Microbial–Host Interactions in Inflammatory Bowel Diseases and Experimental Colitis

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Abstract

The inflammatory bowel diseases (IBD), Crohn’s disease (CD) and ulcerative colitis (UC), are immunologically mediated with genetic and environmental influences. Genetic factors include defective immunoregulation, mucosal integrity/repair and bacterial killing. Commensal bacteria activate pathogenic bacterial antigen-specific effector T cells that cause chronic inflammation in genetically susceptible hosts but induce protective immune responses in normal subjects. Both host and microbial specificities are important. Some bacterial species are aggressive, some are neutral and others protective, but each species has different effects in various hosts. Molecular techniques demonstrate contraction of certain bacterial populations in IBD, especially clostridial subsets, and expansion of others, including Enterobacteriaceae. The balance of beneficial and detrimental bacterial species determines homeostasis vs. inflammation; this balance can be manipulated by antibiotics, probiotics and prebiotics to treat and prevent relapses of IBD. Adherent/invasive Escherichia coli that adhere to and invade epithelial cells and resist killing by macrophages are increased in ileal CD. Hypothesis: Invasive, translocating, intracellular commensal bacteria induce Th1 and Th17 responses that cause CD in susceptible individuals with genetically determined innate immune defects. UC is caused by bacterial metabolic products that induce epithelial injury by blocking epithelial metabolism or overwhelming the genetically susceptible host’s ability to degrade reactive oxygen species.

Clinical Features of Inflammatory Bowel Diseases

Crohn’s disease and ulcerative colitis, collectively referred to as inflammatory bowel diseases (IBD), are chronic, idiopathic immune-mediated
disorders that affect an estimated 1.2–1.5 million patients in the United States [1]. Although ulcerative colitis and Crohn’s disease can occur in the same family and can have overlapping features, these disorders represent a spectrum of distinct diseases with different clinical immunologic and genetic characteristics. Ulcerative colitis is characterized by diffuse inflammation limited to the colonic mucosa that always affects the rectum but may involve all or part of the remaining colon. Its primary clinical symptoms include bloody mucoid diarrhea with abdominal cramping. In contrast, Crohn’s disease is characterized by segmental, granulomatous inflammation that involves the full thickness of the intestinal wall (transmural inflammation) and may affect any part of the gastrointestinal tract, although the distal ileum and colon are preferentially involved. Ulcerative colitis has an atypical Th2 immune profile while Crohn’s disease is characterized by Th1 and Th17 responses with increased production of interferon (IFN)-γ and IL-17 production. Although some genes are shared, for example the IL-23 receptor, the majority of genes are distinct among these disorders [1].

**Table 1. Etiologic hypothesis of Crohn’s disease**

- Chronic intestinal inflammation is due to overly aggressive T-cell responses to a subset of luminal bacteria
- Susceptibility is determined by genes encoding immune responses, mucosal barrier function or bacterial clearance
- Onset/reactivation is triggered by environmental stimuli that transiently break the mucosal barrier and initiate inflammation

**Pathogenesis of IBD**

Both ulcerative colitis and Crohn’s disease occur in areas with the highest concentrations of enteric microbiota, strongly suggesting that commensal bacteria contribute to the pathogenesis of these disorders [2]. We propose that Crohn’s disease is due to overly aggressive Th1/Th17 responses to a subset of luminal commensal bacteria with host susceptibility determined by genes that encode either immune response, mucosal barrier function or bacterial clearance. The onset and/or reactivation of inflammation are triggered by environmental stimuli that transiently break the mucosal barrier and initiate inflammation. This complex theory invokes the interaction of 4 components: genetic susceptibility; environmental triggers; microbial antigens, and adjuvants and the effector immune response (table 1). These factors must intersect since no single component is sufficient to cause chronic relapsing inflammation.

