Intestinal inflammation and the gut microflora

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OVER the past few years there has been a resurgence in interest in the putative involvement of bacteria in the pathogenesis and pathophysiology of chronic idiopathic gut disorders, such as inflammatory bowel disease (IBD) and irritable bowel syndrome. It is clear that the presence of the commensal microflora is critical for normal gut function (1-3). Thus, when considering a role for the enteric microflora in disease, it is important to draw a distinction between pathogenic bacteria that may sporadically gain access to the intestine and functional bowel disorders, and the value of antibiotic therapy to treat gut inflammatory disorders. A variety of experimental evidence from both laboratory model systems and clinical investigations is reviewed with respect to a pivotal role for enteric bacteria in gut inflammation. The voluminous scientific literature on this subject precludes any comprehensive synopsis of the area; instead, pertinent studies are cited to illustrate the ability of bacteria and their products to evoke or exacerbate gut inflammation.

Key Words: Gut inflammation; Inflammatory bowel disease; Microflora

A number of pieces of circumstantial evidence suggest a role for bacteria in enteric inflammation. These include the observations that IBD tends to occur in the region with the greatest bacterial load, that the histological appearance of ulcerative colitis is similar to that caused by Campylobacter, and that genetic predisposition, enhanced enteric permeability and immune dysregulation are also likely key elements in the IBD experienced by cohorts of patients. The following discussion does not discount the value of continued research aimed at defining the role of these other factors in the pathophysiology of enteric inflammatory disease.

ANIMAL MODELS OF GUT INFLAMMATION

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Inflammation intestinale et microflore entérique

RÉSUMÉ : L'idée que la microflore entérique puisse jouer un rôle dans la pathogenèse et la physiopathologie de la maladie inflammatoire de l'intestin (MII) n'est pas nouvelle. En effet, l'étiologie infectieuse possible des MII chroniques et particulièrement de la maladie de Crohn a été au centre de beaucoup de travaux de recherches approfondies. Au cours de l'année 1990, on a noté un intérêt renouvelé à l'endroit du lien entre bactéries et dysfonction intestinale et l'utilisation de l'antibiothérapie dans le traitement des maladies inflammatoires de l'intestin. Diverses expériences tìères de modèles élaborés en laboratoire et en recherche clinique sont passées en revue du point de vue du rôle central des bactéries entériques dans l'inflammation intestinale. L'abondance des documents scientifiques sur le sujet nous empêche de procéder à un synopsis complet du domaine. Nous citons plutôt les études pertinentes afin d'illustrer l'aptitude des bactéries et de leurs produits à susciter ou à exacerber l'inflammation intestinale.
enterocolitis (6) have corroborated the hypothesis that bacteria are elevated in patients with IBD (reviewed in 4,5).

Analyses of a variety of models of spontaneous colitis and enterocolitis (6) have corroborated the hypothesis that bacteria can potentiate and/or initiate enteric inflammation. Mice lacking interleukin (IL)-2 (7) or IL-10 (8), and severe combined immunodeficient mice administered splenic CD45RB+ CD4+ T cells (ie, naive T cells) (9) all spontaneously develop colitis. Despite the varied immune abnormalities, these models have some commonality. First, the pathology is T cell-dependent and is dominated by an IL-12 driven T helper 1 cell (Th1) response (10-14). Second, in all instances the colitis is either absent or significantly reduced in severity if the animals are maintained in a germ-free environment. These findings provide compelling evidence implicating enteric bacteria in the pathophysiology of gut inflammation. A recently reported novel murine model of colitis provided very similar data: up to 50% of mice lacking the multiple drug resistance gene, mdr1a, and housed in specific pathogen-free facilities were found to spontaneously develop colitis (15). The colitic mice had a fourfold increase in mucosal T cells, which were predominantly CD4+ cells, and the incidence of colitis was significantly reduced by antibiotic treatment (15).

Other model systems can also be cited in support of a role for bacteria in gut inflammation. In the dextran sodium sulphate model, colitis is associated with an increase in Enterobacteriaceae and Staphylococcus species in the gut (16,17). The C3H/HeJ/Ilr mouse develops a colitis that appears to be due to Th1 cells that are reactive to conventional antigens derived from the enteric microflora (18). Transgenic rats expressing the HLA-B27 antigen and human beta2-microglobulin display spontaneous colitis that does not develop in germ-free animals (19). Luminally derived Bacteroides species have been implicated as etiological agents in this model (20). Also, 2,4,6-trinitrobenzene sulphonic acid-induced colitis in rats is mild in the absence of gut flora, particularly anaerobic bacteria (21).

