PERSPECTIVE

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Human nutrition, the gut microbiome and the immune system

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Marked changes in socio–economic status, cultural traditions, population growth and agriculture are affecting diets worldwide. Understanding how our diet and nutritional status influence the composition and dynamic operations of our gut microbial communities, and the innate and adaptive arms of our immune system, represents an area of scientific need, opportunity and challenge. The insights gleaned should help to address several pressing global health problems.

Many recent reviews have described the known interactions between the innate and adaptive immune system and the tens of trillions of microbes that live in our gastrointestinal tracts (known as the gut microbiota). In this Perspective, we emphasize how the time is right and the need is great to understand better the relationships between diet, nutritional status, the immune system and microbial ecology in humans at different stages of life, living in distinct cultural and socio-economic settings.

This is a timely topic for many reasons. There is enormous pressure to devise ways to feed healthy foods to a human population whose size is predicted to expand to 9 billion by 2050. The solutions will have to address the challenges of developing sustainable forms of agriculture in the face of constrained land and water resources[1]. There is also a need to develop translational medicine pipelines to define more rigorously the nutritional value of foods that we consume and that we imagine creating in the future. These pipelines are required to evaluate health claims made about food ingredients. Increasing evidence shows that the nutritional value of food is influenced in part by the structure and operations of a consumer's gut microbial community, and that food, in turn, shapes the microbiota and its vast collection of microbial genes (the gut microbiome) (see, for example, refs 2 and 3). Therefore, to define the nutritional value of foods and our nutritional status better, we need to know more about our microbial differences and their origins, including how our lifestyles influence the assembly of gut microbial communities in children, and about the transmission of these communities within and across generations of a kinship[4]. We are learning how our gut microbial communities and immune systems co-evolve during our lifespans, and how components of the microbiota affect the immune system. We are also obtaining more information about how our overall metabolic phenotypes (metabotypes) reflect myriad functions encoded in our human genomes and gut microbiomes. These observations raise the question of how the metabolism of foods we consume by the gut microbial community affects our immune systems.

The link between infections that occur within and outside the gut and the development of nutritional deficiencies has been emphasized for many years. In turn, poor nutrition increases the risk of infection. Nonetheless, there is still a dearth of mechanistic information that explains these observations. Furthermore, only four years remain to achieve the United Nations eight Millennium Development Goals (http://www.undp.org/mdg/). Two of these goals relate to human nutrition: one seeks to eradicate extreme poverty and hunger, and another aims to reduce the under-five mortality rate by two-thirds. Up to 1 billion people suffer from undernutrition of varying degrees, including 'silent' or asymptomatic malnutrition (http://www.fao.org/publications/sofi/en/), making this condition an enormous global health problem. Of the ~10 million children under the age of 5 who die every year, undernutrition contributes in some fashion to more than 50% of these deaths[5]. Sadly, children who survive periods of severe undernutrition can suffer long-term sequelae, including stunting and neurodevelopmental deficits[6]. Moreover, the effects of undernutrition can be felt across generations. Undernourished mothers suffer higher rates of morbidity and mortality, and are more likely to have low-birth-weight children, who have an increased risk of developing type 2 diabetes, hypertension, dyslipidaemia, cardiovascular pathology and obesity as adults[7]. A testable hypothesis is that the gut microbiota may contribute to the risk and pathogenesis of undernutrition through effects on nutrient metabolism and immune function (Fig. 1). Similarly, the experience of undernutrition in childhood could affect the development of metabolic capacities by this microbial 'organ' in ways that result in persistent metabolic dysfunction or inadequate function, thereby contributing to the sequelae of malnutrition. Finally, if we define malnutrition as the inadequate or excessive consumption of dietary ingredients leading to the development of disease, then we also need to consider the alarming epidemic of obesity that is sweeping the world and its relationship to the gut microbiome and the immune system.

The marriage of metagenomics and gnotobiotics

We believe that the 'marriage' of two approaches — one involving culture-independent (metagenomic) methods for describing the gut microbiota or microbiome and the other involving gnotobiotics (the rearing of animals under germ-free conditions, with or without subsequent exposure during postnatal life or adulthood to a microbial species or species consortium) — is a potentially powerful way to address several questions about the relationships between diet, nutritional status, the assembly and dynamic operations of gut microbial communities, and the nature of the interkingdom communications between the gut microbiota and the host (including host–microbial co-metabolism, and the co-evolution of the immune system[3,8,9]). Without dismissing caveats related to the use of gnotobiotic models (see later), in this Perspective we describe ways that may be useful for joining gnotobiotics and metagenomic methods to compare the functional properties of various types of gut microbial community, to explicitly test or generate hypotheses, and to develop new

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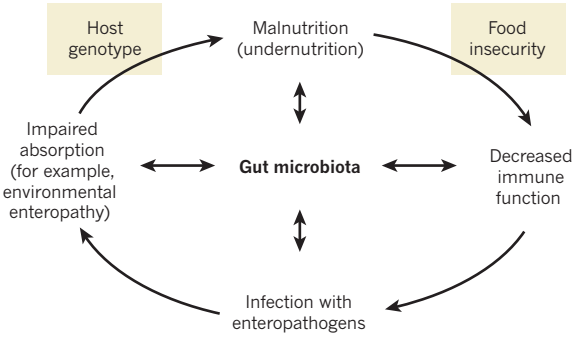


Figure 1 | A schematic of the proposed relationships between the gut microbiota, the immune system and the diet, which underlie the development of malnutrition. Undernutrition is associated with several defects in the innate and adaptive immune systems, which, in turn, are associated with increased predisposition to diarrhoeal illnesses. Recurrent (enteric) infections predispose to macronutrient and micronutrient deficiencies, as well as impaired intestinal mucosal barrier function[77]. These factors lead to a cycle of further susceptibility to infection and worsening nutritional status. A confounding problem is that vaccines designed to protect children from certain pathogens (including enteropathogens) show poor efficacy in areas of the world where poor nutrition is rampant[74]. One testable hypothesis is that the microbiota contributes to disease risk and pathogenesis. Diet shapes gut microbial community structure and function, and the microbiota adapts in ways that promote nutrient processing; the ability of the microbiota to process a given diet affects the nutrient and energetic value of that diet. The microbiota and immune systems co-evolve: malnutrition affects the innate and adaptive immune systems as well as the microbiota. The microbiota acts as a barrier to enteropathogen infection; this barrier function may be disrupted by malnutrition, as well as by perturbations in immune system function. The microbiota affects nutrient processing by the host, including the expression of host genes involved in nutrient transport and metabolism.

experimental (and computational) approaches that together inform the design, execution and interpretation of human studies.

