Diet, gut microbiota and immune responses

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The fields of immunology, microbiology, nutrition and metabolism are rapidly converging. Here we expand on a diet-microbiota model as the basis for the greater incidence of asthma and autoimmunity in developed countries.

Two important advances in the fields of immunology and gut microbiology have emerged in recent years. First, it has been clearly demonstrated that diet has a considerable effect on the composition of the gut microbiota[1,2]. Different human populations can have vastly different intestinal microbiomes, and changes in diet lead to changes in microbiota composition. Second, findings from many laboratories have shown that the composition and products of the gut microbiota have unexpected effects on immune and inflammatory responses[3–5]. Accordingly, diet and the effects it has on the gut microbiota and immune responses are increasingly likely explanations for the greater incidence of inflammatory diseases such as asthma and type 1 diabetes in developed countries. New findings about the gut microbiota and its immunomodulatory abilities fit with persuasive epidemiological data on the connection between obesity and asthma[6] or obesity and type 1 diabetes[7]. We suggest here that a lower intake of fiber (complex plant polysaccharides) adversely affects the makeup of the intestinal microbiota, which leads to less production of immunomodulatory products, in particular short-chain fatty acids (SCFA).

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The gut microbiota can be considered an extension of the self and, together with the genetic makeup, determines the physiology of an organism. For example, two supposedly genetically identical organisms, such as same-sex inbred mice, can have widely different metabolic and inflammatory responses depending on the makeup of their microbiota. The intestinal microbiota is derived at least in part from the mother during the birthing process and is modified thereafter by factors such as diet, antibiotic use, host genetics and other environmental factors. Whereas microbes in the gut were once considered only harmful or pathogenic, it is now clear that commensal bacteria accomplish many beneficial functions, such as vitamin synthesis, the digestion of dietary fiber and the regulation of inflammatory responses. Microbes and vertebrates have evolved together over the millennia, so normal functioning of the digestive and immune systems depends on the presence of nonpathogenic 'beneficial' bacteria (symbionts).

Diet and the gut microbiota

We propose that changes in diet and associated changes in the gut microbiota are driving the increasing incidence of inflammatory disease in developed countries (**Fig. 1**). Similar ideas have been proposed before[8,9]; however, subsequent findings have provided new molecular mechanisms and have made a diet-microbiota hypothesis compelling. The modern Western diet is characterized by food that has been processed, stored and transported and typically contains much less vegetables and fiber than the diets in developing countries or Western diets of ~40 years ago[9]. Diet itself has a considerable effect on the composition of the gut microbiota[1]. For example, changes in the gut microbiota of mice (humanized gnotobiotic mice) have been analyzed after the mice are switched from a diet low in fat and rich in plant polysaccharides to a Western diet high in fat and sugar and low in plant polysaccharides. After just 1 day on the Western diet, mice show changes in their microbial composition, metabolic pathways and gene expression, and within 2 weeks they develop more adiposity[1]. Mice on a Western diet show an increase in bacteria of the Firmicutes phylum and a decrease in those of the Bacteroidetes phylum[1].

There are striking differences between children from rural Africa and those from urban Europe in the composition of their gut microbiota[2]. Children from an African cohort (Burkina Faso) were shown to have a diet very high in fiber, and their microbiota was highly enriched in Bacteroidetes bacteria, with specifically more bacteria known to have genes encoding molecules required for the hydrolysis of complex plant polysaccharides, and had much lower abundance of Firmicutes bacteria than the microbiota of the European cohort had[2]. In fact, the African microbiome in this study contained two bacterial species (*Prevottela* and *Xylanibacter*) completely absent from the Western cohort's microbiome; these species have enzymes necessary for the hydrolysis of cellulose and xylan. In fact, humans and other vertebrates rely completely on the microbiota to digest those otherwise indigestible plant polysaccharides. The fermentation of fiber produces large amounts of SCFA, such as acetate, propionate and butyrate. As we will discuss below, we believe that the amount of SCFA

in the colon and blood is critically important for immunoregulation. It is noteworthy, then, that the production of SCFA changes quickly when organisms are switched to a different diet[10]. The European children discussed above had significantly less SCFA in their feces than did those of the African cohort[2]. Interestingly, allergies and asthma are almost completely nonexistent in certain rural African communities.

