

Bridging immunity and lipid metabolism by gut microbiota

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Activity Objectives

1. To understand the relationship between the microbiota, the immune system, and environmental influences.
2. To identify proposed mechanisms by which metabolism affects the immune system.

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The human gut is a unique organ in which hundreds of different microbial species find their habitat and in which different host physiologic functions, such as digestion, nutrition, and immunity, coexist. Although all these players were studied separately for decades, recently, there has been an explosion of studies demonstrating the essential role for interactions between these components in gut function. Furthermore, new systems biology methods provide essential tools to study this complex system as a whole and to identify key elements that define the crosstalk between the gut microbiota, immunity, and metabolism. This review is devoted to several human diseases resulting from the disruption in this crosstalk, including immunodeficiency-associated and environmental enteropathies, celiac disease, inflammatory bowel disease, and obesity. We describe findings in experimental models of these diseases and in germ-free animals that help us understand the mechanisms and test new therapeutic strategies. We also discuss current challenges that the field is facing and propose that a new generation of antibiotics, prebiotics, and probiotics coupled with novel, systems biology-driven diagnostics will provide the basis for future personalized therapy. (*J Allergy Clin Immunol* 2013;132:253-62.)

Key words: Immunity, immunodeficiency, gut microbiota, intestinal lipid metabolism

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The human gut is a multifaceted organ. The most well-known functions of the gut are digestion, nutrient absorption, and secretion of hormones and enzymes that regulate food intake and metabolism throughout the body. These functions are largely mediated by intestinal epithelial cells, a single layer of epithelium that provides the barrier between the outside world and the body. The gut is also home to millions of resident microbes, which are termed the gut microbiota; as a whole, the microbiota genome has been estimated to contain 150-fold more genes than the host genome.¹ These microbiota contribute to nutrition and metabolism by processing and providing access to nutrients that would normally be inaccessible to the host. In addition to its role in metabolism, the gut is an important immune organ. The gut contains the largest lymphoid tissue mass in the body,² which might not be surprising considering the intestinal bacterial load. Thus in addition to contributing to host metabolism, the gut microbiota provides critical signals for the development of host immunity. Despite taking place in the same organ, historically, gut immune and metabolic functions were viewed and studied independently. More recently, however, systems approaches in research have uncovered intimate interactions between these 2 important functions, most of which involve a mediator: the gut microbiota. Disruptions of this intricate balance between host

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Abbreviations used

CD: Crohn disease
 CVID: Common variable immunodeficiency
 IBD: Inflammatory bowel disease
 SCFA: Short-chain fatty acid
 TLR: Toll-like receptor

and commensal microbes have dramatic effects on human health and disease.

Germ-free animal models, which are completely devoid of microbiota, have been invaluable tools in understanding the contribution of the microbiota to host metabolic function. It has been demonstrated that the microbiota acts at many levels, from lipid processing and absorption to systemic lipid metabolism and storage.³ Germ-free mice have increased fecal lipid and decreased serum lipid levels,⁴ and formation and absorption of lipid droplets was reduced in the gut of germ-free zebrafish.⁵ In addition to revealing these mechanisms of influence on local and systemic lipid metabolism, studies in germ-free mice have long demonstrated that the gut microbiota provides critical signals for the development of local and systemic immunity.^{6–8} Germ-free mice have numerous immune defects, including low expression of antimicrobial peptides in the epithelium, reduced numbers and activation status of T lymphocytes, lower numbers of plasma cells, and impaired production of IgA. Importantly, in general, germ-free and antibiotic-treated mice have enhanced allergic reactions because they have increased numbers of eosinophils and basophils, increased local production of T_H2-associated cytokines, and increased IgE production.^{9,10} In line with the hygiene hypothesis, early-life colonization was important to lessen the symptoms of allergic asthma and inflammatory bowel disease (IBD).¹¹

In this review we will provide examples of clinical conditions involving alterations of both the metabolic and immune functions of the gut, examine animal models for studying these conditions, and discuss how these have helped reveal mechanisms for the interplay between the gut microbiota, immunity, and metabolism. We will also discuss current gaps in our understanding of the complex system that is the gut and how answering these questions will help to understand the pathogenesis and to guide the management of intestinal and systemic pathologies.