What is changing about what we eat?

Changes in dietary consumption patterns affect many aspects of human biology. To fully understand the determinants of nutritional status, we need to know what people are eating and how these diets are changing. Unfortunately, accurate information of this type is hard to obtain, and when available it generally covers a relatively limited time period. As a corollary, searchable databases that effectively integrate information obtained from the surveillance efforts of many international and national organizations (such as the World Health Organization, the UN Food and Agriculture Organization and the United States Department of Agriculture (USDA) Economic Research Service) are needed to monitor changing patterns of food consumption in different human populations. Analysis of USDA data that track the availability of more than 200 common food items between 1970 and 2000 shows that diets in the United States have changed in terms of both the overall caloric intake and the relative amounts of different food items (http://www.ers.usda.gov/Data/FoodConsumption). Linear regression of total caloric intake over time shows that the average number of kilocalories consumed per day increased markedly over this 30-year period ($R^2 = 0.911$, $P < 10^{-15}$). This is consistent with estimates from the US National Health and Nutrition Examination Survey (NHANES), which indicate that adult men and women increased their daily caloric intake by 6.9% and 21.7%, respectively, during the same period[10]. If total caloric intake is analogous to 'primary productivity' in macro-ecosystems, in which primary productivity is used as a proxy for available energy, then increasing the amount of energy input from the diet would be predicted to affect the number of microbial species living in the gut of a single host, as well as the magnitude of the compositional differences that exist between different hosts or even different regions of a single gut (see ref. 11 for discussions about the mechanisms underlying productivity–species richness relationships in macro-ecosystems). Intriguingly, metagenomic studies of bacterial composition in the faecal microbiota of obese and lean twins living in the United States have shown that obesity is associated with decreased numbers of bacterial species[4]. Reductions in diversity could affect community function, resilience to various disturbances and the host immune system.

During the past 30 or so years, the North American diet has also shifted in terms of the relative contributions of different foods to total energy intake. Since 1970, two dietary 'epochs' can be distinguished based on the contribution of grains to overall calories (the mean increase in daily carbohydrate intake for men and women during this period was 62.4 g and 67.7 g, respectively[10]). The consumption of other food items has also changed: Spearman's rank correlations between food availability and time, followed by adjustments of P values to reflect false discovery rates, show that the representation of 177 out of 214 items tracked by the USDA has increased or decreased significantly in US diets since 1970. For example, Americans now eat less beef and more chicken, and corn-derived sweeteners have increased at the expense of cane and beet sugars. Furthermore, methods of food modification and preparation have changed. Comparable data are needed for other countries with distinct cultural traditions, including countries in which people are undergoing marked transformations in their socio-economic status and lifestyles.

We know from metagenomic studies of the human gut microbiota and microbiome that early postnatal environmental exposures have an important role in determining the overall phylogenetic structure of an adult human gut microbiota. The assembly of the microbiota towards an adult configuration occurs during the first three years of life[12], and features of the organismal and gene content of gut communities are shared among family members and transmitted across generations of a kinship[4]. We also know that dietary habits influence the structure of the human genome. For example, populations that consume diets high in starch have a higher copy number of the salivary amylase gene (*AMY1*) than those consuming low-starch diets[13]. We know that these habits also affect the gut microbiome. A wonderful illustration of the latter point is provided by a microbial β-porphyranase in Japanese populations. *Zobellia galactanivorans* is a marine member of the Bacteroidetes that can process porphyran derived from marine red algae belonging to the *Porphyra* genus. Homologues of porphyranase genes from *Z. galactanivorans* are present in the human gut bacterium *Bacteroides plebeius* and are prominently represented in the microbiomes of Japanese but not North American citizens. This finding led to the suggestion that porphyranase genes from *Z. galactanivorans* or another related bacterium were acquired, perhaps through horizontal gene transfer, by a resident member of the microbiota of Japanese consumers of non-sterile food, and that this organism and gene were subsequently transmitted to others in Japanese society[14]. Together, these observations lead to the notion that systematic changes in overall dietary consumption patterns across a population might lead to changes in the microbiome, with consequences for host nutritional status and immune responses.

We also know, from work in gnotobiotic mice that have received human faecal microbial community transplants, that the relative abundances of different bacterial species and genes in the gut microbiota are highly sensitive to different foods[3]. Gnotobiotic mice containing defined collections of sequenced human gut symbionts or transplanted human faecal microbial communities could provide an approach for modelling the effects of different dietary epochs on the gut microbiota and on different facets of host biology. If the desired result is an account of the effects of individual food items or nutrients, then feeding the animals a series of defined diets, each with a different element removed or added, might be an appropriate strategy if the food ingredients for the epoch are known and available. If the focus is on the effects of overall differences in dietary habits within or between groups of humans, then diets should reflect the overall nutritional characteristics of the different groups and not merely be representative of a single individual.

Designing such diets requires detailed accounts of the identity and quantity of each food item consumed, ideally for a large number of people, as well as the methods used for food preparation. The US diet presents a rare opportunity for such an approach, because NHANES data sets (http://www.cdc.gov/nchs/tutorials/Dietary/) provide one-day dietary recall data at several time points since the early 1970s.

Nutrient metabolism and the immune system

The nexus between nutrient metabolism and the immune system occurs at many levels, ranging from endocrine signalling to direct sensing of nutrients by immune cells.

Leptin signalling provides an example of these complex interrelationships. Leptin regulates appetite and is a pleiotropic cytokine, maintaining thymic output and cellularity and promoting the dominance of T helper 1 (T_H1) cells over T_H2 cells[15,16] while inhibiting the proliferation of T regulatory (T_{reg}) cells[17]. Low levels of leptin may account for the decreased cellular immunity associated with periods of nutrient deprivation[16]. Leptin also affects innate immune cells, ranging from the promotion of neutrophil activation and migration to the activation of monocytes and macrophages[15]. Elegant experiments using mice deficient in the leptin receptor in different cellular compartments showed a requirement for leptin signalling in intestinal epithelial cells to prevent severe disease after exposure to *Entamoeba histolytica*. Comparisons of *db/db* mice, which lack a functional leptin receptor, and their wild-type littermates demonstrated that leptin controls infectivity and prevents severe inflammatory destruction of the intestine, thereby affecting mortality[18]. These studies were extended to mice with engineered mutations in the leptin receptor that are found in human populations (Tyr1138Ser and Tyr985Leu, both of which disrupt signalling). These mutations rendered mice more susceptible to *E. histolytica* infection[18]. Leptin levels are significantly reduced in the sera of germ-free mice[19]. Moreover, obese, leptin-deficient (*ob/ob)* mice have marked differences in the taxonomic and genetic content of their gut microbial communities[20]. To our knowledge, the effects of leptin-receptor deficiency on the gut microbiota have not been reported. Nonetheless, leptin-receptor deficiency and *E. histolytica* pathogenesis provide a setting in which the intersections between the endocrine and immune systems, enteric infection and gut microbial ecology can be explored.