Collectively, these models suggest that perturbations in the immune system and reactions directed against commensal bacteria or their products can precipitate as enteric inflammation. In the dextran sulphate model, colitis is associated with an increase in Enterobacteriaceae and Staphylococcus species in the gut (16,17). The C3H/HeJ/Ilr mouse develops a colitis that appears to be due to Th1 cells that are reactive to conventional antigens derived from the enteric microflora (18). Transgenic rats expressing the HLA-B27 antigen and human beta2-microglobulin display spontaneous colitis that does not develop in germ-free animals (19). Luminally derived Bacteroides species have been implicated as etiological agents in this model (20). Also, 2,4,6-trinitrobenzene sulphonic acid-induced colitis in rats is mild in the absence of gut flora, particularly anaerobic bacteria (21).

The animal models discussed above approximate, to some degree at least, Crohn’s disease. The T cell receptor (TcR)-α chain knockout mouse develops a disease that is more like ulcerative colitis in its histopathology and is accompanied by increased IL-4 expression, a Th2 cytokine (26). Appendectomy can protect against the spontaneous colitis developed by the TcR-α knockout mouse, and data have been presented that indicate the interaction of commensal bacteria with the immune system (ie, appendix lymphoid tissue) in the induction of colitis (27).

All of the cited animal models of intestinal inflammation have another striking similarity with human IBD – in no instance has any specific pathogen been identified. Infectious agents, notably the measles virus (paramyxovirus) and M paratuberculosis, have been touted as causes of IBD (4). While infection with these organisms can be correlated with Crohn’s disease in some patients (28), this is certainly not a universal observation (29).

Despite the fact that considerable research efforts have failed to elucidate a cause for IBD (or even a consensus opinion on a putative infectious etiology of IBD), it is nevertheless premature to dismiss the possibility that IBD is due to a specific pathogen(s), particularly in light of the association between Helicobacter pylori and the pathogenesis of an array of gastric disorders (infection with Helicobacter hepaticus or Helicobacter bilis enhances disease in the CD45RB+ CD4+ T cell transfer model of colitis [30,31]; neither agent is suggested as the cause of human IBD) (Figure 1A).

Analyses of animal models of gut inflammation have consistently revealed an intimate association between the enteric microflora and inflammation, and this correlates, at least to some degree, with a variety of clinical observations. However, it would be remiss to overlook a small number of studies that suggest minimal or no involvement of microorganisms in the exacerbation of IBD (32). Because the identification of a specific etiological agent in human IBD has not been forthcoming, it is appropriate to consider more fully the role of bacterial products in the onset or exaggeration of inflammatory diseases in the gut.

**BACTERIAL PRODUCTS AS PROINFLAMMATORY STIMULI**

Numerous bacterial products are potent stimuli of immune activity. Thus, a bacterial component(s), rather than a specific pathogen, may elicit an intestinal inflammatory response that progresses to chronic disease. In this context, suberosal injection of streptococci cell wall-derived peptidoglycan-polysaccharides (PG-PS) results in a granulomatous inflammation in genetically susceptible rats (33); PG-PS have been detected immunocytochemically in the bowel wall of some patients with Crohn’s disease (34), and anti-PG-PS antibodies can be elevated in the serum of some patients with Crohn’s disease (35). Moreover, lipopolysaccharide and the tripeptide formyl-met-leu-phe (fMLP) are ubiquitous bacterial products, and both have been implicated in enteric inflammation (36). For example, neutrophils isolated from patients with Crohn’s disease have...
increased receptor expression for fMLP and are more responsive to fMLP (37). Also, monocytes recruited to the inflamed intestine have enhanced expression of the lipopolysaccharide-receptor, CD14 (38), and monocytes isolated from patients with Crohn’s disease release increased amounts of toxic oxygen metabolites when stimulated (39). In the latter scenario, lipopolysaccharide was implicated as the factor that primed the monocytes for enhanced reactivity. These examples indicate that bacterial products can contribute to the inflammatory process; however, it is unclear whether in the absence of other confounding factors (e.g., a leakier gut to facilitate excessive entry to the mucosa) these products can initiate disease.

