Pathogenesis of IBD

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The Intestinal Microbiota in Inflammatory Bowel Diseases

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Abstract
Abundant clinical and experimental evidence supports a role for resident microbiota in Crohn’s disease and pouchitis, and probably in ulcerative colitis (UC). These disorders occur in areas of highest bacterial concentrations. Pouchitis and Crohn’s colitis respond to antibiotics, while pouchitis and UC can be treated with probiotics. Serologic markers recognizing intestinal bacteria and yeast are present in the majority of Crohn’s disease patients and may predict disease aggressiveness. Abnormal profiles of fecal and mucosally associated enteric bacteria (dysbiosis) occur in Crohn’s disease, UC, pouchitis and experimental enterocolitis, with a proliferation of aggressive species that promote experimental colitis and a corresponding decrease in protective bacterial subsets. Many of these protective bacteria produce short-chain fatty acids, including butyrate, that promote epithelial barrier function, inhibit effector immune responses and induce regulatory T cell subsets. Furthermore, certain Clostridia species stimulate regulatory T cells that can inhibit intestinal inflammation. Animal models of chronic, immune-mediated enterocolitis convincingly demonstrate that enteric resident bacteria stimulate effector immune cells in susceptible hosts and that a subset of enteric bacteria has particularly aggressive activities, with host and bacterial specificity. Recent studies suggest parallel and perhaps complementary roles for enteric viruses, which have only very recently been identified.

Introduction
Humans exist with intestinal bacteria, fungi and viruses, collectively referred to as microbiota, that outnumber our own mammalian cells 10-fold [1–3]. These organisms are metabolically active and reciprocally interact with intestinal mu-
coccus, epithelial cells, and the mucosal and systemic immune systems. These bacteria increase in both concentration and complexity in a spatial gradient from aerobic conditions of the stomach, duodenum and jejunum, where *Streptococcus*, *Lactobacillus* and oropharyngeal species predominate, to the anaerobic environment of the distal ileum and colon, where *Clostridium* and *Bacteroides* species far outnumber other bacterial groups. The aggregate microbial organisms in the intestine total 10–100 trillion. Greater than 85% of enteric bacteria belong to the *Firmicutes* and *Bacteroidetes* divisions, with only 5% or less *Proteobacteria*. Close host/microbial interactions have led to the concept that mammals are supraorganisms, i.e. a composite of mammalian and microbial cells, genomes and metabolomes. Evolution in a preexisting microbial environment helped shape the human genome. The majority of enteric bacteria cannot be cultivated by standard techniques. Recent breakthroughs in understanding the microbiota are a result of culture-independent sequencing analyses that take advantage of a unique property of the bacterial 16S ribosomal RNA gene structure, where species-specific hypervariable regions are flanked by highly conserved regions. PCR primers binding to the conserved regions are used to expand bacterial DNA, then rapidly evolving deep sequencing techniques are used to identify the hypervariable regions, and bacterial families are used to compare sequences with bioinformatic databases. Recent data support an integrated microbiome view in which bacterial function is more important than composition. As confirmed by metagenomic and metabolomic analyses, nutritional, metabolic and other key pathways are shared by overlapping bacterial species, such that the presence of certain bacterial groups can functionally compensate for the absence of other species, and different bacterial groups can synergistically interact. Key metabolic pathways of high relevance to mucosal homeostasis and inflammation include metabolism of nonabsorbed carbohydrates, primarily dietary fiber, that function as prebiotic substrates, to short-chain fatty acids (SCFAs; butyrate, propionate and acetate). These substances function as protective metabolites for colonic epithelial cells. In contrast, dietary and structural sulfites and sulfates are metabolized by other resident bacterial species to produce hydrogen sulfide, which injures epithelial cells.

