved" with a human population and its microbiota. One testable hypothesis is that some of the ingredients in these foods promote the representation of growth-discriminatory taxa in proportions that are age-appropriate. Testing this hypothesis requires databases of food consumption patterns and in vivo models in order to efficiently test different combinations and concentrations of these dietary ingredients for their effects on targeted age- or growth-discriminatory organisms. Fortunately, methods have already been described for performing diet oscillation studies in gnotobiotic mice colonized with defined consortia of bacterial strains and using linear modeling to identify which ingredients correlate with the abundances of given strains (*49*). A related question is whether the effects generalize to strain-level phylotypes obtained from different donors representing a population or populations of interest.

Complementary food-derived "leads" emerging from preclinical models can be advanced to human studies, assuming proper attention is paid to ethical, safety, and regulatory issues involved when enrolling children in vulnerable populations. Children who exhibit persistent MAM after the treatment of SAM represent a more stratified population with a history of severe microbiota immaturity than that of individuals who first present with MAM. Moreover, there is a clear unmet medical need, given the observed failures to progress beyond MAM with current short-term therapeutic protocols (*35*). This issue of what subset of children to enroll in future POC studies emphasizes the need to develop affordable point-of-care diagnostics for defining microbiota maturity.

Until the POC clinical studies are performed, we will not know whether and to what extent these types of food-based interventions can durably repair microbiota immaturity. We will also not know whether there are host factors that operate to regulate the rate of restoration of normal microbiota maturity and what the effects are when an age-appropriate state is achieved over a time frame that is shorter than the extent of immaturity (for example, if rescue occurs over 2 months when the child has a microbiota that at the time of diagnosis is 8 months younger than what is appropriate for his/her chronological age). The finding that microbiota from 6-month-old infants conferred greater growth than those from 18-month-old children in recipient mice has to be repeated by using samples serially collected from multiple individuals representing different populations in order to gain better understanding of the impact of rate (and route) of restoration of a perturbed microbiota on host biology.

In this conceptualization, the primary end-point biomarker for testing lead microbiota-directed food prototypes would be MAZ scores, with enteropathogen burden and assays of gut mucosal immune function (such as IgA responses) serving as secondary end-point markers. A selected affordable lead can then be advanced to studies so as to determine its effects on growth and development outcomes and, if therapeutic efficacy is established, whether it has utility in preventing disease.

It is possible that sustained repair of severely perturbed gut communities will require combinations of food-based and microbial interventions—the latter with defined consortia of cultured strains, including those representing targets of the dietary interventions. The regulatory path for microbial interventions in children, especially for organisms that are not classified as "generally regarded as safe" (GRAS), is uncertain at present, as is the safety (both short and long term) of fecal microbiota transplants in this population in which enteropathogen load is great and barrier function often compromised. To date, clinical trials of probiotics classified as GRAS in children with undernutrition have failed to show efficacy (*50*).

Societal implications of deliberate microbiota-directed interventions in children

The impact of increasing knowledge of the role of the microbiota in defining nutritional status will likely manifest itself in many ways. For these reasons, it is important that a holistic proactive view of the implications of this work be embraced and efforts made to prepare many elements of society through thoughtful engagement (Box 2). As clinical trials of the type described above are being planned, we issue a call for a comprehensive public dialogue about their benefits and perceived risks. Achieving a deep understanding of the biological as well as socioeconomic origins of this pervasive health problem will require "antedisciplinary" people (*51*). The same is true if we are to develop and implement effective treatment and prevention strategies. As such, this area provides an opportunity and need for universities to creatively evolve their educational strategies, policies (including access to their course content), and partnerships (within and across countries) in order to inspire and empower their students to acknowledge, confront, and overcome this daunting 21st-century challenge during their lifetimes.