A group of small bacterial peptides have recently been characterized and designated as superantigens by virtue of their ability to stimulate up to 25% of T cells (40,41). Superantigens are synthesized by a variety of species of bacteria (and other pathogens) (42), and activate T cells by cross-linking an outside domain of the beta-chain of the TcR with the major histocompatibility class II (MHC II) molecule on antigen-presenting cells. This results in a polyclonal expansion of T cells that have a common TcR-beta chain but different antigen specificity. Juxtaposing these data with the pivotal role that T cells play in the induction and regulation of inflammation, bacterial superantigens must be viewed as potential proinflammatory stimuli. Skewing in the expression of the TcR-beta chain implicates bacterial superantigens of unknown identity in the pathophysiology of inflammatory and autoimmune disorders such as rheumatoid arthritis, multisystem vasculitis (Kawasaki disease) and diabetes (43-46).

Recently, a number of investigations have shown T cell clonal expansion in cohorts of patients with Crohn’s disease, suggesting prior exposure to a superantigen(s) (47-49) and the possibility that superantigens may be involved in IBD (50). Enteric effects of the prototypic bacterial superantigen, Staphylococcus aureus enterotoxin B (SEB), were documented in the 1960s when administration of crude S aureus extracts or crude SEB preparations were administered to dogs or Rhesus monkeys (51,52). This resulted in diarrhea and various degrees of intestinal histopathology including disruption of the normal gut architecture, mucus production, epithelial degeneration and a mononuclear cell infiltrate (53). Subsequently, Lionetti et al (54) showed that in vitro application of SEB to human fetal intestinal explants caused significant tissue damage that was correlated with IL-2 and interferon-gamma production, and was prevented by treatment with the immunosuppressive agent tacrolimus (FK 506) (54). It has been shown that T cell activation by SEB (and an unrelated bacterial superantigen, Yersinia pseudotuberculosis mitogen [55]) results in decreased barrier function and diminished ion transport in monolayers of the human colonic T84 epithelial cell line in vitro (56). Interferon-gamma and tumour necrosis factor-alpha were implicated in the epithelial dysfunction, which was partially ameliorated by addition of transforming growth factor-beta or IL-10 to the coculture well (57). These findings implicate Th1-
dominated events in this reductionist model of T cell-driven alterations in epithelial permeability and ion transport function. These data have been complemented by in vivo studies showing that low dose (5 to 100 µg) SEB evokes a murine enteropathy that is characterized by subtle changes in jejunal histopathology, a CD93 T cell infiltrate and reduced responsiveness to proinflammatory stimuli (58,59). Diarrhea was evident in the superantigen-treated mice, and the enteric histopathology and functional abnormalities had resolved by 48 h after treatment.

In summary, a variety of known bacterial products (and others that await characterization [60]) have the ability to elicit acute inflammatory responses and impair normal gut function. Whether these agents initiate disease, convert subclinical conditions to conspicuous disease symptomatology, exacerbate existing disease or evoke relapses in disease activity remains to be determined. The assessment of the impact of proinflammatory bacterial products in laboratory models of spontaneous or induced colitis may provide some insights into these issues (Figure 1B). Additionally, it is essential that the normal and aberrant immunoregulatory pathways evoked in response to agents such as lipopolysaccharide, fMLP and superantigens be precisely defined so that effective therapies can be devised and applied to conditions in which these bacterial products are implicated.

DIRECT BACTERIAL EFFECTS ON THE INTESTINAL EPITHELIUM

Elucidation of the cooperative activity of diverse cell types in homeostatic processes has revealed that nonimmune cells have the capacity to modulate immune responses. The epithelial cell stands as sentinel at the boundary of mucosal surfaces, and abundant evidence shows that this cell is not merely a passive player in innate immunity, but that it can respond specifically to mediators produced in the mucosa (61) and synthesize a plethora of messenger molecules (62). (Other stromal cells such as fibroblasts and muscle cells can also be activated by immune mediators and also have the potential to influence the individual’s response to antigen and/or infection [63,64].)