The ability to use macronutrients is essential for the generation and maintenance of a protective effector immune response. After stimulation through the T-cell receptor (TCR) and co-stimulation through CD28, the metabolic needs of T cells are met by a marked increase in the uptake and use of glucose and amino acids[21,22]. A deficiency in glucose uptake negatively affects numerous facets of T-cell function, with impairment of both proliferation and cytokine expression. Similarly, deficiencies in amino acids such as tryptophan, arginine, glutamine and cysteine reduce immune-cell activation. Furthermore, TCR stimulation in the absence of co-stimulation, which leads to T-cell anergy, has been linked to a failure to upregulate metabolic machinery associated with amino-acid and iron uptake[21,22].

Short-chain fatty acids (SCFAs) provide one of the clearest examples of how nutrient processing by the microbiota and host diet combine to shape immune responses. SCFAs are end products of the microbial fermentation of macronutrients, most notably plant polysaccharides that cannot be digested by humans alone because our genomes do not encode the large repertoire of glycoside hydrolases and polysaccharide lyases needed to cleave the varied glycosidic linkages present in these glycans[23]. These missing enzymes are provided by the microbiome. The luminal concentration of intestinal SCFAs can be modified by the amount of fibre in the diet, which affects the composition of the microbiota[24]. In addition to acting as an energy source for the host, SCFAs exert notable effects on host immune responses. Low levels of butyrate modify the cytokine production profile of T_H cells[25] and promote intestinal epithelial barrier integrity[26], which in turn can help to limit the exposure of the mucosal immune system to luminal microbes and prevent aberrant inflammatory responses. Production of another SCFA, acetate, by the microbiota promotes the resolution of intestinal inflammation by the G-protein-coupled receptor GPR43 (ref. 27). A recent study highlighted the important role of acetate production in preventing infection with the enteropathogen *Escherichia coli* (0157:H7). This effect was linked to the ability of acetate to maintain gut epithelial barrier function[28]. Intriguingly, SCFAs may regulate the acetylation of lysine residues[29], a covalent modification that affects proteins involved in a variety of signalling and metabolic processes. The role of this covalent modification in modulating the activity of proteins intimately involved in innate and adaptive immune responses needs to be explored further. It is tempting to speculate that the covalent or non-covalent linkage of products of microbial metabolism to host proteins produced within the intestine, or at extra-intestinal sites, will be discovered and shown to have important regulatory effects. These different protein modifications could represent a series of mechanisms by which the microbial community metabotype is 'imprinted' on the host.

If nutrients and derived metabolites reflect the functional activity of the microbiota, sensors of nutrient and metabolite availability can be considered akin to microbe-associated molecular patterns (MAMPs) that convey information about microbes to the host. Several families of innate receptors are involved in the recognition of MAMPs: these include Toll-like receptors (TLRs), inflammasomes, C-type lectins such as dectin-1, and RNA-sensing RIG-like helicases such as RIG-I and MDA5. The accompanying review by Maloy and Powrie (page 298) provides an overview of this area. We would like to emphasize that classical innate immune recognition pathways have evolved to survey the nutrient environment. TLR4 can sense the presence of free fatty acids[30], whereas ATP is an important activator of the inflammasome[31]. Several other immune-cell-associated sensors couple information about the local nutrient or metabolite environment to the coordination of local immune responses. Examples are the serine/threonine kinase mammalian target of rapamycin (mTOR)[32], double-stranded RNA-activated protein kinase (PKR)[33], the aryl hydrocarbon receptor (AHR)[34], and various nuclear hormone receptors such as the liver-X-receptor and the peroxisome-proliferator-activated receptors (PPAR-α, PPAR-β and PPAR-γ)[35] (Table 1 and Fig. 2). The mTOR pathway is an example of how energy availability affects immune responses. mTOR is activated by phosphatidylinositol-3-OH kinase and the serine/threonine kinase AKT, and is inhibited by AMP-activated protein kinase, which is a sensor of cellular energy resources. Genetic and pharmacological approaches (the latter using rapamycin) indicate that mTOR signalling affects both the innate and adaptive arms of the immune system — including maturation and effector activity of dendritic cells, inhibition of T_{reg}-cell development, promotion of the differentiation of T_H1, T_H2 and T_H17 cells, regulation of $CD8^+$ T-cell trafficking and inhibition of memory T-cell formation[32,36]. PKR couples the presence of free fatty acids to immune activation, and has been implicated in the pathogenesis of obesity in mice fed a high-fat diet, including their development of immuno-inflammatory and insulin-resistant phenotypes[33] (see below). AHR is activated by several agonists, including kynurenine — a product of tryptophan metabolism by indoleamine-2,3-dioxygenase[37,38]. AHR modulates the differentiation of dendritic cells[39] and promotes T_H17-cell and T_{reg}-cell differentiation and effector activity[40,41]. Withdrawal of tryptophan and arginine controls immune responses[42,43]. The presence of an intact amino-acid starvation response in T cells is essential for the immunosuppressive activity of tryptophan depletion by indoleamine-2,3-dioxygenase[44]. This example illustrates how the ability of T cells to sense levels of a nutrient (tryptophan) in its local environment, rather than using the nutrient solely as a fuel source, is an important determinant of cell fate. If the assessment of local nutrient levels or metabolites is an important feature in the immune decision-making process, and if the products of microbial metabolism are previously unappreciated agonists or antagonists of immune-cell receptors, then an important challenge is to devise *in vitro* and *in vivo* models, including genetically manipulable

gnotobiotic animals (such as mice or zebrafish), to identify the range of metabolites produced by a microbiota (and host) as a function of different defined diets.