At least 40 genes have been implicated in Crohn’s disease and at least 8 have been implicated in ulcerative colitis [3, 4]. These genes can be broadly
defined as those that affect epithelial barrier function, immunoregulation or bacterial killing and/or processing. At least 4 genes associated with Crohn's disease regulate bacterial killing, which could result in defective containment of commensal bacteria (fig. 1). Polymorphisms in NOD2, which was the first gene associated with Crohn's disease, result in defective α-defensin production [5] and clearance of intracellular invasive bacteria [6]. ATG16L1, which is an integral component of the autophagy pathway, contributes to killing intracellular bacteria [7]. Another autophagy pathway gene, IRGM, mediates IFN-γ-induced killing of phagocytosed bacteria and NCF4 mediates NADPH
killing of phagocytosed bacteria [7]. Both NOD2 and ATG16L1 polymorphisms have been associated with Paneth cell dysfunction with defective secretion of antimicrobial peptides, including the α-defensins [5, 8]. Defective mucosal secretion of these antimicrobial peptides in Crohn’s disease may explain the observed dramatic increases in mucosally associated bacteria in active Crohn’s disease [9]. Polymorphisms in these bacterial killing genes and the similarities between clinical and pathologic features of Crohn’s disease and granulomatous inflammation resulting from primary defects in phagocyte function have led to the hypothesis that defective innate immune responses could activate pathogenic T-cell responses in Crohn’s disease [10]. This theory postulates that defective clearance of phagocytosed bacteria by macrophages or defective neutrophil chemotaxis and function result in persistent bacterial antigenic stimulation of compensatory effector Th1/Th17 T-cell responses that cause tissue damage in Crohn’s disease.

Environmental triggers are important modifying factors of genetic susceptibility, explaining the 50% concordance of Crohn’s disease in identical twins and 10% concordance in ulcerative colitis in homozygous twin pairs [11]. Environmental triggers can be broadly characterized as those that alter the gut microflora, such as antibiotics and diet, or those that alter mucosal barrier function and/or immunoregulation, which include acute self-limited infections, nonsteroidal anti-inflammatory drugs, smoking and stress. Dietary influences could help explain the dramatic increase in the incidence of IBD in Western countries from 1950 to 1980 and the more recent increased incidence of ulcerative colitis and Crohn’s disease in Asian countries in the last 2 decades as these countries adopt Western diets. We have observed that dietary sucrose, iron and aluminum potentiates colitis in IL-10 knockout mice and that nonabsorbed oligosaccharides (prebiotics) can attenuate experimental colitis in HLA B27 transgenic rats [12–14]. We hypothesize that dietary components affect enteric bacterial composition, metabolic activity and mucosal immune function, which then induce chronic intestinal inflammation. We postulate that dietary sucrose, fructose and iron preferentially stimulate growth of detrimental enteric microbiota, while poorly absorbed oligosaccharides foster the growth and metabolic activity of beneficial bacteria to increase production of protective short chain fatty acids such as butyrate. In parallel, dietary aluminum, iron and animal fat can stimulate pathogenic mucosal immune responses.

**Microbial Influences in Chronic Intestinal Inflammation**

Crohn’s disease and ulcerative colitis preferentially occur in areas with the highest concentrations of predominantly anaerobic bacteria in the distal ileum and colon (fig. 2). Serologic responses to microbial antigens are present in 80% of Crohn’s disease patients and high titers of antibacterial
serologies are associated with severe disease [15]. Flagellin from a commensal clostridial species is a dominant antigen in experimental colitis in the C3H/HeJ Bir mouse model as well as in human Crohn’s disease, where approximately 50% of patients have serologic responses to this specific flagellin [16]. Furthermore, flagellin-specific CD4 T-cell lines can transfer colitis to immunodeficient recipient mice. Commensal bacterial components can activate both innate and adaptive immune responses, with induction of both regulatory (protective) and effector immune cells. Innate immune responses are stimulated by Toll-like receptors, which are membrane-bound receptors for a wide variety of bacterial and viral products. Ligation of these receptors stimulates signaling through adaptor molecules and kinases in the NF-κB and mitogen-activated protein kinase pathways.

