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Key questions to guide a better understanding of host–commensal microbiota interactions in intestinal inflammation

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Co-evolution with an extremely complex commensal enteric microbiota has helped shape mammalian mucosal immune responses. A yet incompletely defined subset of intestinal bacteria is required to stimulate chronic, immune-mediated intestinal inflammation, including human Crohn's disease, and intestinal microbiota composition is altered in a characteristic manner by the inflammatory response to create a dysbiotic relationship of protective vs. aggressive bacteria. We pose a number of questions regarding host interactions with the enteric microbiota, including influences of inflammation, host genetics, early environmental exposure, and diet on microbial composition and function, and conversely, the effect of bacterial metabolism, enteric fungi and viruses, and endogenous protective bacterial species on host immune and inflammatory responses. These questions are designed to stimulate research that will promote a better understanding of host–microbial interactions in the intestine and promote targeted novel therapeutic interventions.

We have co-evolved with an extremely complex group of bacteria, fungi, and viruses that interact not only with each other but also with the host to help shape our mucosal immune response. These microbial signals activate and differentiate both

effector and homeostatic limbs of the innate and adaptive immune systems, which in turn regulate microbial growth, composition, and function.

Dramatic advances in culture-independent molecular techniques have permitted

a much deeper understanding of the community structure, genetic repertoire, metabolic products, and function of the complex microbiome that outnumber our somatic cells by 10-fold. These advances have depended on the unique structure of the 16s ribosomal RNA subunit, which can be used as a signature of individual bacterial species. These techniques have rapidly evolved from surveys that measure the most common species, such as denaturing gradient gel electrophoresis and terminal restriction fragment-length polymorphism, to sequencing of clone libraries, to the most recent, but still rapidly evolving pyrosequencing techniques using increasingly rapid, first-, second-, and now third-generation parallel sequencers that permit deep exploration of the microbiome. These advances have led to an unprecedented view of the gut microbial composition, now including viral and fungal profiles, as well as bacteria, its adjuvants, antigens, and metabolic products. However, many questions remain regarding how the diverse components interact to shape our immune function for homeostatic and pathophysiological purposes, and how in turn, the diverse components of the mucosal innate and adaptive immune response, immune-mediated inflammation, and host genetics shape the composition and function of the intestinal microbiota.^{1–3}

CURRENT UNDERSTANDING OF THE COMMENSAL MICROBIOTA IN INTESTINAL INFLAMMATION

Commensal intestinal bacteria are essential stimuli for experimental chronic immune-mediated inflammation with strong evidence of their involvement in Crohn's disease and pouchitis, but in

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addition, these agents are almost certainly involved in the pathogenesis of ulcerative colitis, alcoholic liver disease, nonalcoholic steatohepatitis, and metabolic syndrome.^{4–7} Crohn's disease seems to be an inappropriately aggressive TH1 and/or TH17 immune response to a subset of commensal intestinal bacteria in genetically susceptible hosts, which is initiated and reactivated by transient infectious or environmental triggers.^{3,4,8,9} These triggers break the mucosal barrier and activate an initial nonspecific immune response, which becomes chronic because of the constant drive of commensal microbial antigens in hosts that have genetic defects in mucosal barrier function or repair, bacterial killing by innate immune cells or Paneth cells, or immunoregulation.

The profile of both fecal and mucosally associated bacteria exhibit changes described as “dysbiosis” in a subset (~1/3) of inflammatory bowel disease (IBD) patients with active disease and in rodents with experimental colitis that are characterized by decreased bacterial diversity and an altered ratio of beneficial and aggressive bacterial species.^{1,10} These alterations include decreased concentrations of the Lachnospiraceae subset of Clostridiales, and variable changes in Bacteroidetes, with a concomitant increase in Proteobacteria and Actinobacteria (except Bifidobacterides). Bacterial species that are consistently increased in human IBD and experimental colitis include *Escherichia coli*, specifically the B2 and D phylotypic groups and adherent/invasive strains associated with ileal Crohn's disease.^{11–13} In addition, experimental TH1/TH17 activation and colitis in monoassociated interleukin-10-deficient mice alter gene expression in a commensal adherent/invasive *E. coli* strain in preliminary experiments, with upregulation of bacterial stress-response genes.¹⁴ In recent studies, *Ruminococcus gnavus*, *Klebsiella pneumoniae*, and *Proteus mirabilis* have been implicated in Crohn's disease and experimental colitis.^{15,16} Conversely, *Faecalibacterium prausnitzii*, a major representative of the butyrate-producing *Clostridium leptum* group of the Lachnospiraceae family, is decreased in ileal and colonic Crohn's