**Bacterial Regulation of Mucosal Homeostasis versus Inflammatory Bowel Diseases**

Host genetic and environmental factors help determine the composition of intestinal microbiota (fig. 1) [4, 5]. Environmental contributions include colonization by maternal microbiota, with differences in the microbiome of infants who
Microbiota in IBD are delivered vaginally versus Cesarean sections, diet, including breast versus formula feeding, infections and antibiotic exposure (see below). Microbiota community structure in normal individuals differs from that in patients with inflammatory bowel diseases (IBD). Normal resident bacterial communities are characterized by high diversity, with a predominance of Firmicutes and Bacteroidetes and low concentrations of Enterobacteriaceae. In contrast, the abnormal composition (dysbiosis) of patients with IBD is characterized by decreased bacterial diversity, expansion of Enterobacteriaceae, particularly γ-Proteobacteria such as *Escherichia coli*, with parallel contraction of certain *Clostridium* subsets, particularly clades IV and XIVa, that include *Faecalibacterium prausnitzii*. Many of the bacterial species that are decreased in IBD exhibit protective im-

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**Fig. 1.** Conceptual framework for microbial regulation of mucosal homeostasis vs. IBD. Genetic and environmental factors determine intestinal bacterial community structure, which differs in normal individuals and patients with IBD. These bacterial populations and their metabolites reciprocally interact with genetically regulated intestinal mucosal and immune pathways to mediate mucosal homeostasis vs. chronic inflammation. Reproduced with permission from Sartor [4].
munologic, mucosal barrier, and bacteriologic functions that mediate mucosal homeostasis (fig. 1; table 1). High bacterial diversity is responsible for colonization resistance against pathogens, best illustrated by the loss of protection against *Clostridium difficile* colonization following broad-spectrum antibiotic exposure. Resident enteric bacteria in general, and *Clostridium* and *Bacteroides* species subsets in particular, selectively induce regulatory T cells that secrete immunoregulatory cytokines, primarily TGFβ and IL-10 [6, 7]. In addition, *Clostridium* subsets also produce SCFAs that provide essential nutrients for colonic epithelial cells and also induce regulatory T cells [8]. Many bacterial groups activate innate immune defenses that are mediated by bacterial recognition receptors such as Toll-like receptors and NOD2 (through muramyl dipeptide, MDP) as well as the NFκB signaling pathway. Paneth cells are a key innate pathway that regulates mucosal bacterial populations and luminal/mucosal concentrations through production and secretion of antimicrobial peptides, including α-defensins. The net host response to normal resident microbiota by intestinal epithelial and immune cells is mucosal homeostasis and immunologic tolerance (fig. 1).

In contrast, abnormal dysbiotic microbiota activates dysregulated effector TH1 and TH17 cells in genetically susceptible hosts that mediate chronic intestinal inflammation that includes Crohn’s disease, ulcerative colitis (UC) and pouchitis in the absence of appropriate host regulatory responses (fig. 1) [2, 5]. We propose that Crohn’s disease is due to overly aggressive TH1/TH17 cell responses to a subset of luminal bacteria in the background of innate immune dysfunction. Susceptibility is determined by defects in genes that encode either immune responses, mucosal barrier function or enteric bacterial clearance. Finally, the onset and reactivation of disease are triggered by environmental stimuli that transiently break the mucosal barrier and initiate inflammation. This complicated hypothesis depends on the interaction of genetic, environmental, microbial and immune factors (fig. 2), which will be discussed in the following sections.

<table>
<thead>
<tr>
<th>Property</th>
<th>Example</th>
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<tr>
<td>Colonization resistance</td>
<td>prevent infection – <em>C. difficile</em></td>
</tr>
<tr>
<td>Educate the immune response</td>
<td>mucosal homeostasis – IL-10, inducible Treg</td>
</tr>
<tr>
<td>Host nutrition</td>
<td>SCFA metabolism by colonic epithelial cells, vitamin K synthesis</td>
</tr>
<tr>
<td>Activate innate immune defenses</td>
<td>TLR/NF-κB in epithelial cells, stimulation of antimicrobial peptides</td>
</tr>
<tr>
<td>Neuronal development</td>
<td>mediate pain threshold, CNS responses</td>
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Table 1. Protective effects of the normal microbiome
Genetic Susceptibility

One hundred and sixty-three susceptibility loci have been associated with Crohn’s disease and/or UC [9]. Functional abnormalities can be broadly characterized as defective bacterial killing, barrier function and immunoregulation. Several innate genes in the autophagy (ATG16L1 and IRGM), microbial signaling (NOD2) and endoplasmic stress response (XBP-1) pathways are associated with Crohn’s disease. These genes regulate bacterial killing through either intracellular mechanisms (abnormal autophagosome formation or function) or by mediating defective Paneth cell function. These defects most likely lead to accumulation of mucus-associated bacteria and persistence of intracellular bacteria within phagocytic (possibly epithelial) cells (fig. 1). Gulati et al. [10] demonstrated by fecal transplant to germ-free (GF) mice that host genotype (mouse strain) determines composition of intestinal bacteria, but that deletion of the NOD2 gene in mice did not alter luminal microbiota compared with wild-type mice [11]. The latter result is

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**Fig. 2.** IBD pathogenesis is governed by the interaction of genetic and environmental triggers and microbial and immunologic factors. Modified figure used with permission from R.B. Sartor, originally published in *Nature Clinical Gastroenterology & Hepatology*, 3:390–407, 2006.
in contrast with human studies that implicate NOD2 in the regulation of mucosally associated microbiota in the ileum of Crohn’s disease patients [12].