We and others have demonstrated that commensal enteric bacteria are required for chronic experimental intestinal inflammation in a variety of murine, rat, guinea pig and nonhuman primate models [2]. In the absence of bacteria, germ-free (sterile) genetically susceptible hosts exhibit no immune activation and no colitis, while colonization of these germ-free susceptible mouse or rat strains leads to immediate activation of macrophage and effector
T-cell responses and the onset of colitis within 1–4 weeks [17, 18]. Selective colonization of germ-free HLA B27 transgenic rats and IL-10 knockout mice demonstrate that all bacterial species are not equal. For example, in the HLA B27 transgenic rat model, selective colonization with *Bacteroides vulgatus* induces moderate colitis while monoassociation with *E. coli* causes no disease [19]. Feeding the probiotic species *Lactobacillus GG* to transgenic rats colonized with complex cecal bacteria causes protection [20]. Thus in a single model some bacterial species are detrimental, some are neutral, and some are protective. Host specificity is a key feature of these studies. *B. vulgatus* induces colitis in monoassociated HLA B27 transgenic rats but *Escherichia coli* and *Enterococcus faecalis* cause no disease [21]. The opposite result is seen in selectively colonized IL-10-deficient mice where *B. vulgatus* causes no disease but *E. coli* and *E. faecalis* induce chronic colitis with Th1 and Th17 bacterial antigen-specific responses. In a third model, the bone marrow transplanted CD3ε transgenic mouse, no disease occurs with any of these 3 species, but depend on complex enteric bacteria. Finally, different bacterial species can induce different phenotypes of colitis in monoassociated IL-10 knockout mice. In this model, IL-10-deficient mice raised in a germ-free, sterile environmental have no colitis and IL-10−/− mice colonized with commensal bacteria have rapid onset of pancolitis. IL-10 knockout mice colonized with *E. coli* have onset of mild to moderate right-sided colitis within 3 weeks, while those colonized with *E. faecalis* have a slower onset of eventually aggressive distal colitis and duodenal inflammation 10–12 weeks after colonization [22]. The combination of *E. coli* and *E. faecalis*, however, causes aggressive pancolitis onset at 1 week [21, 23].

A continuing controversy in Crohn’s disease is whether a pathogen is responsible for the chronic relapsing nature of the inflammatory response. *Mycobacterium avium* subspecies *paratuberculosis* has been studied for decades due to its similar clinical features to Crohn’s disease [2]. This obligate intracellular pathogen is the cause of Johne’s disease, a granulomatous enterocolitis in ruminants with wasting and diarrhea in adolescent and young cattle. This infection is common in dairy herds in the United States and can be transmitted by milk. Recovery by either culture or PCR is increased in Crohn’s disease in most studies compared with ulcerative colitis and controls. However, inconsistent culture and PCR results, clinical improvement rather than worsening of the inflammatory process with corticosteroids and anti-TNF agents that reproducibly flare traditional mycobacterial infections and lack of long-term responses to triple antibiotic therapies directed toward mycobacteria [24] make it unlikely that this agent causes disease in the majority of Crohn’s disease patients.

Considerable recent evidence implicates functionally abnormal *E. coli* strains in the pathogenesis of ileal Crohn’s disease [2]. Adherent/invasive *E. coli* (AIEC) adhere to and invade epithelial cells and persist within intestinal epithelial cells and macrophages [25]. The virulence factors and molecu-
lar mechanisms that promote epithelial adherence, invasion and persistence are being elucidated by elegant in vitro studies. The initial observations by Darfeuille-Michaud et al. [25] that AIEC are selectively enriched in mucosally associated bacteria isolated from ileal Crohn’s disease samples have been confirmed by Baumgart et al. [26]. In the latter studies fluorescent in situ hybridization analysis demonstrated mucosally adherent \textit{E. coli} and uptake of these organisms by lamina propria macrophage-like cells. We have demonstrated that different \textit{E. coli} strains induce variable degrees of experimental colitis in monoassociated IL-10 knockout mice [27]. In these studies, a randomly isolated murine fecal \textit{E. coli} strain designated NC101 induced moderately severe colitis in monoassociated IL-10 knockout mice but no disease in wild-type controls, while the prototypic human ileal Crohn’s disease AIEC isolate LF82 induced moderate colitis. However, a laboratory \textit{E. coli} strain K12 caused no inflammation. The colitogenic strains induce bacterial antigen-specific Th1 and Th17 responses while K12 elicited similar responses in the CD4+ T cells isolated from the mesenteric lymph nodes of NC101 monoassociated mice with active colitis. These studies indicate that there are no antigenic differences in the 3 strains and no differences in the ability of each strain to colonize the colon of monoassociated mice, suggesting that functional differences must be important. We then demonstrated striking similarities between the phylotype (B2) and expression of virulence factors that promote epithelial attachment, invasion and resistance to killing in the murine and Crohn’s disease isolates. The non-colitogenic K12 (MG1655) is phylotype A and expresses none of the virulence factors that the murine and Crohn’s disease AIEC strains have in common with uropathic and avian pulmonary pathogenic \textit{E. coli} strains.