GUT MICROBIOTA CONTRIBUTES TO THE PATHOGENESIS OF ENTEROPATHY OF THE SMALL INTESTINE

Immunodeficiency-associated enteropathy

Since the first description in 1954, it has been recognized that human immunoglobulin deficiencies frequently present with disruptions in gastrointestinal function.¹² This suggests that there might be a connection between the 2 seemingly independent functions of immunity and absorption in the gut. Up to 50% of patients with common variable immunodeficiency (CVID), the most common symptomatic antibody immunodeficiency, present with gastrointestinal symptoms, including diarrhea, abdominal pain, lipid and sugar malabsorption, and steatorrhea (fatty stools), leading to decreased body mass index and low adiposity.^{13–16} Similar symptoms are observed in many patients with secondary immunodeficiency caused by HIV/AIDS.^{17,18} Histologic examination of the small intestine in both conditions shows intestinal villous atrophy,

epithelial apoptosis, and inflammation.^{13,19–23} In both cases of immunodeficiency, the enteropathy is often found in the absence of detectable pathogenic infections.^{17,19} Interestingly, decreases in microbes that are beneficial to gut health,^{24–26} including bifidobacteria and lactobacilli, are observed in patients with early-stage HIV, which might contribute to gut dysfunction.²⁷

The differential response to therapies also provides evidence for the role of the gut microbiota in immunodeficiency-associated enteropathy. Although highly active antiretroviral therapy decreases the incidence of enteropathy, IgG antibody treatment for patients with CVID rarely ameliorates gut dysfunction, likely because IgA, but not IgG, antibodies are normally produced in response to the microbiota.^{7,13}

The clinical symptoms of human immunodeficiencies suggest a connection between alterations in the immune system and changes in the metabolism and gut microbiota. Our group has recently described enteropathy in mice lacking B lymphocytes (BcKO mice),²⁸ demonstrating how in-depth analysis of a previously established model can reveal new information relevant for a human condition, which was missing an animal model. Indeed, we have found that BcKO mice have defects in fat absorption that are similar to those observed in patients with HIV and CVID, making them a useful model to study the mechanisms of immunodeficiency-associated gastrointestinal syndrome. The severity of this phenotype in mice is much milder than in human subjects, and no significant morphological alterations of the intestine are observed in BcKO mice. In the absence of apparent involvement of pathogens in mice (similar to the situation for CVID patients), the commensal microbiota is the most plausible trigger of immunity and IgA, which is produced by B cells and shown to be required for proper establishment of the gut microbiota.²⁹ Indeed, the investigation of both of these factors (microbiota by means of analysis of germ-free mice and IgA by means of analysis of IgA knockout mice) has confirmed that lack of IgA-mediated regulation of the microbiota is a mechanism behind fat malabsorption. Thus B-cell deficiency is a good example of complex interactions in which, affected by changes in adaptive immunity, the microbiota triggers innate immunity in the epithelium that downregulates fat transport, eventually leading to systemic changes, such as a decrease in overall fat amounts (Fig 1).

Examination of intestinal gene expression profiles revealed 2 clear effects: an increase in immune genes and a decrease in lipid metabolism and transport genes in the intestinal epithelium. Using promoter screening analysis, we revealed that genes from the decreased metabolic profile were enriched for GATA4, a key transcription factor for regulation of fat absorption in the small intestine.³⁰ However, it was unclear whether the metabolic impairment was related to immune activation and which cells were responsible for the latter. To address this question, we used a systems biology approach of gene network reconstruction and analysis. This analysis generated the model of gene regulation that was later supported by *in vitro* experimentation, suggesting that activation of immune (mostly interferon type I- and II-related) pathways in the epithelial cells leads to inhibition of metabolic gene expression (Fig 1). Interestingly, this interconnectedness of immunity and lipid metabolism has also been observed in macrophages in response to viral infection and IFN- γ , in which increased immune demand results in the downregulation of the sterol synthesis pathway.^{31,32}

Furthermore, because of the high similarity in gene expression signatures in the intestine between B cell-deficient mice and