Table 1 Mucosal homeostasis vs. inflammation depends on the balance of beneficial vs. detrimental enteric microbial species

Detrimental bacterial species	Protective bacterial species
<i>Bacteroides vulgatus</i> , <i>Bacteroides thetaiotamicron</i>	Lactobacillus species
<i>Enterococcus faecalis</i>	Bifidobacterium species
Adherent/invasive <i>Escherichia coli</i>	Nonpathogenic <i>E. coli</i>
<i>Klebsiella pneumoniae</i> , <i>Bifidobacterium animalis</i>	<i>Saccharomyces boulardii</i>
<i>Fusobacterium varium</i>	<i>Bacteroides fragilis</i>
Intestinal Helicobacter species	<i>Faecalibacterium prausnitzii</i>
Segmented filamentous bacteria	<i>Roseburia</i> spp.
<i>Ruminococcus gnavus</i>	
<i>Proteus mirabilis</i>	

Abbreviation: IBD, inflammatory bowel disease.

Each species has documented inflammatory or protective activity either in rodent models or human IBD.

disease and has been reported to provide protection in experimental colitis and to predict relapse of postoperative ileal Crohn's disease.^{17,18}

Commensal bacteria have an essential role in driving immune-mediated experimental inflammation in the distal intestine.^{4,6,18} Germ-free genetically susceptible mice and rats exhibit no evidence of chronic colitis under germ-free (sterile) conditions and CD4+ T cells produce interferon- γ and interleukin-17 in response to cecal bacterial lysates and bacterial antigens in specific pathogen-free genetically engineered rodents and after selective colonization with some, but not all, bacterial species in gnotobiotic rodents.^{19,20} It is difficult to predict the pathophysiological effects *in vivo* due to host-specific effects, as segmented filamentous bacterium, a noncultivable commensal that induces TH17 responses in wild-type noninflamed mice²¹ did not induce colitis when monoassociated in the SCID (severe combined immunodeficiency) mouse CD45RBhigh transfer model,²² and *Bifidobacterium animalis*, normally considered to be a protective probiotic strain, can cause colitis in monoassociated interleukin-10-deficient mice.^{23,24} In contrast, other commensal species and their defined components and secreted products have predominantly protective properties.^{18,25} These studies demonstrate both host and bacterial species specificity, as only a relatively small subset of commensal bacterial species will induce experimental colitis,^{19,20} different

hosts respond selectively to the same bacterial species,²⁶ two different commensal bacterial species can induce different phenotypes of inflammation in the same host²⁰ and have additive effects,²⁷ and commensal specific pathogen-free bacterial species that induce colitis in susceptible hosts do not cause inflammation in wild-type hosts.^{19,20} Even different *E. coli* strains have variable abilities to induce experimental, immune-mediated colitis, as adherent-invasive nontoxigenic *E. coli* strains, including a human ileal Crohn's disease isolate, induce chronic colitis, but a nonadherent strain does not.²⁸ These observations lay the foundation for the hypothesis that a subset of nonpathogenic commensal bacteria provide the constant antigenic drive of IBD in genetically susceptible hosts, whereas other endogenous species primarily induce protective regulatory immune responses. The relative balance of these aggressive vs. beneficial organisms determine inflammation vs. homeostasis and potentially can be manipulated for therapeutic benefit (Table 1).

The following key questions to better understand microbial–host interactions remain unresolved. Selected illustrations by currently available data are provided.

IS DYSDIOSIS A CAUSE OR A CONSEQUENCE OF INTESTINAL INFLAMMATION?