Our in vitro functional assays show that NC101 \textit{E. coli} persist within macrophages for up to 72 h, in contrast with the non-colitogenic K12 \textit{E. coli} strain that is rapidly killed by macrophages with very low persistence at 24 h or longer intervals [28]. Likewise, NC101 and LF82 strains that cause colitis showed increased translocation across T84 colonic epithelial monolayers compared with low translocation rates of K12. Functional abnormalities of NC101 and LF82 relative to K12 include prolonged production of IL-12/23 p40 and TNF in macrophages engulfing these \textit{E. coli} strains, NC101 resistance to phagocytosis that is partially mediated by the heat shock/chaperone protein \textit{ibpB} and increased motility in a soft agar in vitro assay.

We hypothesize that persistent exposure to mucosally associated and intracellular commensal enteric bacteria leads to aggressive antibacterial Th1- and Th17-mediated immune responses that result in chronic intestinal inflammation [2]. We propose 2 mechanisms of enhanced bacterial exposure: (1) colonization with commensal bacteria expressing virulence factors that promote luminal growth in an inflammatory milieu, intestinal epithelial cell adherence, invasion and resistance to intracellular killing, and (2) genetically determined host innate defects in bacterial killing and mucosal barrier function. We suggest that susceptible individuals with genetic innate immune defects develop
Crohn’s disease when colonized with *E. coli* or other bacterial strains that possess these virulence factors. Thus, both mechanisms might be relevant to Crohn’s disease with inflammation caused by opportunistic pathogenic *E. coli* strains that are capable of inducing disease in a genetically predisposed host with defects in innate immune function but no disease in a normal host.

**Balance of Beneficial and Detrimental Gut Microbiota**

Animal model studies demonstrate that some bacterial strains are detrimental while others have protective effects, and many are neither aggressive nor beneficial [2]. An active area of investigation in IBD examines the possibility of dysbiosis, which is abnormal microbial composition or function, as a cause of chronic intestinal inflammation. Dysbiosis can induce intestinal inflammation by several mechanisms (fig. 3). Defective production of butyrate and other short chain fatty acids could profoundly affect colonic epithelial function and mucosal barrier characteristics. The relative balance of beneficial and detrimental bacteria as outlined in table 2 may be key to induction of inflammation versus mucosal homeostasis. Recent studies demonstrate that mucosal concentrations of *Clostridium* groups XIVa and IV are decreased in IBD, with selective decreases in *Clostridium leptum* group and *Faecalibacterium prausnitzii* in Crohn’s disease [29, 30]. Sokol et al.
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Table 2. Intestinal inflammation vs. homeostasis depends on the relative balance of beneficial vs. detrimental bacteria

<table>
<thead>
<tr>
<th>Proinflammatory</th>
<th>Protective</th>
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<tbody>
<tr>
<td><em>Bacteroides vulgatus</em>, <em>Bacteroides thetaiotaomicron</em></td>
<td><em>Lactobacillus</em> sp.</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em></td>
<td><em>Bifidobacterium</em> sp.</td>
</tr>
<tr>
<td><em>E. coli</em> – enteroadherent/invasive</td>
<td>Non-pathogenic <em>E. coli</em></td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td><em>Saccharomyces boulardii</em></td>
</tr>
<tr>
<td><em>Bifidobacterium animalis</em></td>
<td><em>Bacteroides thetaiotaomicron</em></td>
</tr>
<tr>
<td><em>Fusobacterium varium</em></td>
<td><em>Faecalibacterium prausnitzii</em></td>
</tr>
<tr>
<td>Intestinal <em>Helicobacter</em> sp</td>
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[31] demonstrated that decreased mucosal concentrations of *F. prausnitzii* at the time of ileal resection predicted the risk of postoperative recurrence of Crohn's disease. Furthermore, secreted products of *F. prausnitzii* have immunomodulatory activity (an increased IL-10/TNF ratio) in vitro and the oral or intraperitoneal administration of this organism or its supernatant decreased the severity of acute experimental colitis. These innovative studies suggest that mucosal concentrations of *F. prausnitzii* could predict postoperative recurrence and the administration of this species or its immunosuppressive product could decrease the risk of relapse.