Host genetics and microbial composition

The innate immune system is another factor that probably influences the composition of the intestinal microbiota. This is best illustrated by a study showing that nonobese diabetic (NOD) mice deficient in the innate signaling molecule MyD88 are protected from the development of type 1 diabetes[3]. Surprisingly, protection is lost when *Myd88*$^{-/-}$ NOD mice are housed under germ-free conditions and are therefore devoid of gut microbiota. The absence of MyD88 in NOD mice leads to an over-representation of bacteria of the Bacteroidetes phylum[3], and this microbiota somehow actively suppresses the development of diabetes, presumably through the production of an immunomodulatory product. Interestingly, Bacteroidetes bacteria are major producers of SCFA. Toll-like receptor 5 (TLR5) is another innate component important for determining the makeup of the microbiota. *Tlr5*$^{-/-}$ mice develop hyperphagia (overeating) and hallmark features of metabolic syndrome, including insulin resistance and greater adiposity[11]. The transfer of *Trl5*$^{-/-}$ microbiota into wild-type mice confers many aspects of the *Trl5*$^{-/-}$ phenotype on the recipient mice, including hyperphagia and obesity[11]. Loss of TLR5 alters the gut microbiota, which somehow 'feeds through' to hyperphagia and other aspects of metabolic syndrome[11]. Given these two studies[3,11], it is reasonable to suggest that any element that affects innate immunity, such as subversion of the immune response by pathogens or polymorphisms in genes involved in innate immunity, might affect the makeup of the gut microbiota.

Disease-associated gut microbiota

There is now mounting evidence that the microbiota is altered in people with allergies or asthma[8]. One of the first studies to demonstrate this examined the intestinal microflora of 76 infants at high risk of atopic diseases at 3 weeks and 3 months of age. Infants in whom atopy was and was not developing had significant differences in microbiota composition, and these microbiota differences preceded the development of atopy[12]. Daily consumption of fermented foods may be important for maintaining the necessary amount of *Lactobacillus* bacteria and may diminish the prevalence of allergic disease[8]. In communities in which consumption of fermented foods is high and antibiotics are not used, cases of allergy and asthma are low. Indeed, a similar idea dates back to the early 1900s, when Elie Metchnikoff reported on a population of Bulgarians who had an extremely long life expectancy, which he attributed to their consumption of yogurt and the requirement for 'good' microbiota to maintain harmony (homeostasis).

A sophisticated analysis of microbiota associated with human inflammatory diseases is only now just commencing. However, differences in the gut microbiota of patients with rheumatoid arthritis have been observed[13,14], particularly in those with erosive rheumatoid arthritis[13], and the differences include a lower abundance of *Bifidobacterium* and *Bacteroides* bacteria[14]. In the KxB/N mouse model of inflammatory arthritis, the removal of anaerobic bacteria (through the use of antibiotics) exacerbates disease[15], presumably via a mechanism that relates to a changed microbiota. The gut microbiota of patients with inflammatory bowel disease is often different from that of healthy people. Again, these changes are seen in the 'beneficial' anaerobic microbes such as the Bacteroidetes and a subgroup of the Firmicutes[16]. It is therefore becoming clear that certain gut microbiota are required for the regulation of immune responses and that perturbations in the microbiota could result in a lack of immunoregulation, the outgrowth of more pathogenic microbes and the promotion of inflammation, particularly in people who are genetically susceptible. We have summarized many factors that potentially affect the composition of the intestinal microbiota (**Fig. 1**).