**Environmental Triggers**

Multiple environmental agents have been implicated in the triggering of IBD or experimental enterocolitis. These include infections, nonsteroidal anti-inflammatory drugs (NSAIDs), diet, smoking, stress, and antibiotics (fig. 2). Others have covered many of these topics in detail. Highly relevant to the concept of high-diversity microbial communities protecting the mucosa, antibiotic use in childhood is a risk factor for developing Crohn’s disease, but not UC [13]. This large epidemiologic study in Denmark showed a relative risk of 3.41 for developing Crohn’s disease following childhood antibiotic exposure, with both time and dose response. It is unclear whether this represents a causal relationship or use of antibiotics for IBD symptoms prior to diagnosis. As discussed by Gary Wu on this issue, diet can profoundly affect bacterial community structure, metabolism and gene expression [14, 15]. Dietary iron and saturated fat potentiate experimental enterocolitis and induce a proliferation of proinflammatory bacterial species [16, 17]. Our preliminary data show similar effects with dietary sucrose and fructose.

**Intestinal Microbiota**

Enteric bacteria provide a constant stimulus that drives innate and adaptive effector immune responses that cause tissue injury. There is abundant, mostly correlative, clinical evidence that microbiota are involved in the pathogenesis of IBD, particularly Crohn’s disease and pouchitis (table 2) [2]. As mentioned earlier, bacterial concentrations are highest in the distal ileum and colon, and mu-

<table>
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<tr>
<th>Table 2. Clinical evidence that enteric bacteria, viruses or fungi induce Crohn’s disease</th>
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<td>Disease located in areas of highest bacterial populations</td>
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<tr>
<td>Increased mucosal association and translocation</td>
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<td>Abnormal composition commensals – dysbiosis</td>
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<td>Certain Crohn’s disease-associated genes alter gut microbiota and bacterial killing</td>
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<td>Infections can induce flares of IBD (C. difficile toxin, CMV)</td>
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<td>Fecal stream diversion prevents disease relapse; disease recurs upon restoration of fecal flow</td>
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<td>Manipulating bacterial populations treats certain subsets</td>
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<td>Microbe-specific serologic and T cell responses</td>
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Cosally-associated bacteria are dramatically increased in active IBD compared with the noninflamed intestine [18]. Frank et al. [19] were among the first to conclusively demonstrate that a subset of patients with Crohn’s disease and UC have abnormal mucosally associated bacterial community structures, with characteristic dysbiosis characterized by decreased *Clostridium* groups IV and XIVa, *Bacteroides* species and a compensatory proliferation of γ-proteobacteria. These abnormalities extend from the ileum to the rectum [20]. An important emerging concept in IBD pathogenesis is that of functional changes of the dysbiotic microbiome [5, 21]. Of note, many of the depleted species, including *F. prausnitzii*, efficiently produce SCFA [19]. As described earlier, SCFAs have important protective effects on mucosal barrier function and in immunosuppression. *F. prausnitzii* is of particular interest because low mucosal concentrations in resected ileal tissues predict postoperative Crohn’s disease recurrence, and this organism has immunoprotective functions in vitro and in several murine colitis models. An important subset of the γ-proteobacteria that proliferates in Crohn’s disease is *E. coli*. Functionally altered *E. coli* with a phenotype of increased epithelial adherence, epithelial invasion and persistence within macrophages, designated adherent/invasive *E. coli* are found in higher concentrations in the ileum of the subset of Crohn’s disease patients (approximately 30 vs. about 5% of controls) [22]. In addition, the majority of Crohn’s disease patients have serologic (antibody) reactivity to enteric bacterial or fungal species [23], and aggregate antimicrobial antibody concentrations have been reported to correlate with Crohn’s disease aggressiveness and complications [24].