The case for micronutrients

The intestinal microbiota can synthesize several vitamins involved in myriad aspects of microbial and host metabolism, including cobalamin (vitamin B_{12}), pyridoxal phosphate (the active form of vitamin B_6), which is involved in several enzymatic interconversions in amino-acid metabolism, pantothenic acid (vitamin B_5), niacin (vitamin B_3), biotin, tetrahydrofolate and vitamin K. In addition to vitamin B_{12}, gut microbes produce a range of related molecules (corrinoids) with altered 'lower ligands', including analogues such as methyladenine and *p*-cresol. More than 80% of non-absorbed dietary vitamin B_{12} is converted to these alternative corrinoids[45,46]. There is preliminary evidence to suggest that syntrophic relationships among members of the human microbiota, and the fitness of some taxa, may be based on the ability to generate, use or further transform various corrinoids[46,47].

Folate and cobalamin produced by the gut microbiota could affect host DNA methylation patterns, whereas acetate produced by the microbial fermentation of polysaccharides could modify chromatin structure and gene transcription by histone acetylation. Thus, the inheritance of a mammalian genotype and intergenerational transmission of a microbiome — together with a complex dynamic in which the microbiome is viewed both as an epigenome and a modifier of the host epigenome during the postnatal period when host, host diet and microbial community co-evolve — could together shape human physiological phenotypes that are manifested during childhood or later in life.

Numerous observational studies indicate that deficiencies in vitamins A, D and E and zinc can adversely affect immune function, particularly T-cell responses. Although a considerable body of work exists detailing the myriad effects of vitamins A, D and E on host immune responses, so far there is little evidence for a role of the microbiota in the biosynthesis or metabolism of these vitamins. However, stimulation of dendritic cells through TLR2 increases the expression of host genes associated with generation of the immunoactive form of vitamin A (retinoic acid), and enteric infection has been linked to vitamin A deficiency[48,49]. Intriguingly, a recent study demonstrated that vitamin A deficiency leads to a complete loss of T_H17 cells in the small intestine of specific pathogen-free mice and an associated significant reduction in the abundance of segmented filamentous bacteria (SFB)[50] — a member of the Clostridiaceae family that drives intestinal T_H17 responses in mice[51,52]. Thus, vitamin A has the potential to modulate immune responses directly, by interacting with immune cells, or indirectly, by modulating the composition of the microbiota.

The microbiota also affects the absorption of key minerals. Perhaps the best characterized micronutrient in terms of its interaction with both the microbiota and the immune system is iron. Iron-deficient mice are resistant to the development of experimental autoimmune encephalomyelitis, and have reduced delayed type hypersensitivity (also known as type IV hypersensitivity) responses and lower levels of IgM and IgG. Iron deficiency also impairs innate immune responses, as it is required for the respiratory burst[53]. Likewise, iron is an essential micronutrient for bacteria. Given the low solubility of Fe^{3+}, microbes have evolved the capacity to produce several high-affinity iron-binding siderophores. Microbes take up soluble Fe^{3+}–siderophore complexes by several active transporters. Early studies in gnotobiotic animals showed a link between the gut microbiota and the development of iron deficiency. Germ-free but not conventionally raised rats become anaemic when fed a low-iron diet. The germ-free rats also show increased loss of iron in their faeces compared with their conventionally raised counterparts[54]. The iron balance that exists between host and microbiota is disturbed in a mouse model of Crohn's disease in which tumour-necrosis factor-α (TNF-α) expression is dysregulated: oral (but not parenteral) iron supplementation in these animals causes a shift in the gut microbial community composition, as defined by 16S ribosomal-RNA-based surveys, and exacerbates their ileitis[55].

Metagenomic methods need to be applied to delineate further the role of the microbiota in micronutrient deficiencies. Several questions remain, such as how iron deficiency affects the configuration of the gut microbiota and microbiome, including the production of siderophores. Iron repletion could return the microbiota/microbiome to a normal, pre-deficient state; alternatively, there may be persistent structural and functional perturbations that require continued nutritional supplementation to correct. There may be particular configurations of the microbiota/microbiome that predispose the host to iron or other types of micronutrient deficiency. The iron content of mother's milk during postnatal life could also affect the assembly and metabolic operations of the microbiota. In principle, these questions can first be addressed in gnotobiotic mouse models, and also extended to macronutrient-deficient states.

The microbiota and the immune system in obesity

Obesity, metabolic syndrome and diabetes illustrate the role that the diet–microbiota–immune axis has in shaping human systems biology. Although the marked increase in obesity worldwide can be linked to an ever-growing trend towards excessive caloric intake, the microbiota has also been implicated in obesity. Studies of a cohort of twins living in the United States indicate that the bacterial phylogenetic composition of the faecal microbiota and the representation of microbial genes involved in several aspects of nutrient metabolism in the faecal microbiome are different in lean versus obese twin pairs[4]. Different research groups using different primers to amplify bacterial 16S ribosomal RNA genes for culture-independent analyses of gut microbial ecology, and studying different human populations consuming different diets, have reported varying results concerning the bacterial phylogenetic composition of the microbiota in lean versus obese individuals[56].

Evidence that a link exists between the microbiota and obesity comes from transplant experiments in gnotobiotic mice. Gut communities from leptin-deficient, *ob/ob,* mice or mice with diet-induced obesity induce a greater increase in adiposity when transferred to germ-free recipients than do communities from wild-type littermates or mice that have been given a healthy, calorically less-dense diet[20,57]. Germ-free mice are resistant to diet-induced obesity. Further studies have shown that the gut microbial community regulates the expression of genes that affect fatty-acid oxidation and fat deposition in adipocytes. For example, production of the secreted lipoprotein lipase (LPL) inhibitor angiopoietin-like protein 4 (ANGPTL4; also known as fasting-induced adipose factor) is suppressed by the microbiota: studies of germ-free and conventionalized wild-type and *Angptl4*$^{-/-}$ mice established that microbiota-mediated suppression of gut epithelial expression of this secreted LPL inhibitor results in increased LPL activity and fat storage in white adipose tissue[19,58]. Moreover, *Tlr5*-deficient mice have a gut microbiota with a distinct configuration from that encountered in wild-type littermate controls. When their gut microbiota is transplanted to wild-type, germ-free recipients, food intake is increased compared with recipients of microbiota transplants from wild-type mice: increased adiposity and hyperglycaemia ensue[59]. The mechanism underlying the increase in food consumption remains to be defined, although the authors of the study speculate that inflammatory signalling may desensitize insulin signalling in ways that lead to hyperphagia.