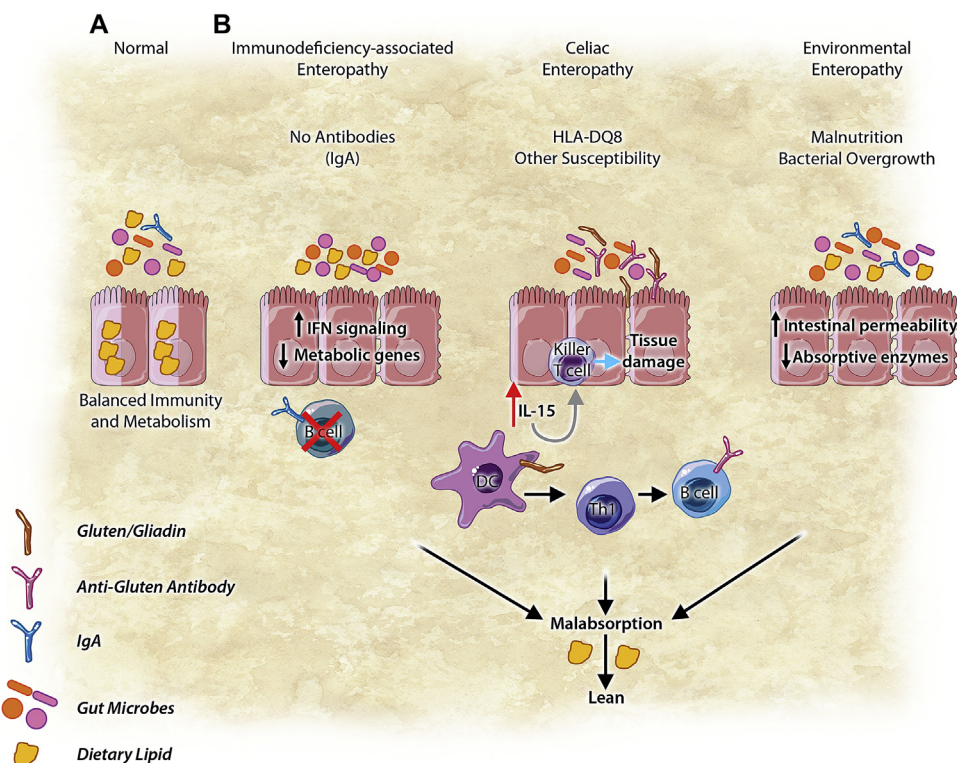


FIG 1. Mechanisms of intestinal enteropathy in patients with immunodeficiency, celiac disease, and environmental enteropathy. **A**, Under normal conditions, immune and metabolic functions are balanced in the intestinal epithelium. *Light and dark pink colors* represent epithelial metabolic and immune functions, respectively. **B**, Alterations in immunity and gut microbiota composition result in enteropathy and defects in metabolism. In the absence of B cells or IgA, the gut microbiota drives the interferon-related immune response in the epithelium at the expense of lipid metabolism. During celiac disease, the immune response mounted to gluten antigen results in cytotoxic killer T-cell activation and epithelial tissue damage. Increased IL-15 expression by the epithelium contributes to tissue damage by signaling to intraepithelial lymphocytes. It is unclear whether metabolic gene expression is reduced in the epithelium but epithelial barrier function is disrupted. This compromises epithelial function, affecting overall metabolism. In patients with environmental enteropathy, malnutrition and bacterial overgrowth result in increased microbiota interaction with the epithelium. We hypothesize that this leads to an increase in immunity and a decrease in metabolism in the epithelial cells, leading to malabsorption, as seen in patients with B-cell deficiencies. DC, Dendritic cell.

patients with HIV/CVID,²⁸ this animal model holds great promise for translational research. In this regard selecting and testing new therapeutic targets among regulatory genes that show similar behavior in mice and human subjects would increase the probability of successfully translating the results of preclinical studies performed in animals to human subjects.

Celiac disease

The symptoms of CVID-associated enteropathy often resemble those of another enteropathy of the small intestine, termed celiac disease or celiac sprue,¹³ although the significant infiltration of plasma cells in patients with celiac disease sets it apart. The small intestine of patients with celiac disease is characterized by villous hypertrophy along with intestinal inflammation, including the accumulation of intraepithelial cytotoxic T cells and an increase in plasma cells and upregulation of IL-15 in the lamina propria.^{33,34} These symptoms are due to an immune response to gliadin, a breakdown product of the wheat protein gluten. Celiac disease has a strong genetic component, most commonly specific variants of the MHC locus.^{35,36} However, genetics do not explain the

whole story because environmental inputs are also important. Recent work has highlighted that patients with gastrointestinal symptoms associated with celiac disease have reduced microbial diversity and a different composition, such as a decrease in total bifidobacteria and *Bifidobacterium longum*, compared with that seen in unaffected subjects and even patients with celiac disease without gastrointestinal symptoms.^{37,38} This suggests a role for the gut microbiota in the manifestation of gastrointestinal symptoms in susceptible patients. Indeed, it has been revealed that *Bifidobacterium* species affect the inflammatory response to gluten by affecting the variants of gliadin peptide formed from gluten breakdown and suppressing the inflammatory response.^{39,40} The ultimate result of this inflammatory response in the small intestine is malabsorption, as observed with immunodeficiency-associated enteropathies. Therefore different defects in immunity, together with alterations of the gut microbiota, can lead to similar outcomes in terms of metabolism (Fig 1).