REFERENCES AND NOTES

1. Z. A. Bhutta *et al*., Evidence-based interventions for improvement of maternal and child nutrition: What can be done and at what cost? *Lancet* **382**, 452–477 (2013). doi: 10.1016/S0140-6736(13)60996-4; pmid: 23746776
2. M. de Onis, A. W. Onyango, J. Van den Broeck, W. C. Chumlea, R. Martorell, Measurement and standardization protocols for anthropometry used in the construction of a new international growth reference. *Food Nutr. Bull.* **25** (suppl.), S27–S36 (2004). doi: 10.1177/15648265040251S104; pmid: 15069917
3. UNICEF, World Health Organization, W. B. Group, Levels and trends in child malnutrition, 1–6 (WHO, 2015).
4. A. J. Prendergast, J. H. Humphrey, The stunting syndrome in developing countries. *Paediatr. Int. Child Health* **34**, 250–265

(2014). doi: 10.1179/2046905514Y.0000000158; pmid: 25310000
5. R. Martorell, A. Zongrone, Intergenerational influences on child growth and undernutrition. *Paediatr. Perinat. Epidemiol.* **26** (suppl. 1), 302–314 (2012). doi: 10.1111/j.1365-3016.2012.01298.x; pmid: 22742617
6. M. I. Smith *et al*., Gut microbiomes of Malawian twin pairs discordant for kwashiorkor. *Science* **339**, 548–554 (2013). doi: 10.1126/science.1229000; pmid: 23363771
7. World Health Organization, Infant and young child feeding; Fact sheet no. 342, 1–5 (WHO, 2016).
8. B. L. Horta, C. G. Victora, Short-term effects of breastfeeding. *World Health Organization*, 1–54 (2013).
9. J. T. Smilowitz, C. B. Lebrilla, D. A. Mills, J. B. German, S. L. Freeman, Breast milk oligosaccharides: Structure-function relationships in the neonate. *Annu. Rev. Nutr.* **34**, 143–169 (2014). doi: 10.1146/annurev-nutr-071813-105721; pmid: 24850388
10. M. N. Huda *et al*., Stool microbiota and vaccine responses of infants. *Pediatrics* **134**, e362–e372 (2014). doi: 10.1542/peds.2013-3937; pmid: 25002669
11. M. Weng, K. Ganguli, W. Zhu, H. N. Shi, W. A. Walker, Conditioned medium from Bifidobacteria infantis protects against Cronobacter sakazakii-induced intestinal inflammation in newborn mice. *Am. J. Physiol. Gastrointest. Liver Physiol.* **306**, G779–G787 (2014). doi: 10.1152/ajpgi.00183.2013; pmid: 24627567
12. S. Fukuda *et al*., Bifidobacteria can protect from enteropathogenic infection through production of acetate. *Nature* **469**, 543–547 (2011). doi: 10.1038/nature09646; pmid: 21270894
13. M. R. Charbonneau *et al*., Sialylated milk oligosaccharides promote microbiota-dependent growth in models of infant undernutrition. *Cell* **164**, 859–871 (2016). doi: 10.1016/j.cell.2016.01.024; pmid: 26898329
14. D. M. Muoio, Metabolic inflexibility: When mitochondrial indecision leads to metabolic gridlock. *Cell* **159**, 1253–1262 (2014). doi: 10.1016/j.cell.2014.11.034; pmid: 25480291
15. J. Liu *et al*., A laboratory-developed TaqMan Array Card for simultaneous detection of 19 enteropathogens. *J. Clin. Microbiol.* **51**, 472–480 (2013). doi: 10.1128/JCM.02658-12; pmid: 23175269
16. A. M. Acosta *et al*., The MAL-ED study: A multinational and multidisciplinary approach to understand the relationship between enteric pathogens, malnutrition, gut physiology, physical growth, cognitive development, and immune responses in infants and children up to 2 years of age in resource-poor environments. *Clin. Infect. Dis.* **59** (suppl. 4), S193–S206 (2014). doi: 10.1093/cid/ciu653; pmid: 25305287
17. P. S. Korpe, W. A. Petri Jr., Environmental enteropathy: Critical implications of a poorly understood condition. *Trends Mol. Med.* **18**, 328–336 (2012). doi: 10.1016/j.molmed.2012.04.007; pmid: 22633998
18. S. McKay, E. Gaudier, D. I. Campbell, A. M. Prentice, R. Albers, Environmental enteropathy: New targets for nutritional interventions. *In Health* **2**, 172–180 (2010). doi: 10.1016/j.inhe.2010.07.006; pmid: 24037697