The primary vs. secondary nature of the characteristic pattern of decreased Clostridial groups IV (including *F. prausnitzii*)

and XIVa, and in some studies, Bacteroidetes, with increased concentrations of Proteobacteria (including *E. coli*), *R. gnavus*, and Actinobacteria that is associated with IBD is unclear. Transmission of colitis^{16,29} and metabolic syndrome⁵ by fecal transplants from affected to wild-type recipient mice that are normally not susceptible provides evidence for an etiological role for abnormal commensal bacteria. However, the finding of very similar dysbiosis patterns in intestinal inflammation in widely diverse hosts, including mice, humans, and dogs,^{13,30,31} disparities between the microbiota in active vs. quiescent IBD,^{17,32,33} and similarities of microbiota alterations in infections, and chemically and genetically induced gut inflammation^{34,35} strongly support nonspecific alterations as a consequence of the inflammatory milieu. This fundamental issue has profound implications for proposed therapeutic strategies, such as fecal transplants.

CAN THE COMMENSAL MICROBIOTA BE ALTERED PERMANENTLY BY THERAPEUTIC INTERVENTIONS?

This question is integral to the duration of microbial-based therapies, including fecal transplant. Although an individual's fecal microbiota changes daily with dietary and environmental shifts, there is a remarkable long-term stability of the intestinal bacterial population.³⁶ Probiotic administration only transiently alters fecal bacterial composition due to a lack of long-term colonization, with reversion to baseline within 2 weeks of ceasing therapy.³⁷ However, despite earlier evidence to the contrary, recent data indicate long-term changes in microbial composition and diversity after antibiotic therapy, particularly if administered cyclically.^{38–40} These observations, along with transfer of colitis^{16,29} and metabolic syndrome⁵ by fecal inoculation and activation of ileal interleukin-17 responses by colonization with a specific bacterial species (segmented filamentous bacterium),²¹ suggest that immunological effects of commensal bacterial transfer may be sustained over at least several months. The prospect of permanently changing the intestinal microbiota in

older children and adults remains speculative at present.

WHAT ARE THE RELATIVE INFLUENCES OF GENETICS VS. ENVIRONMENTAL FACTORS IN SHAPING THE INTESTINAL MICROBIOME?

Multiple factors contribute to the development of the intestinal microbiota, including maternal transmission, early life exposures, diet, and genetics.^{1,41} However, the relative roles of environmental vs. genetic influences are not yet well understood. In a seminal metagenomic study, Turnbaugh *et al.*⁴² demonstrated that the degree of similarity between monozygotic and dizygotic twin pairs was not significantly different, although monozygotic twins had a trend toward greater similarity, and that bacterial communities were more similar within family members than between different families, suggesting a primary role for early shared exposure rather than genetic direction of microbial composition. In contrast, Frank *et al.*⁴³ reported that the NOD 2 (nucleotide-binding oligomerization domain containing 2) composite genotype, ATG 16L1 polymorphisms, and ileal disease location were associated with mucosal dysbiosis in patients with Crohn's disease. Similarly, NOD 2 deficiency in mice resulted in decreased *F. prausnitzii* ileal mucosal concentrations.⁴⁴ Similarly, monoassociation of 23 inbred mouse strains with the defined altered Schaedler flora indicated important genetic effects,⁴⁵ although the lack of differences in the fecal microbiota in two different mouse strains implanted into a foster mother demonstrated lasting effects of maternal microbiota transmission.⁴⁶ These and other studies have revealed that both genotype and environmental influences, mostly diet and early microbial exposure, combine to shape enteric microbiota composition, which remains quite stable once developed.

DOES INITIAL BACTERIAL COLONIZATION AND EARLY ENVIRONMENTAL EXPOSURES DETERMINE LIFE-LONG BACTERIAL COMPOSITION?

Transition from the sterile amniotic sac to the external environment is accompanied by progressive transition to increasingly