Rodent models provide compelling evidence of a primary role of resident enteric bacteria in the pathogenesis of chronic, immune-mediated intestinal inflammation [2]. We and others have demonstrated that in the absence of intestinal bacteria, GF mice on multiple genetically susceptible backgrounds such as IL-10-deficient mice have no immune activation and no clinical, visible or histologic evidence of colitis [2, 25]. However, GF IL-10–/– mice colonized with specific pathogen-free enteric bacteria develop bacterial antigen-specific TH1 and TH17 cell activation and grossly intact colitis within one week of colonization. Exceptions to this rule are SAMP1/Yit mice, which have attenuated ileitis in bacteria-free conditions, and dextran sodium sulfate-treated mice, which exhibit potentiated colitis in GF conditions [26]. Gnotobiotic rodent studies indicate differential ability of various intestinal residential bacterial species to induce colitis. For example, HLA B27 transgenic mice have no colitis when GF, aggressive bacterial antigen-mediated colitis when colonized with complex resident intestinal bacteria, moderate colitis when monoassociated with *Bacteroides vulgatus*, but no inflammation when colonized with a resident *E. coli* strain and diminished colitis when *Lactobacillus rhamnosus* GG are mixed with complex resident microbiota.
These studies demonstrate that all resident bacteria are not equal – some are aggressive, some are neutral, and yet others are protective. Parallel studies demonstrate host-specific responses since opposite results are seen in IL-10−/− mice where B. vulgatus induces no inflammation, while the same E. coli strain induces cecal-dominant colitis and Enterococcus faecalis causes distal colitis [28]. These gnotobiotic IL-10−/− studies are examples of how different bacterial species can induce different phenotypes of disease in the same host [28, 29].

Recent work has demonstrated that different strains of E. coli have functional differences that impact disease pathogenesis and have identified some of the virulence genes that mediate these functional properties. Various E. coli strains have differential abilities to induce chronic, bacterial antigen-specific enterocolitis in monoassociated IL-10−/− mice [30, 31]. Induction of colitis correlates with the ability of E. coli strains to adhere to the mucosa, persist within macrophages and translocate across the mucosal barrier. Mechanisms of these pathogenic functions are being elucidated, with an example of a relevant virulence gene being the chitin-binding domain of chiA, which binds to N-glycosylated chitinase 3-like-1 on intestinal epithelial cells [32]. Multiple other bacterial species, including Enterococci and Bacteroides exhibit fundamental variations that affect protective versus pathogenic properties.

**Future Directions**

The relative balance of beneficial versus detrimental bacteria (table 3) strongly contributes to intestinal homeostasis versus inflammation, along with genetic and environmental factors. It is evident from the heterogeneous results in human disease and observations in animal models that this balance is unique in each individual and each person responds differently to various bacterial spe-
cies. Functional changes of enteric microbiota must be considered in parallel with compositional changes. Identification of functionally relevant bacterial genes, virulence factors and metabolic profiles represents important areas of future investigation to define protective and detrimental activities of resident bacteria. Very recent studies of enteric viruses and fungi promise to expand our understanding of the host/microbe interactions that mediate this chronic dysregulated immune activation that causes IBD (fig. 1).

This dysregulated balance of protective and proinflammatory resident intestinal microbial components offers an attractive target for novel approaches to more effectively and physiologically treat IBD, with an effort to restore a more homeostatic profile of bacteria, fungi and viruses. In parallel, intestinal microbiota offers considerable potential for diagnostic tests that can identify clinically relevant subsets of patients with more predictable responses to therapeutic manipulation and clinical outcomes. Current therapeutic approaches that use standard antibiotics and probiotics have not been as successful as one would anticipate, given the strong influence of resident bacteria in IBD pathogenesis. Applying customized approaches to correcting an individual’s unique bacterial profile and augmenting levels and function of endogenous, resident protective bacterial species rather than standard probiotics that do not persist in the human intestine are areas of high therapeutic investigative priorities (table 4). Incorporating these novel microbial approaches should revolutionize treatment of IBD in parallel with the dramatic advances in immunotherapy provided by biologic agents.

**Disclosure Statement**

Dr. Sartor is a member of the Advisory Board of the North American Probiotic Council, sponsored by Danone and Yakult. He has been on nutrition Advisory boards from General Mills, Abbott and GSK and Advisory panels for Salix, Vertex and Merck.
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