19. M. Kosek *et al*., Assessment of environmental enteropathy in the MAL-ED cohort study: Theoretical and analytic framework. *Clin. Infect. Dis.* **59** (suppl. 4), S239–S247 (2014). pmid: 25305293
20. M. Kosek *et al*., Fecal markers of intestinal inflammation and permeability associated with the subsequent acquisition of linear growth deficits in infants. *Am. J. Trop. Med. Hyg.* **88**, 390–396 (2013). doi: 10.4269/ajtmh.2012.12-0549; pmid: 23185075
21. C. Naylor *et al*., Environmental enteropathy, oral vaccine failure and growth faltering in infants in Bangladesh. *EBioMedicine* **2**, 1759–1766 (2015). doi: 10.1016/j.ebiom.2015.09.036; pmid: 26870801
22. E. M. Brown *et al*., Diet and specific microbial exposure trigger features of environmental enteropathy in a novel murine model. *Nat. Commun.* **6**, 7806 (2015). doi: 10.1038/ncomms8806; pmid: 26241678
23. S. E. Winter *et al*., Host-derived nitrate boosts growth of E. coli in the inflamed gut. *Science* **339**, 708–711 (2013). doi: 10.1126/science.1232467; pmid: 23393266
24. E. Quévrain *et al*., Identification of an anti-inflammatory protein from *Faecalibacterium prausnitzii*, a commensal bacterium deficient in Crohn's disease. *Gut* **65**, 415–425 (2016). doi: 10.1136/gutjnl-2014-307649; pmid: 26045134
25. S. Guo *et al*., Secreted metabolites of *Bifidobacterium infantis* and *Lactobacillus acidophilus* protect immature human enterocytes from IL-1β-induced inflammation: A transcription profiling analysis. *PLOS ONE* **10**, e0124549 (2015). doi: 10.1371/journal.pone.0124549; pmid: 25906317
26. S. Isanaka *et al*., Routine amoxicillin for uncomplicated severe acute malnutrition in children. *N. Engl. J. Med.* **374**, 444–453 (2016). doi: 10.1056/NEJMoa1507024; pmid: 26840134
27. I. Trehan *et al*., Antibiotics as part of the management of severe acute malnutrition. *N. Engl. J. Med.* **368**, 425–435 (2013). doi: 10.1056/NEJMoa1202851; pmid: 23363496
28. E. K. Gough *et al*., The impact of antibiotics on growth in children in low and middle income countries: Systematic review and meta-analysis of randomised controlled trials. *BMJ* **348** (apr15 6), g2267 (2014). doi: 10.1136/bmj.g2267; pmid: 24735883
29. P. C. Papathakis, L. N. Singh, M. J. Manary, How maternal malnutrition affects linear growth and development in the offspring. *Mol. Cell. Endocrinol.* (2016). doi: 10.1016/j.mce.2016.01.024; pmid: 26820126
30. L. H. Allen, B vitamins in breast milk: Relative importance of maternal status and intake, and effects on infant status and function. *Adv. Nutr.* **3**, 362–369 (2012). doi: 10.3945/an.111.001172; pmid: 22585913
31. R. A. Salam, C. MacPhail, J. K. Das, Z. A. Bhutta, Effectiveness of Micronutrient Powders (MNP) in women and children. *BMC Public Health* **13** (suppl. 3), S22 (2013). pmid: 24564207
32. S. Bartz *et al*., Severe acute malnutrition in childhood: Hormonal and metabolic status at presentation, response to treatment, and predictors of mortality. *J. Clin. Endocrinol. Metab.* **99**, 2128–2137 (2014). doi: 10.1210/jc.2013-4018; pmid: 24606092
33. C. Y. Chang *et al*., Children successfully treated for moderate acute malnutrition remain at risk for malnutrition and death in the subsequent year after recovery. *J. Nutr.* **143**, 215–220 (2013). doi: 10.3945/jn.112.168047; pmid: 23256140
34. L. M. Lenters, K. Wazny, P. Webb, T. Ahmed, Z. A. Bhutta, Treatment of severe and moderate acute malnutrition in low- and middle-income settings: A systematic review, meta-analysis and Delphi process. *BMC Public Health* **13** (suppl. 3), S23 (2013). doi: 10.1186/1471-2458-13-S3-S23; pmid: 24564235