complex microbiota over time.^{47,48} Initial colonization depends on mode of delivery, with vaginally delivered infants acquiring microbial communities resembling their mother's vaginal microbiota dominated by *Lactobacillus*, *Prevotella*, or *Sneathia* spp., whereas infants delivered by Cesarean section acquired skin organisms, including *Staphylococcus*, *Corynebacterium*, and *Propionibacterium* spp.⁴⁹ Initial communities are aerobic, transitioning gradually to the anaerobic complex microbiota. Maternal and paternal influences are evident in twin studies, with shared environment more important than genetic influences.⁴² Dominant maternal influences on transmitted bacterial communities are confirmed by mouse studies.⁴⁶ Dietary regulation is important, with domination of the preweaning microbiota by the lactose-responsive *Bifidobacterium* and *Lactobacillus* spp. and rapid diversification of bacterial communities after weaning.⁵⁰ Somewhat surprisingly, cultivatable bacterial communities do not seem to be influenced heavily by breast vs. bottle feeding, with the exception of *Lactobacillus rhamnosus*.^{47,48} As atopic disorders appear to be linked to early (<6 months of age) microbiota composition,⁵¹ multiple investigators have explored early probiotic intervention in infants or even mothers with atopic conditions in the third trimester of pregnancy, usually with little success.⁵² Although most authors state that the human microbiota is stabilized by 12 months of age, transplantation of the human microbiota to germ-free (sterile) adult mice shows that the absence of competing organisms can obviate age effects.⁵³

These studies suggest that the potential of targeting infants at high risk for developing immunological diseases for constitution with the "healthy" microbiota to prevent the onset of disease has not yet been achieved. This potentially important area of investigation needs to be further explored in rodent models, in which consequences of experimentation are acceptable and mechanistic studies can be performed. Early intervention in infancy or during pregnancy could be a very physiological and nontoxic approach to altering life-long risk of

familial inflammatory/allergic conditions, given the apparent importance of early infant colonization on adult microbial communities.

INFECTIOUS AGENTS: TRIGGERS OR CHRONIC STIMULI?

Although the essential role for the commensal microbiota as antigenic stimuli of effector immune responses in chronic intestinal inflammation is broadly accepted, the importance of traditional and opportunistic pathogens in IBD remains quite controversial. An etiological role for MAP (*Mycobacterium avium* subspecies *paratuberculosis*) in Crohn's disease is not widely supported, but the intriguing possibility that an obligate intracellular opportunistic pathogen such as MAP or adherent/invasive *E. coli* selectively persists within macrophages of individuals with genetic defects in intracellular microbial clearance, such as NOD 2, ATG 16L1, and IGRM, must be considered.⁴ Chronic infections can cause persistent enterocolitis in normal hosts, with potentiation of injury in susceptible hosts, as illustrated by enterotoxigenic *Bacteroides fragilis*.⁵⁴ Transient infection by traditional enteric pathogens can trigger chronic, immune-mediated experimental intestinal inflammation⁶ and is linked to human IBD in epidemiological studies.⁵⁵ Potential mechanisms include breaking the mucosal barrier and/or activating pathogenic immune responses that are subsequently perpetuated by commensal enteric antigens in genetically susceptible hosts who are unable to repair epithelial breaches or downregulate the inflammatory response. An alternative mechanism is priming of adaptive immune responses to commensal bacteria, as documented for *Helicobacter bilis*⁵⁶ and cytomegalovirus⁵⁷ infection. Cadwell *et al.*⁹ recently provided powerful proof of the concept that infectious triggers can interact with host genetic susceptibility to induce disease. Noravirus infection induced a characteristic abnormality in Paneth cell granules in ATG 16L1 mutant mice, similar to those in Crohn's disease patients with ATG 16L1 polymorphisms, and potentiated acute toxin-induced colitis, which was driven in part by commensal bacteria. Neither the genetic defect

nor viral infection alone was sufficient to induce the Paneth cell phenotype or potentiate toxin-induced colitis; only the interaction of the viral trigger with genetic defect enhanced colitis.

CAN ENDOGENOUS PROTECTIVE COMMENSAL BACTERIAL SPECIES MORE EFFECTIVELY TREAT AND PREVENT INFLAMMATORY DISEASES THAN WILL TRADITIONAL PROBIOTIC SPECIES?