Obesity in mice and humans is associated with the infiltration of adipose tissue by macrophages, $CD8^+$ T cells[60] and $CD4^+$ T cells[61,62], and with the expression of inflammatory cytokines and chemokines such as interleukin-6 (IL-6), IL-17, TNF-α, CC-chemokine ligand 2 (CCL2) and interferon-γ[60,62,63]. By contrast, adipose tissue in lean mice is home to a population of immunosuppressive T_{reg} cells that prevents inflammation[64]. Mice deficient in CC-chemokine receptor 2 (*Ccr2*) and with obesity induced by consumption of a high-fat diet have reduced macrophage infiltration of the adipose tissue and improved glucose tolerance relative to *Ccr2*-sufficient controls[60], highlighting the role of factors in recruiting

Table 1 | Metabolite sensors associated with immune cells

Sensor	Agonist	Immune response affected
mTOR	S1P	Inhibits T_{reg}-cell differentiation and maintenance[88]
	Leptin	Promotes T_H1-cell differentiation[16]
	Leptin	Inhibits T_{reg}-cell proliferation and function[17,89]
AHR	6-formylindolo[3,2-b] carbazole	T_H17-cell differentiation and IL-22 production by T_H17 cells[40,41]
	2,3,7,8-tetrachlorodibenzo-*p*-dioxin	Promotes T_{reg}-cell induction[40]
	Kynurenine	Promotes T_{reg}-cell induction[38]
PKR	Free fatty acids; palmitic acid	Promotes insulin resistance through inhibitory phosphorylation of IRS-1 (ref. 33)
RAR–RXR	Retinoic acid	Promotes intestinal T-cell homing[90]; promotes T_{reg}-cell generation[91]; promotes T-cell proliferation[92]; promotes T_H2-cell differentiation over T_H1 cells[93]
VDR–RXR	1,25(OH)$_2$ vitamin D_3	Inhibits lymphocyte proliferation[94]; inhibits interferon-γ, IL-17 and IL-2 expression[95]; promotes emergence of T_{reg} cells[96]; drives antimicrobial peptide expression[97]; promotes T-cell expression of CCR10 (ref. 98)
GPR120	ω-3 Fatty acids	Inhibits inflammatory cytokine production and chemotaxis by macrophages[99]
GPR43	Acetate	Promotes resolution of intestinal inflammation[27]
P2X receptors	ATP	Promotes T_H17-cell generation[100]

AHR, aryl hydrocarbon receptor; mTOR, mammalian target of rapamycin; PKR, double-stranded RNA-dependent protein kinase; RAR, retinoic acid receptor; RXR, retinoid X receptor; S1P, sphingosine-1-phosphate; VDR, vitamin D receptor.

inflammatory immune cells and their associated pro-inflammatory products in the pathogenesis of metabolic abnormalities associated with obesity. Blockade of TNF-α[65] or expanding T_{reg} cells using anti-CD3 monoclonal antibody[62] prevents the onset of obesity-associated insulin resistance in a mouse model of diet-induced obesity.

Inflammation drives the development of insulin resistance through the phosphorylation of insulin receptor 1 by TNF-α activation of c-Jun amino-terminal protein kinase 1 (JNK1), and perhaps inhibitor of nuclear factor-κB kinase-β (IKK-β), protein kinase C and mTOR. Whereas signalling by the adaptor protein MyD88 promotes the development of type 1 diabetes in pathogen-free NOD (non-obese diabetic) mice, germ-free *Myd88*$^{-/-}$ NOD animals are susceptible to this disorder[66]. These findings suggest that particular intestinal microbial configurations can promote or prevent inflammatory immune responses that drive metabolic dysfunction.

Mice fed a high-fat diet have increased serum levels of lipopolysaccharide[67]. Furthermore, genetically obese mice that are deficient in leptin or its receptor have reduced intestinal barrier function[68]. As noted earlier, SCFAs produced by microbial fermentation affect the barrier. Thus, it will be important to assess whether obese humans show similar reductions in barrier function. A high-fat diet alters the structure of the intestinal microbiota, potentially leading to a reduction in gut barrier integrity. The enhanced translocation of microbes and/or their antigens may result in increased microbial antigen load at extra-intestinal sites, enhanced immune stimulation and the development of insulin resistance. Furthermore, nutrients are known to activate inflammatory arms of the immune system directly[69]. The capacity of the intestinal microbiota to shape immune responses outside the intestine is well documented. Studies have highlighted the ability of the microbiota and specifically SFB to support the development of autoimmune arthritis[70] and experimental autoimmune encephalomyelitis[71], both of which have been linked to excessive T_H17 responses.

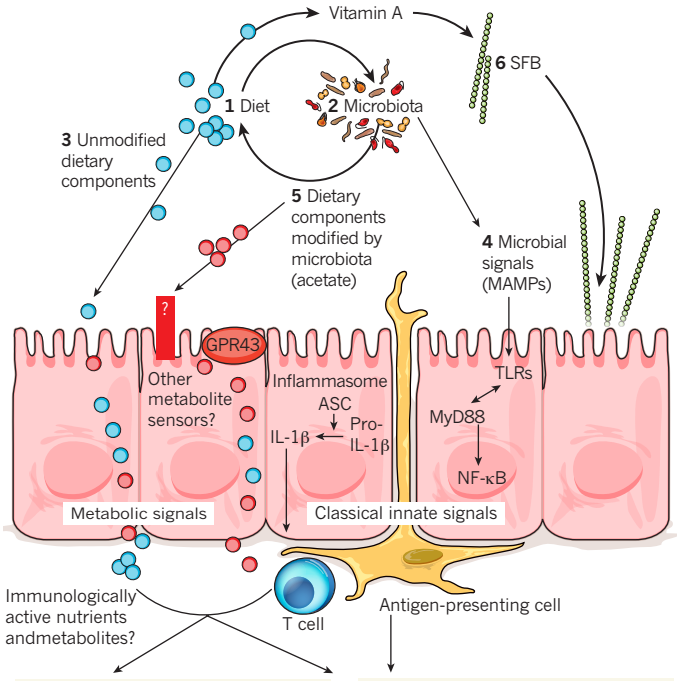
Unfortunately, the spatial relationships between members of the microbiota and their proximity to elements of the gut-associated immune system in healthy individuals or individuals with mucosal barrier dysfunction are not well understood. Gnotobiotic mouse models of obesity may help to provide important insights about the biogeography of microbial communities along the length and width of the gut, including whether microbial consortia occupy ectopic sites that could affect the development and perpetuation of barrier dysfunction (such as in the crypts of Lieberkühn, where multipotential gut stem cells reside — as described by Medema and Vermeulen (page 318)). Newer methods, such as combinatorial labelling and spectral imaging fluorescence *in situ* hybridization (CLASI-FISH)[72], offer a great deal of promise for characterizing the spatial features of microbe–microbe and microbe–host cell interactions in the gut mucosa, especially if they are applied to gnotobiotic models composed of defined collections of sequenced microbes.