The ability of enteric microbiota to produce toxic metabolites may be relevant to the pathogenesis of ulcerative colitis (table 3; fig. 3). Anaerobic bacteria can produce nitric oxide and hydrogen sulfide from dietary nitrate and sulfate, respectively [32]. Both of these substances can block β-oxidation of butyrate and interfere with lipid and protein synthesis by colonic epithelial cells, which could induce mucosal injury and permeability. This mucosal injury could then lead to bacterial translocation and activation of lamina propria immune cells, particularly atypical in natural killer (NK) T cells that produce IL-13, which has been postulated to mediate inflammation in ulcerative colitis patients [33]. These metabolic abnormalities in concert with primary genetic defects in epithelial barrier function or healing and loss of tolerance to commensal bacteria or self antigens could lead to ulcerative colitis (table 3). Intestinal bacteria can also produce reactive oxygen species that can cause tissue injury, particularly in a host with defective degradation of oxygen metabolites. Finally, abnormal endosomal stress responses caused by polymorphisms in the XBP1 gene could induce epithelial injury and have been linked to ulcerative colitis [34]. Furthermore, experimental colitis occurs in XBP1-deficient mice [34] The final common pathway of ulcerative colitis may be enhanced mucosal permeability, uptake of luminal antigens and activation of overly aggressive atypical Th2 responses by NK T cells to commensal bacterial or fungal lipoprotein with subsequent exaggerated production of IL-13.
Conclusions and Therapeutic Implications

Both Crohn’s disease and ulcerative colitis appear to be complex interactions between host, microbial and environmental factors that lead to inappropriately aggressive antibacterial T-cell effector responses that promote chronic relapsing tissue injury. Bacterial antigens and metabolic products are key promoters of the chronic inflammation and barrier dysfunction in both disorders. Therefore, therapeutic manipulation of intestinal bacteria by selectively altering beneficial versus detrimental species by antibiotics, probiotics and/or prebiotics could reverse the inflammatory response and restore mucosal homeostasis (table 2). Although probiotic and prebiotic administrations offer great promise in treating and preventing ulcerative colitis and Crohn’s disease, current evidence of efficacy is limited by inconsistent and underpowered studies. Results of probiotics in IBD can be summarized as follows: certain probiotics, particularly *E. coli Nissle* and VSL3, may be effective in decreasing relapse of quiescent ulcerative colitis. VSL3, a combination of 8 individual probiotic species, can prevent relapse and perhaps the onset of pouchitis after colectomy. To date, results in Crohn’s disease have been disappointing. It is likely that individualizing treatment with various probiotic species and strains based on profiles of microbiota composition and metabolic activity will optimize results. Large prospective studies in carefully defined patient subsets will be necessary to achieve the potential of these cost-effective and nontoxic approaches to treating IBD patients.

References

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Sartor


Discussion

Dr. Cerf-Bensussan: How do you see dysbiosis; as a secondary or primary phenomena in Crohn's disease or something which is actually some kind of vicious cycle?

Dr. Sartor: That is a critical question. I think it could be primary, although much of what we see is probably secondary. It could be primary because there are probably some host genetically determined signals that govern which bacteria can attach to the mucosa. Older studies in identical versus non-identical twins showed differences in bacterial composition with closer composition in the identical twins. So there can be a host genetic determination of the bacterial population. I think, however, that diet, environmental factors, all the things we talked about yesterday, can profoundly influence gut bacteria. Even inflammation can affect bacteria. We have done two sets of studies that I will briefly mention. In one we took specific pathogen-free bacteria from a normal 129 wild-type mouse, both fecal and cecal, we divided them into two aliquots and identically colonized germ-free IL-10 knockout versus wild-type mice. We did this twice with two different molecular microbiologists and clearly showed some bacterial species increased in the presence of inflammation and some decreased. Those that increased were Enterobacteriaceae, Escherichia coli, Klebsiella and, surprisingly, Bifidobacterium animalis, which was quite surprising. We showed that if you monoassociate IL-10 knockout mice with that particular species you could get mild inflammation [1, 2]. In addition, we have shown that inflammation per se influences bacterial gene expression. We did E. coli microarray with our murine E. coli that causes colitis in the IL-10 knockout but not in the wild-type. The gene array of E. coli in the wild-type versus the knockout mouse with colitis showed a large number of genes differentially regulated by the inflammatory response. If you give IFN-γ, TNF and stress the oxygen situation you can actually see in vitro, some bacterial genes, such as IbpB, are stimulated [3].

Dr. Cerf-Bensussan: This means that you are using inflammation to select genes which have the capacity to express proteins that will protect them against host
inflammation, and get rid of competitors and perhaps beneficial bacteria which may not be equipped with these genes?