The immunostimulatory or proinflammatory effects of bacterial products and how these impinge on gut function in general, and epithelial function in particular, are well known (65). Moreover, it is increasingly apparent that the gastrointestinal epithelium can respond directly to bacterial attachment and contact with bacterial toxins. Thus, infection with a variety of pathogens, including enteroinvasive bacteria (eg, Escherichia coli, Salmonella species, Yersinia enterocolitica) elicits the production of chemokines and proinflammatory cytokines from cultured human gastrointestinal epithelial cell lines (66-68). Similarly, Salmonella dublin infection can result in epithelial nitric oxide production via mobilization of nuclear factor KB and tyrosine kinase activity (69). In addition, bacterial infection can elicit increased transepithelial neutrophil migration (70) and increased expression of adhesion molecules (eg, intracellular adhesion molecule 1) (71), and a gastric epithelial cell line when infected with H pylori has been shown to express the immune costimulatory molecules B7-1 and B7-2 (72). Furthermore, infection with noninvasive enteropathogenic E coli results in an epithelial signal transduction cascade and a rearrangement of the enterocytic cytoskeleton to form an actin-rich area underneath the site of bacterial attachment (73). Functional correlates of this are increased paracellular permeability and deranged epithelial electrolyte transport (74-76). Both effects can affect directed water movement, and 'loosening' of the paracellular permeation pathway may evoke inflammatory responses via enhanced uptake of luminal material that has bypassed normal epithelial processing. The increased paracellular permeability is not due to epithelial cell death, but rather to focal dilations in the perijunctional ring of filamentous actin and the tight junction-associated protein, zonula occludens-1 (74).

In examining epithelial cell interactions with bacteria, an intriguing observation has been reported where the bacterium carries its own receptor to facilitate epithelial attachment. The enteropathic E coli bacterium binds to the surface of epithelial cells via a tyrosine-phosphorylated protein designated Hp90. This sets in progress a cascade of events that leads to the development of a pedestal-structure, the attaching and effacing lesion and ultimately to increased epithelial permeability (74). It now seems that the Hp90 expressed in the host epithelial cell is of bacterial origin (77). If similar scenarios are applicable to other species of bacteria, this will have a radical effect on how epithelial cell interactions with bacterial pathogens are viewed.

Bacterial toxins (and certain other products) can evoke a spectrum of signal transduction events in epithelial cells. For instance, Clostridium difficile toxins A and B, Vibrio cholerae zonula occludens toxin and Bacteroides fragilis enterotoxin can increase the permeability of confluent epithelial monolayers in vitro and/or elicit the production of proinflammatory chemokines (78-82). Phospholipase C from Clostridium perfringens can modulate the arachidonic acid cascade in intestinal epithelial cells, leading to increased production of platelet-activating factor (83). When epithelial MHC II expression is increased, as is typical during active inflammation, the enterocyte is capable of presenting bacterial superantigens to T cells, leading to activation as characterized by increased proliferation and cytokine production (84).

The above data are from in vitro studies that allow direct assessment of the effect of the bacteria/bacterial products on the enterocyte in the absence of other cell types. Caution must be used when extrapolating in vitro results to in vivo results (85); however, in situ studies have clearly shown epithelial activation (ie, production of messenger molecules and increased expression of accessory molecules) during inflammation. Consequently, the enterocyte is identified as an active component in immune responses, responding to bacterial pathogens (invasive and noninvasive) and bacterial products, and with the potential to elicit an inflammatory response.

Recognition of the immune function of the epithelium, in conjunction with its ideal location for oral drug delivery,
highlights the enterocyte as an attractive target for anti-inflammatory therapies. Extrinsic modulation of the gut epithelium’s response to bacteria requires precise knowledge of the signalling cascades that occur in response to infection, and strategic points therein that can be modulated by pharmacologic interventions. For example, both protein kinase C and myosin light chain kinase are important in the loss of epithelial barrier function following exposure to the verotoxin-producing E. coli strain O157:H7 (86). Indeed, infection with this organism can be confused with Crohn’s disease (87). Additionally, bacterial infection or immune mediators evoked by bacterial products have been found to activate epithelial nuclear factor κB (88); increase cytosolic calcium, inositol-triphosphate and phosphatidylserine protein levels (89); activate mitogen-activated protein kinase pathways (90); and mobilize signal transducers and activators of transcription proteins (91, 92) in in vitro model systems. If epithelial cell function is important in the regulation of enteric inflammatory diseases, and considerable data are available to support this hypothesis (93), then knowledge of the signalling pathways evoked in response to bacteria or bacterial products will be critical to understanding the pathophysiological mechanism(s) of these disorders (Figure 1C).

As a final example of the intricate interplay that can occur among the epithelium, inflammation, cytokine signalling and bacteria, it has been shown that transgenic mice that overexpress IL-7 develop colitis (94). Also, IL-7 is an important mediator in the microflora-dependent colitis that develops in animals that lack functional T and B cells (ie, RAG-2 knockout mice) (95). Moreover, in vitro studies show not only that epithelial cells are a source of IL-7 (that can modulate mucosal T cell responses) (96), but also that epithelial IL-7 receptor expression can be upregulated following bacterial invasion (97).