Traditional probiotic species are an attractive potential treatment of intestinal inflammation, but results to date have not lived up to their promise, despite well-documented immunosuppressive effects.^{58,59} In addition to their immunoregulatory activity, at least one probiotic species strongly influences gene expression and metabolism of commensal bacteria.⁶⁰ An important limitation of the currently available probiotic preparations, which are traditionally derived from fermented foods rather than commensal enteric bacterial species, is their inability to colonize the distal intestine and persist after administration ceases.^{4,59} A potential solution is to use commensal protective bacterial species, such as *F. prausnitzii*, *B. fragilis*, host-derived *Lactobacillus*, *Bifidobacterium*, and *E. coli* strains, which exhibit protective activities in experimental murine studies. This strategy is particularly appealing as a means to correct specific deficiencies in protective commensal bacterial concentrations in an individual, such as selective replacement of low numbers of mucosally adherent *F. prausnitzii*, which is a risk factor for postoperative recurrence of Crohn's disease.¹⁸

WHAT IS THE ROLE OF DIET IN SHAPING AND MANIPULATING ENTERIC BACTERIAL COMPOSITION, FUNCTION, AND METABOLISM?

An extremely important yet vastly underexplored field is the effect of diet on the gut microbiome. Nascent studies clearly demonstrate the ability of various human diets to alter enteric bacterial composition, gene expression, and metabolic activity in reconstituted gnotobiotic mice.^{61,62} Dietary manipulation, which includes the use of prebiotic substances that foster the growth and metabolic

activity of endogenous butyrate-producing bacterial species, provides great promise for an extremely physiological and cost-effective approach to modify the intestinal microbiome in order to treat and prevent inflammatory diseases in high-risk individuals.^{4,63,64}

WHAT ARE THE EFFECTS OF MICROBIAL METABOLITES ON EFFECTOR AND REGULATORY MUCOSAL IMMUNE FUNCTION AND INFLAMMATION?

Intestinal bacteria are metabolically active and greatly contribute to the gut metabolome. These organisms use either dietary or host-derived carbohydrate substrates, the availability of which can almost immediately alter bacterial gene expression to upregulate or downregulate various bacterial genes and metabolic pathways.⁶² Nonabsorbed dietary carbohydrates (fiber, prebiotics) are substrates used by subsets of enteric anaerobic bacteria to produce beneficial short-chain fatty acids, most notably butyrate, which is the primary energy substrate for distal colonocytes and suppresses immune activation. Recent studies indicate multiple mechanisms and immunosuppression by butyrate, including inhibition of histone deacetylases and induction of apoptosis.^{65,66} In contrast, bacterial production of toxic metabolites, such as hydrogen sulfide, nitrous oxide, and reactive oxygen metabolites, can injure epithelial cells and potentially induce chronic intestinal inflammation.⁶⁷

HOW DO COMMENSAL FUNGI AND VIRUSES AFFECT INTESTINAL IMMUNE FUNCTION, INFLAMMATORY PROCESSES, AND BACTERIAL COMPOSITION AND FUNCTION?

Another virtually unexplored frontier of the gut microbiota is the lush fungal and viral elements that can directly affect intestinal epithelial cells and mucosal immune responses and can influence intestinal bacterial populations and function. Bacteriophages offer an opportunity to modulate specific bacterial populations because of their selective tropism. Recent molecular surveys identify abundant commensal viruses mainly targeting commensal bacteria.⁶⁸ As mentioned above, noravirus can induce aberrant Paneth cell

Figure 1 Chronic IBD depends on an interaction of at least four components, which alone are not sufficient to cause disease. IBD, inflammatory bowel diseases; NSAID, nonsteroidal anti-inflammatory drug.

granule morphology and potentiate acute experimental colitis.⁹

CONCLUSIONS AND FUTURE DIRECTIONS

Commensal enteric bacteria provide an essential role in the pathogenesis of Crohn's disease and probably other chronic, immune-mediated inflammatory conditions in the intestine and liver, and also provide obligate stimuli for the normal development and differentiation of mucosal immune responses. IBD requires interactions between genetic susceptibility, environmental triggers, enteric microbiota, and effector and regulatory immune responses, as outlined in **Figure 1**. Although recent breakthroughs in molecular techniques have led to a rapid evolution in our understanding of the enteric bacterial composition, many questions remain regarding their function and their complex mutualistic interplay with the host. Answering the questions posed in this commentary will begin to address some of the most obvious gaps in our knowledge of host-microbial interactions that determine chronic immune-mediated inflammation vs. mucosal homeostasis and peaceful coexistence with our complex gut microbiota. This knowledge

will lay the foundation for novel therapeutic interventions that extend beyond our current reliance on probiotics arising from fermented dairy products.

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DISCLOSURE

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