Dr. Sartor: Yes, that's one possibility. There are all kinds of things going on in inflamed gut: there is increased permeability from the host to the lumen; secretion of oxygenated blood products; iron is increased with red blood cells, all of which influence metabolism. Secondary phenomena can happen as well. Crohn's patients, even without histologic inflammation, have increased expression of CCAM6 which is the binding factor for enteroadherent \textit{E. coli} \cite{4}. These investigators postulated that there was an intrinsic defect in Crohn's patients that would lead to increased attachment and this is even further upregulated with TNF and inflammation.

Dr. Cerf-Bensussan: Regarding your very interesting data showing that, depending on the germ used to colonize the mice, the form of colitis, right or left colitis, do you mean that these bacteria may have some way to adhere?

Dr. Sartor: That is our hypothesis, and we are now using FISH to look at mucosal adherence in the very early pre-inflammatory phase, looking at the first 3 days for example. We have some preliminary evidence that our \textit{E. coli} preferentially attach to the right colon versus the distal colon, but we need to replicate these findings. There is no difference in luminal concentrations, so \textit{E. coli} grows equally on the left and right, but I do think there is differential mucosal attachment. The other possibility is that there are regional responses in epithelial cell differentiation of the epithelial cells. The left and right colon are unlikely, and differential T-cell responses or innate responses from the macrophage dendritic cell population are, I think, unlikely.

Dr. Björkstén: I have two questions for you. One regarding short-chain fatty acid changes in ulcerative colitis and Crohn's disease because I am interested from an ecological point of view. You mentioned butyrate as fodder for the cells but what about the other short-chain fatty acids? Are there changes for example in isocaproic acid and other markers suggesting an altered ecology of microbiota rather than a specific strain?

Dr. Sartor: This is not work that I have done personally. Most people have looked at butyrate. The reason for this is that, looking in vitro at the growth requirements of the distal colonocyte and epithelial colonic cell lines, butyrate contributes most to its metabolic use with propionate second and acetate the third. I am not aware of careful examination of all those fatty acids, only butyrate. Total short chain fatty acids tend to be decreased in Crohn's disease and osteocolitis. It's a murky area; there is no real definitive evidence.

Dr. Björkstén: If one was looking at the pattern of the fatty acids, it is conceivable at least that the changes would actually give you an ecologic balance rather than, as now, looking at single strains.

Dr. Sartor: Yes, work has been done in normals but not in inflammatory bowel diseases (IBD).

Dr. Björkstén: My second question is related to the clinical effects of various probiotics. You mentioned briefly some of the strains that have been used, but it seems to be fairly inconsistent and a meta-analysis by the Cochrane Institute was negative with the possible exception of pouchitis. Would you like to comment on this?

Dr. Sartor: I have written far too many reviews, and my conclusion in every single one is that there is vast potential in this area that has not been reached, primarily because good studies haven't been done with large numbers of patients. In the United States there is no burning reason for companies to do expensive clinical trials because these are food products, not drugs, which don't require large properly designed studies. It is not that they have been done and are negative, they just haven't been done. In Crohn's disease there is not much evidence. There was an unpublished study in
Canada showing that VSL3, a combination of 8 different probiotic species, was negative in the prevention of relapse after surgery at 6 months analysis.

Dr. Björkstén: So it could be a question of underpowered studies.

Dr. Sartor: Absolutely, the trouble is they didn't get much of a trend. The absence of evidence isn't the same as a negative report.

Dr. Belli: Enteral nutrition can induce and maintain remission in Crohn's disease. However, the results on changes in microflora on enteral nutrition are conflicting in the literature. Can you comment on this and especially on the difference that can be seen between polymeric and elemental diets in terms of modification of microbiota?

Dr. Sartor: There are people in the audience who probably know more about this than I do. But you are absolutely correct that enteral feeding with either polymeric or elemental diets is bowel rest with surgical bypass, or total parenteral nutrition and bowel rest are effective in Crohn's disease. They are not so effective in ulcerative colitis. In our experience it is more effective in small intestinal disease than colonic disease. With refeeding the inflammation tends to recur usually fairly promptly, so it's not a long-lasting effect. Is there an effect on bacteria; I just don't know. The studies really haven't been very compelling. Again we need better before and after studies in these types of patients. Molecular analysis is clearly the only way to go. The older studies were culture-driven and you only see the tip of the iceberg with cultures. I would really like to see molecular analysis, and I haven't seen that well done. One diet therapies working by bacterial compositional changes, bacterial functional changes, the absence of some detrimental dietary effect, an antigen, a barrier breaker? Carageenan for example, causes colitis, and this red seaweed extract used as an agent to keep ice cream and many other products together causes colitis in guinea pigs. Are there some detrimental elements in the diet or conversely is there some protective benefit from the elemental diet?