Mouse models of celiac disease are not fully developed, but those that currently exist provide evidence that the microbiota is an important contributor to the inflammatory response to gluten. A model based on a common susceptibility locus, HLA-DQ8,

displays a strong immune response and altered secretion of acetylcholine and altered neuromuscular response when fed with gluten, but this inflammation does not result in enteropathy.^{41,42} However, moderate enteropathy is observed when HLA-DQ8 is expressed in the autoimmune-sensitive NOD (nonobese diabetic) mouse background.⁴³ Additionally, overexpression of IL-15 from an enterocyte-specific promoter recapitulates both T- and B-cell pathologies of celiac and blunted villi but is not dependent on sensitivity to gluten.^{44,45} Currently, the model that best reproduces multiple aspects of celiac disease is sensitization with IFN- γ treatment combined with gliadin feeding.⁴⁶ This model has demonstrated the protective effect of bifidobacteria in patients with celiac disease, although it is unclear whether bifidobacteria are reduced in this model, as observed in patients. The addition of *B longum* CECT 7347 to this model reduces the extent of villus stunting and decreases the levels of TNF- α while increasing IL-10.⁴⁷ Refinement of these models will be critical for our understanding of the contribution of the communication between the gut microbiota and the immune system for the progression of celiac disease.

Environmental enteropathy

Other environmental effects can also result in low weight gain and symptoms of malabsorption, such as diarrhea. Known as environmental enteropathy or tropical sprue, this condition represents a major health problem, resulting in underweight status and stunting of children in the developing world, affecting approximately 178 million children.⁴⁸ Surprisingly, attempts to provide nutritional intervention for these children are unsuccessful approximately 70% of the time,⁴⁹ suggesting that lack of nutrition is not the sole cause of stunting and low weight. Major sanitation and hygiene difficulties, key characteristics of impoverished environments, lead to a high rate of infectious diarrhea, intoxications, and bacterial contaminations of fecal origin. Thus together with poor nutrition, microbial factors have been implicated in the pathogenesis of this chronic enteropathy. The microbial component is further supported by a variant of this disease called "traveler's diarrhea." This condition is frequent in visitors exposed to contaminated water or food but generally subsides on return to places of improved sanitation.⁵⁰

Therefore it appears that chronic subclinical infections of different causes (bacterial, protozoal, and viral) in the gut are the major contributors to the pathogenesis of environmental enteropathy. However, reduced nutritional status itself can be also associated with changes in normal gut microbiota in the absence of infection, such as in anorexic patients who have increases in methanogenic bacteria.⁵¹ Thus the case of environmental enteropathy reveals a vicious cycle of malnutrition leading to increased susceptibility to infection, infection leading to malnutrition through decreased absorption, and both malnutrition and infection disrupting the normal gut microbiota.⁵² This results in damage to the intestine manifested by blunted villi, inflammatory infiltrates, decreased production of absorptive enzymes, and high intestinal permeability (Fig 1).⁵⁰ The example of environmental enteropathy demonstrates how disturbances in the microbiota and immunity through undernutrition, infections, or overgrowth of commensals in the small intestine can affect intestinal function and specifically absorption so that malnutrition persists even with nutritional interventions.

The current hypothesis is that environmental enteropathy develops from a combination of malnutrition, chronic bacterial

infection, and population of the small intestine with commensals normally living in the large intestine because of fecal contamination. The last causal factor is difficult to model in laboratory animals, specifically in mice, because of their coprophagic nature (ie, mice are adapted to chronic fecal contamination). A malnutrition-associated enteropathy model that reproduces many features of the human condition has also been established and is independent of pathogenic infection. In this model weanling mice are fed an isocaloric diet designed to mimic the endemic diet of Northeastern Brazil. After 6 weeks on this diet, decreased villous height, increased intestinal permeability, and epithelial apoptosis are observed in the intestine.⁵³ Although the mechanisms of these alterations have not been studied, it is likely that they are also driven (as in immunodeficiency) by interactions between immunity, increased bacterial load, and metabolic function of the intestinal epithelium. Another method to model environmental enteropathy was introduced very recently by means of transplantation of microbiota from healthy and malnourished children into germ-free animals. Colonization by microbiota from malnourished children resulted in a weight loss phenotype in 2 of 3 cases tested, providing experimental support for a causal role of the microbiota.⁵⁴ Thus despite large interspecies differences and potential limitations,⁵⁵ this approach represents a next step in experimental models of microbiota-associated human diseases.