**CLINICAL PERSPECTIVE**

Given the long-standing postulate that Crohn’s disease is caused by an infectious agent, it is not surprising that antibiotics have a similar history as that of anti-inflammatory therapy (98, 99). Much interest has been and continues to be shown in metronidazole (Flagyl, Rhône-Poulenc Rorer, Ville St Laurent, Quebec) (100) as a treatment for Crohn’s disease. The effectiveness of other antibiotics such as ciprofloxacin [Cipro, Bayer Inc, Etobicoke, Ontario] and clarithromycin [Biaxin, Abbott Laboratories, St Laurent, Quebec] are being explored.

Currently, the merits of antibiotic therapy in IBD is a highly controversial issue with advocates both for and against antibiotic use as first line therapy in treating Crohn’s disease (100). This debate is typified by a pair of recent commentaries where the authors reviewed virtually identical clinical data and drew diametrically opposed conclusions. One assessment indicated that in the light of current treatments, “the use of antibiotics as primary therapy should strongly be considered” (101). Contrarily, Dr Feagan’s (102) interpretation of the same data (considering study design, statistical analysis and change in Crohn’s disease activity index) led to the conclusion that while “we should not abandon the concept of targeting drug therapy towards the enteric flora...[the current] data suggest that antibiotics have little efficacy” in the treatment of Crohn’s disease. Bearing in mind the risk of evolving bacterial antibiotic resistance and the chance of antibiotic-associated diarrhea caused either by disruption of the natural gut flora (103) or possibly by direct effects on the epithelium (104), antibiotics do appear to be effective, at least in alleviating disease symptomatology in a subpopulation of sufferers of Crohn’s disease. However, the available data are insufficient to allow firm conclusions about the global effectiveness of antibiotic treatment in Crohn’s disease. It is generally acknowledged that larger randomized controlled trials are required to resolve this controversy. Furthermore, the value of antibiotic therapy needs to be considered in terms of the clinical goal—that is, are antibiotics a cure for gut inflammation, a treatment for specific aspects or complications associated with active disease (eg, perineal disease [105]), a method to alleviate disease symptomatology or a maintenance therapy to reduce the recurrence of disease relapse?

In modulating gut flora to benefit the patient, the use of probiotics (natural flora with low or no pathogenicity) is an additional therapeutic strategy to combat food allergy (106), various bacterial infections (107) and gut inflammation (108, 109). Preliminary data have been presented showing that *Lactobacillus* species can significantly reduce the spontaneous colitis that develops in IL-10-deficient mice (110). Probiotics are a novel and attractive alternative to traditional antibiotic therapies; however, the study of probiotics is in its infancy, and there are obvious problems in attempting to manipulate extrinsically a complex ecosystem such as the human gut lumen by introducing foreign organisms.

**CONCLUSIONS**

In this overview, data have been presented that illustrate an association among bacteria, bacterial products, aberrant immune responses and enteric inflammation. However, the chicken and egg conundrum persists, and it remains unclear whether idiopathic gut disorders are of bacterial etiology or whether another defect facilitates an inappropriate response to the commensal microflora and thereby exaggerates existing disease. Resolution of this issue may ultimately be central to defining a cure for IBD. Moreover, there is increasing support for the use of antibiotics as an alternative or complement to current therapies, particularly in the context of treating complications associated with IBD and alleviating the disease symptoms in a cohort of patients with Crohn’s disease. Furthermore, while it is premature to abandon the pursuit of an infective, transmissible cause of IBD, it must be acknowledged that the search thus far has been unsuccessful. Consequently, it may be appropriate to de-emphasize identification of a specific pathogen and focus research efforts on assessing the impact of ubiquitous commensal bacterial products on gut form and function, and consider the effect of these agents on nontraditional immune cells such as the epithelium (Figure 1). Finally, knowledge of how bacteria and
bacterial products can influence intestinal physiology, combined with data relating to epithelial permeability defects, immune dysfunction and any genetic propensity to develop disease, may be the key to understanding chronic disorders such as IBD and irritable bowel syndrome, on a patient-by-patient basis.

ACKNOWLEDGEMENTS: Studies referred to from the authors laboratory were conducted with operating grants from the Medical Research Council of Canada and the Crohn’s and Colitis Foundation of Canada. The comments of Dr G Tougas (McMaster University, Hamilton), Dr PM Sherman (Hospital for Sick Children, University of Toronto) and Mrs B McGlinchey (McMaster University) on this manuscript are gratefully acknowledged.

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Can J Gastroenterol Vol 13 No 6 July/August 1999
Bacteria and inflammation

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