Dr. Tobin: I think you are absolutely right that it is the molecular analysis of enteral nutrition that we really need very badly right now. There is some suggestion that we might get some investment in this because the great enthusiasm from the pharmaceutical companies for the newer monoclonal agents has now been tempered by some of the long-term studies (2 years) which are not very exciting. If I had Crohn's disease, I would not be happy to think that I had only a 25% chance of long-term remission. So clearly that's only one element of the equation and we really do need to see the mechanisms in enteral nutrition because that is really the issue that has held it up the most, apart from the different issues about whether you should use it for remission induction or for maintenance, and whether a smaller amount could maintain remission for patients.

Dr. Sartor: Very importantly, can dietary modifications be used in the genetically susceptible population? If we could identify at birth those people who carry associated genes, might we alter the diet in a totally innocuous way and prevent the onset?

Dr. Tobin: The other issue is that changing the bacteria also changes the motility of the gut. When these children take 2 liters or so of enteral nutrition, their motility changes very profoundly and that cannot be underestimated in terms of what it actually does to bacterial concentrations. If transit is sped up, is doubled, then the bacterial concentrations are halved. It is very interesting in these studies that it usually takes a couple of weeks for the children to go into remission, but they have very profound benefits at that time, and that's what is really good to see.

Dr. Sartor: Clearly we need to get better evidence of efficacy, what is the active ingredient, and what mechanism, because obviously anything can be improved.

Dr. Tobin: Following up on Dr. Björkstén's comment about the use of probiotics and of course the meta-analysis will always conclude these studies because we have
used different products. You wouldn’t really want to start with VSL3 if you have 8 different bacteria in genetically different people; it’s a recipe for a non-conclusion of your study.

**Dr. Isolauri:** Some time ago in allergic children we made the same observation as you on the distinct effects of different bifidobacteria strains. We first compared the detection of fecal bifidobacteria in allergic children and healthy children on the same diet, even on breast milk, and it was found to be different [5]. We also compared the immunomodulatory effects of specific bifidobacteria strains. These strains adhere to different parts of the intestine, some adhere better in the upper parts and some in lower parts, and that is why we should be very cautious when we evaluate gut microbiota from fecal samples [6–8].

**Dr. Sartor:** I totally agree with the idea that there is a regional population of bacteria and the best way of looking at what is happening in the epithelium and the lamina propria is most likely, not for sure, due to the things that are inherent and certainly those are the translocating. I forgot to mention that *Clostridia* and *Bifidobacteria* seem to be the populations that make most of the short-chain fatty acids and those are the ones that are decreased in IBD fairly reproducibly.

**Dr. Cerf-Bensussan:** You made a very interesting observation about the inducing role of aluminum, which reminded me of a talk I recently heard where it was mentioned that not related molecules like NALP are in fact receptors for ALUM. Does this mean that in our environment we may have some other factors, not bacteria but some pollens or metals, which could be seen by the innate system and NALP proteins?

**Dr. Sartor:** We chose aluminum based on report of granulomatous enterocolitis occurring in horses situated downstream from aluminum smelting plant, and aluminum was found in the granulomas in the gut. Alum, or aluminium hydroxide, induces a good vigorous vaccine response. If it is injected under the skin or if it is inhaled, granulomas occur. So it seemed like an interesting thing to look at in Crohn’s disease. We did show worsening inflammation in IL-10 knockout mice, the mechanism to be determined. Iron I think may be even more interesting. Aluminum is ubiquitous in the aluminum-canned drinks we use, as preservatives and almost any packaged item including vitamins, and that might account for the increase in the epidemiologic risk of IBD in the second half of the 20th century. RAMP-1 is a siderophore in the phagocyte that sequesters iron within the phagocyte and pulls it away from a replicating intracellular bacteria. In RAMP-1 it has been associated with mycobacterial susceptibility in TB as well as leprosy. It has recently been implicated in Crohn’s disease as one of the genetic polymorphisms. Again it could be a host–microbial interaction where there is continuous fight between the host and the bacteria for essential iron. So I think heavy metals are ripe for investigation.