SYSTEMIC METABOLISM AND OBESITY ARE GOVERNED BY GUT MICROBIOTA FUNCTION

Clinical findings

The World Health Organization estimates that 1.5 billion adults and 42 million children worldwide are overweight or obese.⁵⁶ Obesity is driven by a complex combination of dietary, genetic, and socioeconomic factors. Additionally, obesity rarely occurs in isolation; it is often associated with additional metabolic symptoms, such as insulin resistance and increased serum lipid levels, which are termed metabolic syndrome.

In addition to overnutrition and genetic factors, the gut itself plays an important role in the development of obesity and metabolic disorder. Several clinical observations are supportive of this idea. For example, bariatric surgery often involves bypassing the duodenum and at least some portion of the jejunum, resulting in both food intake restriction and malabsorption.^{57,58} Patients who undergo bariatric bypass have significantly improved markers of metabolic syndrome, even before weight loss, including reduced serum lipid levels and improved insulin sensitivity. Interestingly, bypass surgery results in a higher percentage of patients with significant weight loss and metabolic improvement than laparoscopic banding, which only restricts the stomach volume.^{59,60} These results suggest that intestinal mechanisms, including the microbiota, are involved in the decrease in fat uptake and improvement in metabolic parameters in response to bariatric surgeries.

Recently, the gut microbiota has been recognized as an important player in metabolism and obesity. One of the most evident clinical observations that drive this idea is related to response to antibiotics. Treatment of infants with antibiotics was found to be associated with increased body mass.⁶¹ Additionally, in an African population *Helicobacter pylori* positivity was correlated with higher weight, and decreased cholesterol levels were reported after its eradication by antibiotic treatment.⁶² However,

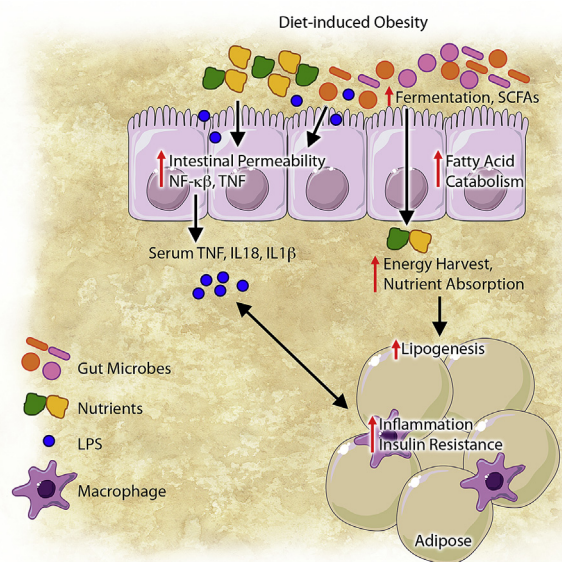


FIG 2. Microbiota regulation of systemic metabolism, obesity, and obesity-associated inflammation. In the presence of increased dietary lipids, changes in the microbiota include enhanced fermentation and production of SCFAs, which contribute to increased energy harvest and lipid absorption in the intestine. The microbial dysbiosis and nutrient overload result in increased intestinal permeability and increased inflammation in the intestinal epithelium and adipose tissue, which contribute to insulin resistance. *NF-κB*, Nuclear factor κB.

eradication of *H pylori* with antibiotic treatment in an American population increased weight gain over time,⁶³ and no change in cholesterol levels was observed.⁶⁴ Therefore these results suggest that the composition of the gut microbiota might predetermine the metabolic response. Indeed, changes in the composition of the gut microbiota have been reported in obese patients.^{65,66} However, the most recent metagenomics studies in patients with type 2 diabetes indicate that changes in microbial functions rather than in taxa might present more consistent perturbations, although they can still vary between populations.^{67,68} Although these particular results alone do not qualify the microbiota changes as a cause or effect of obesity and metabolic syndrome, they provide an independent demonstration of the strong relationship between the gut microbiota and fat metabolism.