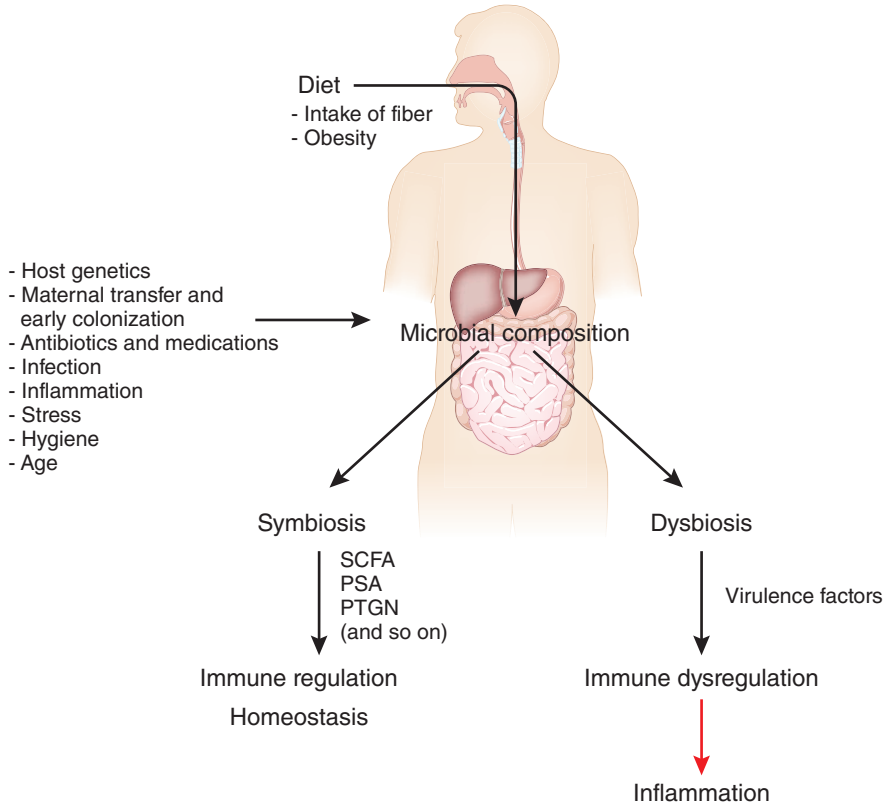

Figure 1 Diet, microbial composition and regulation of the immune system. Diet and other environmental and host factors have a major effect on gut microbial composition. Our model would suggest that balanced microbial composition results in symbiosis; this provides regulation of immune and inflammatory responses through anti-inflammatory and/or immunomodulatory products such as SCFA, polysaccharide A (PSA) and PTGN, which helps maintain homeostasis. Dysbiosis would lead to dysregulation of the immune system through lack of beneficial microbial products and an increase in virulence factors, which could leave the host susceptible to inflammation. Dysbiosis could occur through the consumption of a Western diet, as well as through changes induced by factors such as host genetics, maternal transfer and antibiotic use.

Gut microbiota and the immune system

It has been recognized only recently that the gut microbiota can influence immune function beyond the gut. Mice deficient in a single G protein–coupled receptor, GPR43, have a profoundly altered inflammatory response.