**Dr. Conway:** My comment relates to the various populations in the different regions of the colon and your suggestion that it may be adhesion to the mucosa that’s important. Considering the number of organisms that are in the contents versus those that are on the wall of the colon, it is quite significantly larger, and the metabolism of that population can be very important, I wonder if diet can actually be a bigger contributing factor than adherent organisms? We know that prebiotics, if they are short chains, are very rapidly degraded and therefore will have an effect in the early part of the colon, than if they are very long chain and much more resistant to degradation. So you get very different population composition and metabolism because of the oligosaccharide composition of the diet.

**Dr. Sartor:** You are probably quite correct. I think that probably the luminal bacteria are quite important as far as antigenic stimulation and adjuvant stimulation are concerned. Obviously antigens have to come in contact with a dendritic cell, rather APC and T or B cell. Dr. Brandtzaeg showed this mucous layer; how impervious is the
mucous layer to bacterial antigens? We know that it sequesters antimicrobial peptides and secretory IgA and IgM to form a physical barrier that then either kills the bacteria or keeps it from attaching to the epithelial cell. How permeable is that mucous layer to aqueous antigens? I don't know. Horseradish peroxidase gets across, but how representative that is for the bacterial antigens, I really don't know. But your point is well taken, and clearly one of the presumed mechanisms of action of prebiotics is a substrate for a short-chain fatty acid production.

Dr. Brandtzaeg: Yesterday I showed how you can reach the point of no return with regard to a break in intestinal homeostasis. You mentioned that in experimental situations you can have a phenotype of inflammation reflecting the actual bacteria – the opportunistic strain which is doing the job. Then thinking about Crohn's disease, the preference of enteritis is still ileum. Could this inflammation be explained by NOD mutations in Paneth cells and a lack of defensin production? Then comes the real question: how can you explain the patchy initial lesions in Crohn's disease, all the way up to the oral cavity? It was sometimes thought that the initial lesion in Crohn's disease could be sitting on top of the lymphoid aggregates. That hasn't been really followed up.

Dr. Sartor: No, not to my knowledge. There are two features of IBD that disturb me: one is the patchy distribution, the segmental inflammation, the very distinct aphthous ulceration with histologically normal mucosa in between, and the second thing is the sharp demarcation in enterocolitis. What can explain this, I don't know. If I give indomethacin to rats, a reproducible aphthous-type ulcer, a discrete ulcer, occurs, and it is my impression a small number of sections tends to be over a lymphoid aggregate; Peyer's patch in the small intestine, lymphoid aggregate in the colon. That has been described in older histology works, but I am not sure it has been followed up. This initial focus could then spread; a mucosal barrier dysfunction is created and the bacteria translocate.

Dr. Brandtzaeg: We know that the M cells are extremely vulnerable to infections and distributed as you say.

Dr. Sartor: One thing that might perpetuate that segmental inflammation is the secondary vasculitis and even lymphangitis that occur. Many of the granulomas tend to occur in the lymphatics and maybe that's how bacteria are taken up. Then there is a relative ischemia or a relative stasis of blood flow in lymphatic edema in that area that might then alter barrier function.

Dr. S. Koletzko: Most of the animal models are based on colitis. If we look at humans and especially children with Crohn's, a very high proportion of children have upper GI involvement including the esophagus without a mucous layer. How does this fit with these theories, and do we have an animal model for upper GI involvement?

Dr. Sartor: Actually we do. IL-10 knockout mice colonized with Enterococcus faecalis get gastric and duodenal involvement, and duodenal strictures in the late stage, although the predominant region is in the colon. HLA-B27 transgenic rats have antral and duodenal involvement in SPF conditions but not with Bacteroides vulgaris. So there are some animal models but clearly the colon is the predominant area. Particularly in mice and rats, which are coprophagics, there is a large amount of bacteria coming downstream. Clearly the diet we eat has a lot of dietary antigens. We have demonstrated that dead bacteria can induce inflammation as well; they don't have to be viable. Then as Dr. Cerf-Bensussan mentioned, there is a secondary proliferation of bacteria once you break that barrier. So in indomethacin-induced injury in the mid small bowel of our Lewis rat population, you get a very impressive overgrowth of anaerobic bacteria; metronidazole treats the lesion. So I think that there can be primary and secondary effects here.
References