Because of the potential implications for therapy, a large proportion of research about this relationship (microbiota and fat metabolism) is focused on the effects of the microbiota on metabolism. However, this is clearly not a 1-way road. Indeed, it was shown that dietary input can affect the composition of the microbiota both transiently and in a more stable manner, suggesting that the microbiota responds to optimize use of available nutrients.⁶⁹⁻⁷¹

Models and mechanisms of obesity and metabolic disease

Given the increasing incidence of obesity, it is not surprising that there are many well-developed and deeply studied animal models for obesity and associated metabolic syndrome. A very popular model, diet-induced obesity, mimics many of the complex phenotypes of human obesity, including inflammation and metabolic disease. Rodents fed a diet that is extremely high in fat (typically

40% to 60%) become dyslipidemic and obese. They also have symptoms of insulin resistance, have systemic inflammation, and, depending on the strain, might have type 2 diabetes.⁷²

Increased ingestion of dietary fat has significant effects on lipid absorption, even before the development of obesity. Short-term feeding with a high-fat diet (3-4 weeks) increases proliferation of the intestinal epithelium, resulting in hypertrophy of the terminal ileum, which in turn increases oleate uptake⁷³⁻⁷⁵ and predisposes mice to increased lipid absorption, even after return to a normal diet and in the absence of increased body mass.⁷⁶ Interestingly, we observed that once mice become obese, there is a significant shortening of the small intestine by approximately 15% (unpublished data), probably reflecting compensatory mechanisms in the gut trying to resist the nutrient overload. Another adaptation mechanism is suggested by a study of gene expression on a high-fat diet showing a dose-dependent decrease in expression of several cholesterol transporter genes and increased expression of fatty acid catabolism genes (Fig 2).⁷⁷

The chronic low-grade inflammation that accompanies obesity is characterized by increased levels of circulating TNF-α, IL-18, IL-1β, and other proinflammatory cytokines believed to be produced by immune cells infiltrating adipose tissue, muscles, and the liver.⁷⁸ However, the intestine might be added to the list of organs that contribute to this inflammatory state. The dysregulation of immune genes in the intestine⁷⁷ and the activation of TNF-α and nuclear factor κB in the ileum in response to a high-fat diet even before the development of obesity (Fig 2)⁷⁹ lead us to speculate that the chronic inflammation present in obesity might be initiated in the gut. Remarkably, this ileal inflammation in response to fat requires the presence of the gut microbiota, as it is not observed in germ-free mice.⁷⁹ The role of the microbiota in obesity and the accompanying metabolic syndrome is further corroborated by the fact that elimination of the microbiota with antibiotics improves metabolic parameters, such as weight, adiposity, and insulin resistance.⁸⁰⁻⁸² This seems to be related to the decrease in endotoxemia and inflammation resulting from increased intestinal permeability on a high-fat diet.^{81,83}

Mucosal immunity contributes to the regulation of development of both dysbiosis and obesity in response to a high-fat diet. For example, mice lacking lymphotoxin, a critical molecule in gut immunity, are resistant to diet-induced obesity because of the maintenance of microbial diversity that is normally reduced in response to a high-fat diet. This resistance can be eliminated simply by cohousing with lymphotoxin-competent control mice, which provided constant exposure to the microbiota of the obese siblings. This demonstrates that it is the presence of an obesity-promoting microbiota that confers sensitivity to obesity.⁸⁴ However, defects in immunity can also result in increased fat mass, as is reported in Toll-like receptor (TLR) 2 and TLR5 knockout mice. These phenotypes are transmissible to wild-type mice through microbiota transfer, indicating that their alteration is causative of increased adiposity.^{85,86} Intriguingly, TLR2- and TLR5-deficient mice can present with immune activation in the colon and spontaneous colitis, respectively,^{87,88} although the TLR5 knockout phenotypes vary from absence of colitis⁸⁹ to protection of colitis in a T-cell transfer model.⁹⁰ These results might be related to the complex polygenic nature of IBD, which involves other microbial sensing molecules, such as NOD2, autophagy pathways, and T-cell responses contributing to aberrant immune responses and tissue damage (Fig 3).^{91,92} Interestingly, reports on another sensing pathway leading to inflammasome