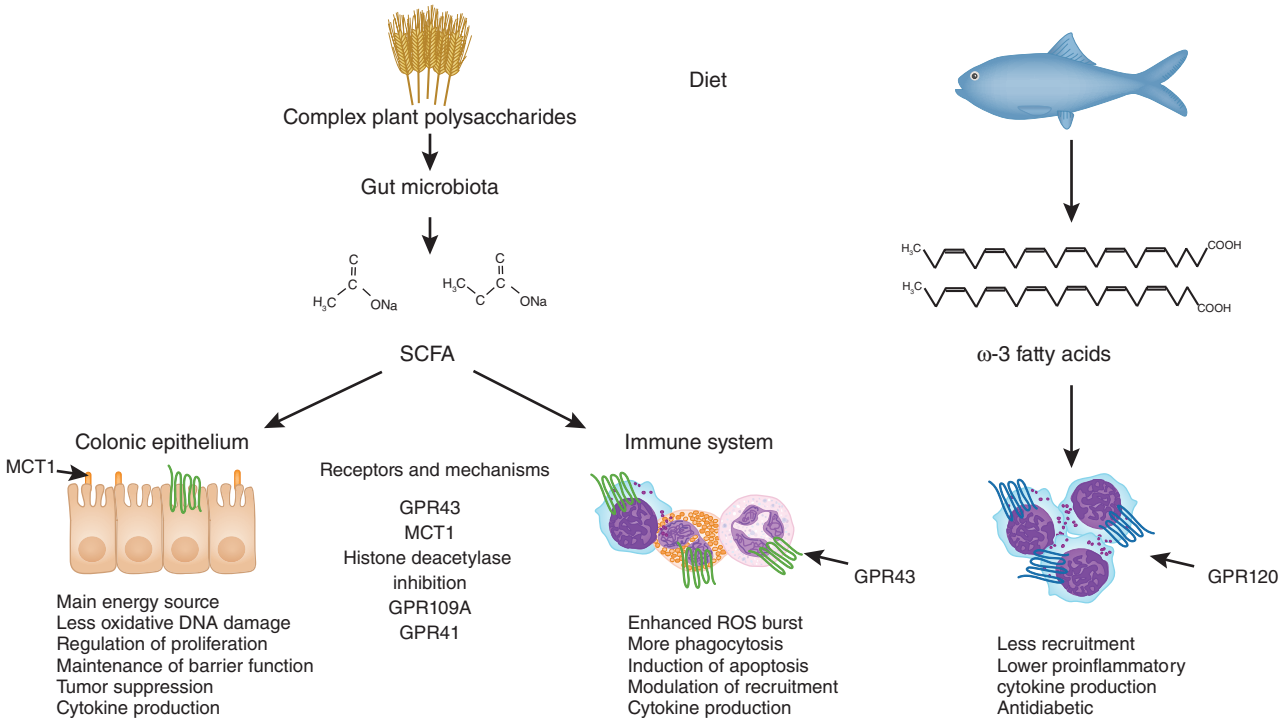

Figure 2 Diet, fatty acids and the actions of anti-inflammatory GPCRs. SCFA (derived from complex plant polysaccharides) and ω-3 fatty acids regulate inflammation through GPR43 and GPR120, respectively. SCFA are produced by the gut microbiota as a byproduct of fermentation of dietary fiber and have several beneficial effects. In the colonic epithelium, butyrate is the main energy source of colonic epithelial cells and is transported into cells via monocarboxylate transporters (such as MCT1 and SLC5A8). SCFA are important for maintaining epithelial barrier function, regulating proliferation and tumor suppression. SCFA also diminish oxidative DNA damage and regulate cytokine production. The effects of SCFA on epithelial cells relate mostly to their role as an energy source and also their inhibition of histone deacetylases. SCFA could also operate through GPR41, GPR43 and GPR109A. In the immune system, SCFA have several anti-inflammatory effects but are also important for stimulating immune function, and their role therefore seems to be important for the regulation of timely immune responses and in resolution of inflammation. Acetate enhances the production of reactive oxygen species (ROS) and phagocytosis but also induces apoptosis and modulates neutrophil recruitment. Many of these anti-inflammatory effects are mediated through GPR43 (ref. 4). The ω-3 fatty acids have anti-inflammatory and antidiabetic effects through their binding of GPR120 expressed on macrophages[28].

The only known ligands of GPR43 are SCFA, particularly acetate and propionate, which are mainly a product of the metabolism of fiber by gut microbes. GPR43-deficient mice (*Ffar2*$^{-/-}$; called '*Gpr43*$^{-/-}$' here) have exacerbated and poorly resolving inflammation in the KxB/N serum-induced arthritis model and a model of allergic airway inflammation induced by ovalbumin plus aluminum hydroxide, as well as in colitis models, and *Gpr43*$^{-/-}$ neutrophils have an intrinsic hyper-reactive phenotype[4]. GPR43 is expressed mainly on cells of the innate immune response and inflammatory cells, such as neutrophils, eosinophils and activated macrophages.

SCFA are also beneficial in other ways. Butyrate is the main energy source for colonocytes and is thus associated with maintenance of the epithelium. SCFA can also bind other GPCRs, including GPR41 (but with different affinity and SCFA preference)[17], and SCFA, particularly butyrate, inhibit histone deacetylases and inhibit activation of the transcription factor NF-κB[18]. Germ-free mice devoid of microbiota have very low concentrations of SCFA[19] and also show exacerbated or poorly resolving responses in many inflammatory models[4,20], similar to the responses of *Gpr43*$^{-/-}$ mice. We have summarized the many different aspects of the actions of SCFA on cells of the immune response and epithelial cells (**Fig. 2**).