Undernutrition and environmental enteropathy

Undernutrition can have many clinical manifestations ranging from mild, asymptomatic micronutrient deficiencies to severe, life-threatening conditions such as kwashiorkor and marasmus. Estimates indicate that the implementation of current 'best practice' interventions — including increasing the time of breastfeeding, supplementing diets with zinc and vitamins, hygiene measures such as improving hand washing, and optimizing the treatment of acute severe malnutrition — could reduce mortality during the first three years of life by only 25%, even if there is almost perfect compliance[5]. Several environmental and genetic factors have long been postulated to influence the development of moderate to severe forms of malnutrition[73], but the underlying mechanisms remain poorly defined. Food availability, although a major factor, is not the only contributor. For example, in Malawi, the concordance for severe malnutrition between twins within the same household who are fed similar diets is only 50% (M. Manary, personal communication). This observation raises several questions. Do different configurations of the gut microbiota predispose one co-twin to kwashiorkor or marasmus? The effect of nutrient deficiency, in either the mother or her child, on the configuration of the microbiota and microbiome in the developing gut is not clear. It is possible that nutrient deficiency in the mother affects the assembly of the microbiota by causing changes in the mother's gut microbiota or in the nutrient and immune content of her breast milk. Both the microbiota and milk are transmitted to the infant, yet we have much to learn about how the biochemical and immunological features of breast milk change, and how breast milk and the infant microbiota 'co-evolve' during the suckling period when a mother is healthy or malnourished (see below).

Identifying how malnutrition affects the gut's microbiome may prove to be very important for improving many associated clinical disorders. Malnutrition could delay the maturation of the gut's microbial metabolic organ or skew it towards a different and persistent configuration that lacks the necessary functions for health or increases the risk of diseases, including immuno-inflammatory disorders. Nutrient repletion may return the microbiota/microbiome to a 'normal' pre-deficient state; alternatively, structural and functional perturbations may persist, which require continued nutritional supplementation to correct. There may be microbiome configurations that correlate with vaccine responsiveness[74].

Studies of severe forms of malnutrition indicate that these patients often have many characteristics of environmental enteropathy[75]. Also known as tropical sprue or tropical enteropathy, environmental enteropathy is a poorly characterized chronic inflammatory disease that mainly affects the small intestine. The disorder afflicts individuals who reside in areas with poor sanitation and who have high exposure to faecal-contaminated water and food. As an example, Peace Corps volunteers returning to the United States from such areas would report a history of diarrhoeal disease and have signs and symptoms of chronic



T cell

mTOR
Promotes T_H1, T_H2, T_H17 cell differentiation; inhibits T_{reg} cell differentiation

RAR–RXR
Promotes intestinal T-cell homing; promotes T_H2 and T_{reg} cell differentiation

VDR–RXR
Promotes T_{reg} cell differentiation; inhibits T_H1 and T_H17 cell differentiation

AHR
Promotes T_H17 and T_{reg} cell differentiation

LXR and PPAR
Control T-cell differentiation

Antigen-presenting cell

TLRs

Inflammasomes

mTOR
Modulates DC function and differentiation

RAR–RXR and VDR–RXR

AHR
Modulates DC differentiation

PKR
Regulates inflammatory responses

GPR120
Inhibits inflammatory responses in macrophages

Figure 2 | Metabolite sensors that help to coordinate immune responses. Immune-cell-associated sensors use information about the local nutrient or metabolite milieu to organize local immune responses. (1) The dietary intake of macronutrients and micronutrients shapes microbial community structure (2), which, in turn, changes the nutritional value of the consumed food. (3) Unmodified dietary components are absorbed in the intestine, where they can interact with immune cells. (4) Microbial signals in the form of microbe-associated molecular patterns (MAMPs) modify local mucosal immune responses through innate signalling pathways such as the inflammasome or TLRs. Inflammasomes recruit the adaptor protein apoptosis-associated speck-like protein containing a CARD (ASC), which promotes binding of caspase, which in turn, cleaves pro-IL-1β to IL-1β. (5) Microbe-modified dietary components (such as acetate produced by the fermentation of polysaccharides) provide signals by which the immune system can monitor the metabolic activities of the microbiota. (6) Vitamin A can modify the representation of segmented filamentous bacteria (SFB) in the mouse gut microbiota, and is an example of a micronutrient directly modifying intestinal microbial ecology. SFB induce the differentiation of T_H17 cells. DC, dendritic cell; LXR, liver-X-receptor; RAR, retinoic acid receptor; RXR, retinoid X receptor; VDR, vitamin D receptor.

malabsorption and nutritional deficiencies[76]. The malabsorption associated with environmental enteropathy is often subtle in children, manifesting itself clinically only as stunting due to chronic undernutrition[76]. The breakdown in intestinal mucosal barrier function in this disorder can lead to increased susceptibility to enteropathogen infections. Recurrent infections predispose individuals to nutritional deficiencies and further compromise barrier function, leading to a vicious cycle of further susceptibility to infection and worsening nutritional status[77].

Efforts to break this cycle have focused on vaccines that could prevent infection. However, there is significant heterogeneity in the responses to vaccination between children living in highly Westernized societies and children living in certain developing countries. Oral rotavirus vaccine elicits responses in more than 95% of children living in Westernized societies but in only 49% in Malawi[78]. Lower oral polio vaccine efficacy has been reported in populations with greater enteric disease burden[79]. Studies in Chilean children have demonstrated a negative correlation between oral cholera vaccine responses and small bowel bacterial overgrowth[80]. In addition, patients with coeliac disease, which shares phenotypic features with environmental enteropathy, can have a blunted response to parenteral hepatitis B vaccination, but only when their disease is active[81].