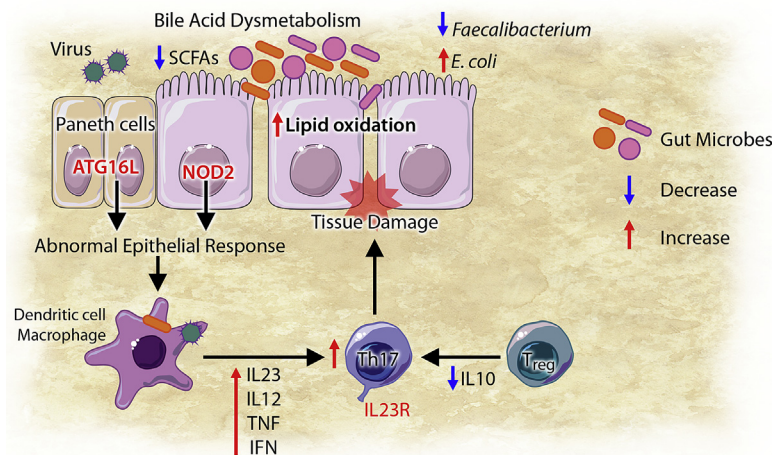


FIG 3. Mechanisms of microbiota and immune-mediated injury and metabolic changes in patients with IBD. Disruption of bacterial sensing by defective NOD2 or ATG16L (in the presence of norovirus) alters the immune responses to the gut microbiota, resulting in an increase in levels of proinflammatory cytokine, including IL-12, IL-23, TNF, and interferon (*IFN*). In turn, these cytokines activate a T-cell response that leads to tissue damage. Reduction in IL-10 levels reduces regulatory T (T_{reg}) cell numbers, further promoting the inflammatory response. Alterations in gut microbiota composition lead to decreases in anti-inflammatory SCFA levels and to bile-acid dysmetabolism. The end result of this inflammatory response is epithelial tissue damage and changes in lipid metabolism, such as an increase in lipid oxidation. Red gene labels indicate established IBD-associated loci.

activation also show opposite metabolic phenotypes in different studies.^{93,94} These seemingly discrepant results suggest that common factors, such as genetic background, local microbiota, or food supply, can contribute to observed differences in phenotype. The involvement of microbial sensing in both obesity and IBD demonstrates the intimate connections between the microbiota, immunity, and metabolism.

As discussed previously, dysbiosis of the gut microbiota has been observed in obese patients. Obese mice that lack leptin (*ob/ob* mice) have an increased ratio of Firmicutes to Bacteroidetes, as observed in some studies of obese patients.⁹⁵ Even a single species can have dramatic effects on obesity and metabolic disease. *Akkermansia muciniphila* levels are decreased in obese and diabetic mice, and treatment to restore levels protects against obesity and hyperglycemia development.⁹⁶ Conversely, changes in diet, such as an increased intake of saturated milk fat, can promote the expansion of a typically low-abundance “bile-loving” bacterium that leads to colitis on a susceptible IL-10 knockout background.⁹⁷ Interestingly, a recent study further underscored the importance of bile-acid dysmetabolism by showing that certain microbial transformations of bile acids occurring in patients with IBD might potentiate inflammation.⁹⁸

Another hypothesis is that changes in the core microbiome, but not in specific microbes, are what confers propensity to obesity. Metagenomic sequencing of the microbiota from lean and obese patients, as well as mice, has revealed a shift in the core microbiome that is characterized by an increase in energy harvest potential by increased expression of genes required for fermentation of complex polysaccharides (Fig 2).⁹⁹⁻¹⁰¹ Microbiota-driven breakdown of complex polysaccharides into short-chain fatty acids (SCFAs), such as propionate, butyrate, and acetate, in the large intestine provides a previously unavailable additional source of energy,^{102,103} most significantly to colonocytes but also to peripheral tissues.¹⁰⁴ Importantly, the increase in potential energy harvest capacity has true functional effects on host metabolism because transplantation

of the obesity-associated microbiota into lean mice increases adiposity of the recipients in the absence of increase food intake.⁹⁹ However, another study demonstrates that butyrate supplementation prevents obesity in mice receiving a high-fat diet,¹⁰⁵ possibly suggesting a dose-dependent differential effect of SCFAs on lipid metabolism and obesity development.

A mechanism involving SCFAs also seems to underlie the increase in fat mass after disruption of the microbiota by low doses of antibiotics. Historically, low-dose antibiotics have been used in agricultural settings and have been correlated to the increasing size of farm animals.^{106,107} Treatment of young mice with low-dose antibiotics confirms these observations; these mice have increased overall adiposity and altered composition of the gut microbiota without a decrease in total bacterial load. Examination of the gut microbiome uncovers increases in SCFA production capability similar to those observed in obese animals.¹⁰⁸ Although not examined, we hypothesize that mice receiving low doses of antibiotics might also have improved lipid absorption because of decreased microbial pressure on the intestinal epithelium, allowing for enhancement of metabolic function. Essentially, this is the opposite action of the same mechanism that leads to a decrease in absorption in the setting of immunodeficiency and environmental enteropathies (Fig 2).