SCFA-GPR43 is probably just one of several pathways by which the microbiota regulates inflammatory responses in the gut and elsewhere. Bacteria of the phylum Bacteroidetes also use fiber for glycan synthesis. The commensal bacteria *Bacteroides fragilis* produce a particular glycan, polysaccharide A, which has strong anti-inflammatory effects. Colonization of germ-free mice by *B. fragilis* or treatment with purified polysaccharide A protects mice from the induction of experimental inflammatory bowel disease. Polysaccharide A increases local production of interleukin 10 by inducing regulatory T cells[21]. Peptidoglycan (PTGN) is another example of a bacterial product that can modulate peripheral immune function. PTGN derived from the gut microbiota enters the blood and primes the innate immune system, promoting the killing of certain bacterial pathogens[5]. Depletion of the microbiota in mice results in much lower systemic PTGN concentrations, and this leads to less killing of *Streptococcus pneumoniae* and *Staphylococcus aureus* by neutrophils[5]. PTGN signals via the pattern-recognition receptor Nod1, which recognizes meso-diaminopimelic acid–containing PTGN found predominantly in Gram-negative bacteria. All these data support the idea that certain beneficial bacteria have developed molecules that induce protective intestinal immune responses and also regulate systemic immune responses.

It is likely that the gut microbiota influences the adaptive and innate immune systems in completely different ways. The microbiota is well recognized for its role in the proper development of the immune system. For example, germ-free mice have poorly developed lymphoid tissues and show perturbations in the development of T cell and B cell subsets, and in some cases germ-free mice do not develop the diseases present in conventional mice. This probably relates to an inability to mount adaptive immune responses due to defects in the adaptive immune system in the absence of microbiota, rather than to a lack of microbes *per se*[20]. However, components of the innate

immune response in germ-free mice show hyperactivity; for example, macrophages from germ-free mice have higher basal concentrations of lysozymal enzymes than do those of conventional mice[22]. The presence of segmented filamentous bacteria is important for the development of interleukin 17–producing helper T cells, and these bacteria are necessary for the development of autoimmunity in the T cell–transgenic K/BxN model of arthritis[15]. However, this model has an easily distinguishable initiation stage (dependent on T cells and B cells) and an effector stage dependent on mast cells, complement activation and neutrophils. The effector stage is exacerbated in germ-free mice, as well as in *Gpr43*$^{-/-}$ mice[4], probably because it is inflammatory-type cells that selectively express GPR43.

Does diet affect inflammatory disease?

If diet affects the composition of the microbiota, and the microbiota regulates immune and inflammatory responses, then diet should have easily quantifiable effects on the immune response. Although the results of studies in this area are highly promising, most of the evidence so far has been indirect or has been derived from studies with limited numbers of trial subjects. The effect of diet on asthma and allergies has been reviewed[9]. One of the differences noted in Western diets has been less consumption of dietary fiber (complex plant polysaccharides). Human populations that consume adequate or large amounts of dietary fiber have a lower incidence of inflammatory disease, including colitis, type 2 diabetes and colon cancer[23]. In one study, 1,861 children were monitored from birth to assess whether nutrient intake by their mothers during pregnancy correlated with the development of asthma in the children at 5 years of age. The mothers of children who later developed childhood wheeze and asthma had a distinctly different dietary intake[24]. Perhaps one of the strongest associations has been the linkage of obesity with the development and severity of asthma in both children and adults, as shown in numerous epidemiological studies[6]. Direct assessment of the intake of dietary fiber and inflammation has been studied mostly in inflammatory bowel disease, with encouraging results[25]. In addition, some trials have reported positive effects of SCFA in patients with inflammatory bowel conditions; in fact, such patients often have much lower concentrations of SCFA[25]. Several studies have demonstrated benefits of a vegan diet (which is high in fiber) on diminishing the severity of arthritis[26], and although such effects are indirect, these results could suggest that a high-fiber diet might benefit patients with rheumatoid arthritis.

Although the gut microbiota may be one mechanism for the regulation of immune responses, it is also likely that dietary substances also directly affect immunity. Placebo-controlled trials of fish oil, which contains omega (ω)-3-fatty acids, have demonstrated a substantial benefit of fish oil in chronic inflammatory disease, including less disease activity and less use of anti-inflammatory drugs[27]. Only recently has a possible molecular mechanism emerged. The ω-3 fatty acids exert anti-inflammatory effects through binding to GPR120, a GPCR[28] (**Fig. 2**). GPR120 is expressed mostly by macrophages, and the binding of ω-3 fatty acids to this GPCR represses the production of tumor necrosis factor and interleukin 6 and macrophage-induced tissue inflammation. This has been shown to occur through coupling to β-arrestin-2 and inhibition of downstream signaling mechanisms, including the kinase TAK1 (ref. 28). Thus, the binding of fatty acids to GPR120 and GPR43 represents an additional mechanism for immune regulation, and at present these GPCRs are the two leading molecules that could be the source of the effects of diet on inflammatory responses.