Traditionally, the most definitive test for environmental enteropathy has been small intestinal biopsy. Biopsies typically show reductions in small intestinal villus height, increased numbers of intraepithelial lymphocytes, and increased infiltration of the underlying lamina propria by T cells with a predominant T_H1 phenotype[75]. Some of these features are found in patients with coeliac disease, in which a luminal antigen (gliadin) drives a T-cell response that, in turn, results in epithelial destruction, reduced absorptive surface area and malabsorption[76]. Unlike coeliac disease, the antigens that drive the host immune response in environmental enteropathy are unknown, but there may be an association with certain HLA alleles, such as HLA-Aw31 (ref. 82).

The pathological events that lead to the development of environmental enteropathy are poorly understood, in part because of the absence of a robust set of readily assayed biomarkers that would improve the ability to diagnose, classify and potentially subcategorize individuals that show the broadly defined clinical manifestations that define this disorder. Epidemiological data showing a strong association of environmental enteropathy in areas with poor sanitation, as well as the occasional epidemic spread of the disease and its responsiveness to antibiotic treatment, reinforce the long-standing belief that there is an 'infectious' aetiology. Although cultures of jejunal aspirates from individuals with environmental enteropathy have suggested contamination of the proximal small bowel by aerotolerant Gram-negative bacteria[83], no single pathogen or set of pathogens has been identified in the gut microbiota of most affected individuals. There is a distinct possibility that this enteropathy is not the result of a single pathogen but rather the result of colonization with microbial consortia that are inflammogenic in the context of a susceptible host. In fact, what constitutes a normal immune repertoire in a healthy gut probably varies considerably depending on environmental exposures and the configuration of a microbiota. Moreover, most metagenomic studies of the microbiota have focused on members of the superkingdom Bacteria, which dominate these communities. Other tools need to be developed so that they can be extended to viral, archaeal and eukaryotic components. The latter group includes parasites that compete for nutrients within the intestines of infected individuals. Parasites can interact directly with bacterial members of the microbiota during their life cycle in ways that promote hatching of parasite eggs, and can shape immune function through factors such as excretory–secretory products, which have been shown to modulate cytokine production, basophil degranulation and immune-cell recruitment and to interfere with TLR signalling[84].

It seems reasonable to posit that individuals living in regions with high oral exposures to faecal-contaminated water and foods, and/or with a eukaryotic component of their gut community that includes parasites, will have gut-associated immune systems with significantly different structural and functional configurations than those without these exposures. In this sense, including the term environmental together with enteropathy is logical and emphasizes the need to place a host's immune and gut microbiome phenotypes in the context of their various exposures.

The representation and expression of microbiome genes in the gut communities of affected individuals compared with healthy controls may correlate with environmental enteropathy. Comparative metagenomic studies could thus provide important new diagnostic tools in the form of microbial taxa and microbiome gene functions. In addition, they could provide pathophysiological insight about relationships between host diet, enteropathogen representation in the microbiota, and microbiome gene composition and expression (including expressed metabolic functions). A major challenge will be to correlate this data with the results of quantitative phenotyping of the innate and adaptive immune systems of the human gut. This will require new and safe approaches for the sampling of immune system components, especially in the gut mucosa. Similarly, as noted earlier, the spatial relationships between members of the microbiota, as well as their proximity to elements of the gut-associated immune system in healthy individuals or in individuals with mucosal barrier dysfunction, is not well understood.

Microbiota assembly and breast milk

Breast milk is known to protect newborns from infection, in part because of the copious quantity of maternally generated antibodies that it contains. Although these antibodies have specificity for components of the microbiota, the microbial targets are not well defined for given maternal–infant dyads, or as a function of time after delivery. In addition to antibodies, breast milk contains other immunoactive compounds, including cytokines such as IL-10, growth factors such as epidermal growth factor and antimicrobial enzymes such as lysozyme. The effect of maternal nutritional status on the glycan, protein, lipid and cytokine landscape of breast milk needs to be defined further. This analysis should have a temporal axis that explores co-evolution of the immunological and nutrient properties of mother's milk and the postnatal assembly and maturation of the infant gut microbiota and of the innate and adaptive immune systems. Important feedback systems may be revealed. Similarly, knowledge of the vaginal and cutaneous microbiota of mothers before and after birth, as a function of their nutritional status, could be very informative. For example, common configurations of microbial communities that occupy these body habitats could correlate with the development of environmental enteropathy in mothers and their offspring.

Personalized gnotobiotics and culture collections

As noted above, studies have demonstrated the ability of intestinal microbial communities to reshape themselves rapidly in response to changes in diet. These observations raise the question of whether and how malnourished states affect (1) the spatial/functional organization of the microbiota and the niches (professions) of its component members; (2) the capacity of the community to respond to changes in diet; (3) the ability of components of the microbiota to forage adaptively on host-derived mucosal substrates; and (4) the physical and functional interactions that occur between the changing microbial communities and the intestinal epithelial barrier (including its overlying mucus layer). One way of developing the experimental and computational tools and concepts needed to examine these challenging questions in humans is to turn to gnotobiotic mice that have been 'humanized' by the transplantation of gut communities from human donors with distinct physiological phenotypes, and to feed these mice diets that are representative of those of the microbiota donor.

Personalized gnotobiotic mouse models

We have used metagenomic methods to show that gut (faecal) communities can be efficiently transplanted into germ-free mice, and the mice can then be fed diets that resemble those consumed by the human microbiota donors, or diets with ingredients that are deliberately manipulated[3,85]. Transplanted human gut microbial communities can be transmitted from gnotobiotic mothers to their pups. In principle, mice humanized with microbiota from individuals residing in different regions of the world, and given diets that are representative of those cultural traditions, can provide proof-of-principle global 'clinical trials' of the nutritional value of foods and their effect on the microbiota and the immune system.

Transplantation of a human faecal microbiota into germ-free mice can be viewed as capturing an individual's microbial community at a moment in time and replicating it in several recipient gut ecosystems. The humanized mice can be followed over time under highly controlled conditions in which potentially confounding variables can be constrained in ways that are not achievable in human studies. This type of personalized gnotobiotics also provides an opportunity to determine the degree to which human phenotypes can be transmitted via the gut microbiota as a function of diet. Moreover, the documented responses of microbial lineages and genes encoding metabolic pathways in the transplanted, replicated communities may provide mechanistic insight into differences in the adaptations of healthy versus diseased gut microbiomes (and the host immune system) to changes in diets, plus new biomarkers of nutritional status and the effect of various therapeutic interventions, including those based on dietary manipulations. Putative microbial biomarkers obtained from studies of these mice can, in turn, be used to query data sets generated directly from the human donor(s).