35. S. Subramanian *et al*., Persistent gut microbiota immaturity in malnourished Bangladeshi children. *Nature* **510**, 417–421 (2014). pmid: 24896187
36. L. V. Blanton *et al*., Gut bacteria that prevent growth impairments transmitted by microbiota from malnourished children. *Science* **351**, aad3311 (2016). doi: 10.1126/science.aad3311; pmid: 26912898
37. J. Planer *et al*., Development of the gut microbiota and mucosal IgA responses in twins and gnotobiotic mice. *Nature* 10.1038/nature17940 (2016). doi: 10.1038/nature17940
38. A. Reyes *et al*., Gut DNA viromes of Malawian twins discordant for severe acute malnutrition. *Proc. Natl. Acad. Sci. U.S.A.* **112**, 11941–11946 (2015). doi: 10.1073/pnas.1514285112; pmid: 26351661
39. J. K. Pfeiffer, H. W. Virgin, Transkingdom control of viral infection and immunity in the mammalian intestine. *Science* **351**, aad5872 (2016). doi: 10.1126/science.aad5872; pmid: 26816384
40. M. Schwarzer *et al*., Lactobacillus plantarum strain maintains growth of infant mice during chronic undernutrition. *Science* **351**, 854–857 (2016). doi: 10.1126/science.aad8588; pmid: 26912894
41. A. L. Kau *et al*., Functional characterization of IgA-targeted bacterial taxa from undernourished Malawian children that produce diet-dependent enteropathy. *Sci. Transl. Med.* **7**, 276ra24 (2015). doi: 10.1126/scitranslmed.aaa4877; pmid: 25717097
42. T. Hashimoto *et al*., ACE2 links amino acid malnutrition to microbial ecology and intestinal inflammation. *Nature* **487**, 477–481 (2012).
43. M. Gomez de Agüero *et al*., The maternal microbiota drives early postnatal innate immune development. *Science* **351**, 1296–1302 (2016). doi: 10.1126/science.aad2571; pmid: 26989247
44. M. Wu *et al*., Genetic determinants of in vivo fitness and diet responsiveness in multiple human gut Bacteroides. *Science* **350**, aac5992 (2015). doi: 10.1126/science.aac5992; pmid: 26430127
45. A. Hsiao *et al*., Members of the human gut microbiota involved in recovery from *Vibrio cholerae* infection. *Nature* **515**, 423–426 (2014). doi: 10.1038/nature13738; pmid: 25231861
46. H. J. Kim, H. Li, J. J. Collins, D. E. Ingber, Contributions of microbiome and mechanical deformation to intestinal bacterial overgrowth and inflammation in a human gut-on-a-chip. *Proc. Natl. Acad. Sci. U.S.A.* **113**, E7–E15 (2016). doi: 10.1073/pnas.1522193112; pmid: 26668389
47. J.-L. Casanova, Severe infectious diseases of childhood as monogenic inborn errors of immunity. *Proc. Natl. Acad. Sci. U.S.A.* **112**, E7128–E7137 (2015). pmid: 26621750
48. C. O. Marginean, C. Banescu, S. Voidazan, C. Duicu, IL-6 572 C/G, 190 C/T, and 174 G/C gene polymorphisms in children's malnutrition. *J. Pediatr. Gastroenterol. Nutr.* **59**, 666–673 (2014). doi: 10.1097/MPG.0000000000000492; pmid: 25023587
49. J. J. Faith, N. P. McNulty, F. E. Rey, J. I. Gordon, Predicting a human gut microbiota's response to diet in gnotobiotic mice. *Science* **333**, 101–104 (2011). doi: 10.1126/science.1206025; pmid: 21596954
50. M. Kerac *et al*., Probiotics and prebiotics for severe acute malnutrition (PRONUT study): A double-blind efficacy randomised controlled trial in Malawi. *Lancet* **374**, 136–144 (2009). doi: 10.1016/S0140-6736(09)60884-9; pmid: 19595348
51. S. R. Eddy, "Antedisciplinary" science. *PLOS Comput. Biol.* **1**, e6 (2005). doi: 10.1371/journal.pcbi.0010006; pmid: 16103907

ACKNOWLEDGMENTS

Work cited from the authors' laboratory was supported in part by a grant from the Bill & Melinda Gates Foundation. J.I.G. is cofounder of Matatu, a company characterizing the role of diet-by-microbiota interactions in animal health. Upon completion of his Ph.D., M.R.C. has joined Matatu as a research scientist.

10.1126/science.aad9359