Interestingly, in addition to microbial sensors, changes in SCFA production represent another intersection between obesity and IBD. Reduced microbial diversity, especially a decrease in Ruminococcaceae in patients with Crohn disease (CD), a decrease in Leuconostocaceae in patients with ulcerative colitis, and reduced *Faecalibacterium* species in patients with both forms of IBD,^{109,110} has been associated with a decrease in the production of SCFAs (Fig 3).^{109,111} A decrease in metagenomic abundance of SCFA metabolism genes in patients with CD with ileal involvement supports this reduction in SCFA production ability.^{109,111} The anti-inflammatory properties of SCFAs and their importance in overall health have been well described.¹¹²⁻¹¹⁵ These SCFAs

appear to contribute to the inflammatory response in patients with IBD because treatment of both patients with ulcerative colitis and those with CD with butyrate improves symptoms and disease scores.^{116,117}

Another mechanism of gut microbe regulation of systemic lipid metabolism has been also described.^{4,118,119} Germ-free mice have increased expression of the circulating lipoprotein lipase inhibitor angiopoietin-like 4 (Angptl4, also known as Fiaf) in the intestine, and this expression is repressed on colonization.^{118,120} The decreased expression of angiopoietin-like 4 in the intestine results in increased circulating lipoprotein lipase, which promotes increased fatty acid metabolism in the liver and storage of triglycerides in adipocytes.¹¹⁸ Thus there is significant evidence from the germ-free models that the gut microbiota influences both fat metabolism and immunity in the gut. However, additional mechanisms of direct cooperation between these 2 functions, as demonstrated in the case of B-cell knockout mice,²⁸ remain to be further clarified.

FUTURE PERSPECTIVES

Clinical and experimental data indicate that gut function requires a fine balance of cross-regulation between the intestinal epithelium, the immune system, and the gut microbiota. Whether it is immunity or environmental factors that lead to disturbances in lipid metabolism or alterations in lipid metabolism that lead to immune disorders, the microbiota is almost always the one that transmits the signal to other side.

There seem to be 2 key emerging events that are currently moving the field forward. First is the integration of the gut in terms of both metabolism and immunity, which is critical to our understanding of disease and development of new therapeutic strategies. Second is a tsunami of technological and conceptual advances in the research on the microbiome that puts the commensal microbiota in the center of many physiologic processes, especially in the gut. Here we discuss some questions that should promote research and translation of basic science knowledge into clinical practice.

First, we need to be able to discriminate between situations in which a specific microbial species is influencing the host phenotype versus those in which a change in a specific function of the microbiota shared across the taxa contributes to disease. Another question that is awaiting an answer is why some host phenotypes can be reproduced by transplantation of microbiota into a genetically different host^{85,86,100} and why others cannot.^{28,121} In other words, what makes alterations in the microbiota self-sustainable and able to persist and alter the phenotype in hosts with different genotypes? Finally, there are several animal models of gut-associated human diseases, but the critical question is as follows: Can we translate findings about mouse microbes into human subjects? Perhaps a more effective approach would be to work in the opposite direction, from human patients back into animal models. The challenge then is the development of reliable animal systems allowing not only colonization with human microbiota but also actual reproduction of the physiologic processes taking place in the human gut.

On a clinical note, the analysis of the microbiome should mature from being the tool of basic science into a new “microscope” for clinicians, providing diagnostic and prognostic information that would allow for proactive measures before the establishment of outright disease. Additionally, given the importance of the gut microbiota in health, there is a great need for

development of more pathogen-specific antibiotics in an effort to spare the gut microbiota while still treating infection. If depletion of commensals cannot be avoided, we need to learn which specific prebiotics, probiotics, or their components can be beneficial, depending on which antibiotics are administered. In addition, prebiotic/probiotic use should not be restricted to simple recovery after antibiotic use. Rather, their unique ability to shape microbial function in a specific manner¹²²⁻¹²⁴ should be applied to remedy a diverse array of conditions associated with changes in the microbiota, ranging from the gut diseases and obesity discussed here to clinical depression^{125,126} and late-onset autism.^{127,128} Along these same lines, it might be possible to target a specific microbial gene present across different taxa in cases in which changes in this gene in the overall microbiome contribute to disease instead of changes in specific taxa/species.

Finally, is it all about microbes in the gut? Probably not. There is evidence of a direct connection between immunity and metabolism not always dependent on microbiota. Indeed, activation of immunity led to decreased expression of metabolic genes in cell cultures devoid of microbes and microbial byproducts.²⁸ Interestingly, similar crosstalk between the interferon-dependent pathway and cholesterol metabolism was also reported in different cell types, suggesting that this mechanism is highly conserved.^{31,32} Perhaps this fundamental mechanism can be borrowed from nature for therapeutic purposes. For example, we might try to dampen immune activation within the epithelium to restore metabolic function. Overall, the integration of immunity, metabolism, and the microbiota in the gut makes it an important player in human health and an exciting target for therapeutic interventions.

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