Despite the potential power of using humanized mice to study interactions between the host immune and metabolic systems and the intestinal microbiota under highly controlled conditions, this approach has caveats. Recent work on T_H17 responses suggests that unlike the mouse microbiota, which contains SFB, a faecal microbiota from a human donor is not sufficient to drive the expression of immune-related genes in the small intestine of previously germ-free mice[52]. This raises the possibility that humanization may not fully recapitulate the capacity of a mouse microbiota to mature the intestinal immune system in mice. However, earlier studies on the effects of human microbiota on the mouse immune system showed that the ability of *E. coli* heat-labile enterotoxin to break oral tolerance to ovalbumin in germ-free mice can be inhibited by transplantation of either a human or a mouse microbiota during the neonatal period[86]. Furthermore, a single component of a human gut symbiont, the polysaccharide A component of *Bacteroides fragilis*, can mature components of the $CD4^+$ T-cell response in mice[87]. Finally, we have observed a similar increase in the frequency of TCR-β^+ cells among lymphocytes in the mesenteric lymph nodes of gnotobiotic recipients of a human or mouse microbiota, compared with germ-free controls (P.P.A., V. K. Ridaura and J.I.G., unpublished observations). This suggests that although not all components of the mouse immune system will be matured by a human gut microbiota, the immune system is not likely to remain ignorant of these communities. In addition, any differences detected in direct comparisons of the effects of two different human gut communities may represent responses relevant to the human immune system.

Personalized bacterial culture collections

We have recently shown that the human faecal microbiota consists largely of bacteria that can readily be cultured[3]. Metagenomic analysis indicates that most of the predicted functions of a human's microbiome are represented in its cultured members. In gnotobiotic mice, both complete and cultured communities have similar properties and responses to dietary manipulations. By changing the diet of the host, the community of cultured microbes can be shaped so that it becomes enriched for taxa suited to that diet. These culture collections of anaerobes can be clonally arrayed in multiwell formats: this means that personalized, taxonomically defined culture collections can be created from donors representing different human populations and physiological phenotypes in which the cultured microbes have co-evolved within a single human donor's gut habitat.

Together, these advances yield a translational medicine pipeline for examining the interactions between food and food ingredients, the microbiota, the immune system and health. The goals for such a human translational medicine pipeline are to identify individuals

with interesting phenotypes, to assess the transmissibility of their phenotypes by human microbiota transplants into gnotobiotic animals, to select candidate disease-modifying taxa (retrieved from clonally arrayed, taxonomically defined personal bacterial culture collections), to sequence selected taxa and to reunite them in various combinations in gnotobiotic mice as defined model gut communities. The interactions of disease-modifying taxa with one another, their effects on host biology, and how these effects are influenced by diet can be further explored using methods such as high-throughput complementary DNA sequencing (RNA-Seq), mass-spectrometry-based proteomics and metabolomics, multilabel fluorescence *in situ* hybridization (for biogeographical studies of the microbiota), whole-genome transposon mutagenesis (to identify fitness factors for microbes under various dietary contexts[46]), and immune profiling and other measurements of mucosal barrier function. Knowing the degree to which tractable bacterial taxa can influence host physiology, and how dietary components can be used to affect specific organisms in the microbiota[3,85] in ways that provide benefit to the host may be useful for discovering new generations of probiotics and prebiotics.

Looking ahead

With massive prospective national surveys planned and being implemented — such as the National Institutes of Health National Children's Study, which will follow a representative sample of 100,000 children from before birth to age 21 — the time is right for an initiative to evaluate the relationships between our diets, nutritional status, microbiomes and immune systems. Many components could constitute this initiative. We can readily foresee several of these.

Dietary databases

As noted earlier, there is a need to create more and improved databases for monitoring changing patterns of food consumption, in which the surveillance efforts of several organizations can be integrated. This tool and other interdisciplinary approaches could be used to define a set of study populations that represents established and emerging food consumption patterns in distinct cultural and socio-economic settings. An emphasis could be placed on comparing humans living in Westernized societies with those living in developing countries that are undergoing marked transitions in lifestyles and cultural traditions. New, reliable, cost-effective and generalized methods will be needed to acquire quantitative data about the diets consumed by individuals in these study populations, and the resultant data will need to be deposited in searchable databases using defined annotation standards. Moreover, guidelines related to the ethical and legal aspects of human-subject research involving observational and interventional nutritional studies of pregnant women and their offspring need to be further developed.

New biomarkers of nutritional status

Readily procured human biospecimens could be used together with high-throughput, targeted and non-targeted (quantitative) profiling of metabolites in comprehensive time-series studies to define the relationship between diet, nutritional status and microbiome configuration in healthy individuals at various stages of life (for example, in women before, during and after pregnancy, and in their children during the first five years after birth). This could be accompanied by studies of malnourished individuals before, during and after well-justified, defined nutritional interventions. In addition to these data, genomes (genotypes), epigenomes and microbiomes could be characterized in these study cohorts together with a variety of clinical parameters (such as vaccine responses) and environmental parameters (such as water sanitation). The resultant data sets would be deposited in annotated searchable databases. A translational medicine pipeline that includes relevant cellular and animal models would help to guide the design and interpretation of these human studies.

Quantitative phenotyping of the immune system

As noted earlier, a major challenge is to obtain cellular and molecular biomarkers for quantitative profiling of the innate and adaptive immune systems, including biomarkers of mucosa-associated barrier function. Given the small quantities of biomaterials available from certain body sites, this initiative should help to advance 'miniaturizing technology' for quantitative measurements of cells and biofluids. Non-invasive imaging-based biomarkers are also needed.

Goals include identifying new host and microbial biomarkers and mediators of nutritional status, determining the nutritional value of various foods, and characterizing the function of the human adaptive and innate immune systems (including mucosal barrier integrity and mucosal immunity) and the dynamic operations of the microbiota. This information would be used for demonstration projects that rigorously define nutritional health and test preventive or therapeutic recommendations for micronutrient and macronutrient consumption, for example in pregnant women and infants/children, and their effect on the assembly and operations of the immune system. The microbiome component could also help to define a previously uncharacterized axis of human genetic evolution (our microbiome evolution), reflecting in part our changing dietary habits. It could also produce testable hypotheses about unappreciated aspects of the pathophysiology of Western diseases, and yield new microbiome-based strategies for disease prevention or treatment. ■

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