The hygiene hypothesis revisited

The hygiene hypothesis[29] is now the prevailing explanation for the increase in asthma and atopic disorders in Western countries. It suggests that excess cleanliness in the environment has led to a decrease in the number of infectious stimuli needed for proper development of the immune system. The prevalence of asthma and allergies is lower for people who are raised on a farm and those who belong to larger families or are in a lower birth order in such families and is related to socioeconomic status. Some of the observations that gave rise to this hypothesis may be equally relevant to a 'diet hypothesis'. For example, children who live on farms and children from urban environments probably have different diets. An interesting case is that of Japan, where there is a high degree of hygiene and considerable urbanization but much less asthma than in Australia or the USA. The diet in Japan and other Asian countries typically includes large amounts of rice, beans and fermented or pickled foods, which yield high concentrations of SCFA, and fish, which has large amounts of ω-3 fatty acids. Greenland Eskimos, who have a very large intake of ω-3 fatty acids in their diet, have extremely low rates of heart disease and also chronic inflammatory diseases. Another observation that would challenge the hygiene hypothesis is that the urban poor in the USA have a higher incidence of infectious diseases such as tuberculosis but still have a high incidence of asthma, which seems to correlate better with diet and obesity than with hygiene in these populations.

Manipulation of the microbiota

If the microbiota does have a substantial bearing on immune responses, as the preliminary reports discussed above would suggest, then this opens up new avenues for therapy. Probiotics (live microorganisms thought to be healthy for the host) have been tested in many clinical trials, with some notable successes[8], although few large-scale trials of humans with inflammatory disease have been undertaken. It may be true that clinical trials with probiotics will also need to incorporate dietary considerations, because probiotics require fiber for their metabolism, and it is likely that SCFA represent one of the mechanisms by which probiotics can be beneficial. Moreover, people may vary considerably in their capacity to support the expansion of newly introduced microbes in their gastrointestinal tract. In some clinical trials of probiotics, it has not been clear whether oral administration allows probiotics to survive during their transit through the stomach. It may also be necessary to provide constant dietary support for these microbes[8] or to select microbes that are better at surviving passage through the stomach and colonizing the colon. The results of preliminary trials of fecal transplantation (the transfer a fecal sample from a healthy person to treat another) for colitis caused by *Clostridium difficile* have been very promising, and this could be used to treat severe cases of dysbiosis (microbial imbalance)[30]. Fecal transplantation also represents a promising approach for the treatment of metabolic syndrome disorders such as type 2 diabetes and may be of benefit for inflammatory diseases.

Future directions

The recent flurry of research articles on diet and its effects on gut microbiota, together with the new findings on the regulation of immune responses by microbiota, opens up an entirely new approach to the understanding and treatment of human inflammatory disease. Likely suspects in this equation are SCFAs, but there may be numerous molecules produced by gut microbes, or dietary molecules themselves, that affect immune responses. Rather than developing new anti-inflammatory drugs, it might be more cost-effective to devote more effort to new approaches, such as monitoring the human intestinal microbiota and manipulating it if required through the use of probiotics and/or prebiotics (nondigestible food ingredients that stimulate the growth and/or activity of bacteria). The opportunity exists to develop new probiotics on the basis of emerg-

ing knowledge of the mechanisms by which the microbiota modulates inflammatory responses. Another possibility is to develop probiotics from microbiota derived from human communities in which allergies and asthma are almost completely nonexistent. It will also be important to determine how antibiotics change the composition of the gut microbiota and how relevant this is to inflammatory diseases. Finally, if diet is a major contributing factor to the prevalence of allergies, asthma and even autoimmune disease, should humans consciously alter their intake of fiber, ω-3 fatty acids or other foodstuffs? Carefully controlled trials are needed to establish whether diet directly affects inflammatory disease and, if so, at what point in human development it operates, as well as through what cellular and molecular mechanisms. Increasing knowledge emerging from the human microbiome project and an increasing ability to sequence the metagenome will allow systematic analysis of the intestinal microbiota in human inflammatory disease.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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