Host–bacterial interactions in inflammatory bowel disease

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ABSTRACT

Large numbers of different bacterial species are resident in the lumen of the distal gastrointestinal tract. The normal intestinal host–microbial interactions are not well understood, but the relationship is generally believed to be either mutually beneficial or beneficial to one without disadvantage to the other. Animal model and clinical studies suggest that IBD (inflammatory bowel disease) may develop in a susceptible individual when the normal host–bacterial relationship is dysregulated. In addition to rodent models, this article reviews studies that have investigated the cellular and molecular mechanisms of interactions between intestinal mucosal cells and the resident luminal bacteria in healthy individuals and patients with ulcerative colitis and Crohn’s disease. Mechanisms by which the intestinal mucosa is able to avoid pro-inflammatory responses to commensal bacteria (and their products) but able to respond appropriately to luminal pathogens is currently an area of active investigation. Such studies are beginning to provide important clues regarding possible alterations in the mucosa that lead to the development of pro-inflammatory responses to resident bacteria in patients with IBD. Approaches to alter the intestinal microflora for therapeutic purposes and their potential mechanisms of action are also discussed.

INTRODUCTION

Both types of IBD (inflammatory bowel disease), ulcerative colitis and Crohn’s disease, can present at any age, but the peak incidence is in the second and third decades of life. Ulcerative colitis only affects the colon, whereas Crohn’s disease usually involves the distal small intestine and colon. Both diseases are characterized clinically by relapses and remissions (the latter often following treatment with corticosteroids), with associated histological changes in the mucosa. During a relapse, there is mucosal infiltration by circulating polymorphonuclear cells, lymphocytes and monocytes and resolution of inflammation and mucosal repair/remodelling occurs during remission.

An increasing number of experimental and clinical studies incriminate bacteria in the pathogenesis of IBD. Disease may occur due to a loss of tolerance to resident intestinal bacteria in susceptible individuals, but to date there is no compelling evidence of an aetiological role for any singular pathogenic micro-organism. Cellular and molecular aspects of communications between the resident luminal bacteria and cells of the intestinal mucosa are considered first, with an emphasis on potential mechanisms by which the mucosal cells may mediate differential responses to pathogenic bacteria and the resident micro-organisms. The concept of ‘protective’ (or beneficial) resident bacteria and those with a capacity to induce inflammation (‘pro-inflammatory’) is considered. Such work has led to studies to investigate the role of putative ‘beneficial’ bacteria (probiotics) in the treatment of chronic inflammation in animal models and in patients.

Key words: Crohn’s disease, host–bacterial interaction, inflammatory bowel disease, mucosa, probiotics, therapeutics, ulcerative colitis.

Abbreviations: IBD, inflammatory bowel disease; IL, interleukin; LPS, lipopolysaccharide; NF-κB, nuclear factor-κB; PAMP, pathogen-associated molecular pattern; sIgA, secretory IgA; TLR, Toll-like receptor; TGF-β, transforming growth factor-β.

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NORMAL HOST–BACTERIAL RELATIONSHIP

Normal luminal microflora

The gastrointestinal tract is resident to a complex, active and dynamic microbial population. In the stomach and proximal small intestine, acid secretion, secretion of bile and phasic ‘house keeping’ motility patterns hinder colonization. However, numbers of bacteria dramatically increase in the distal small intestine rising to an estimated $10^{15}$ to $10^{12}$ bacterial cells/g content in the colon [1], which contribute to 60% of the faecal mass [2]. Over 400–500 species of bacteria are represented, belonging to 30 genera [1,3], although individuals will exhibit much variation in the types and numbers of species within their flora. The use of molecular techniques show that these figures, which are based on conventional culturing techniques, could be underestimated in terms of numbers and diversity [4].

In the infant, the establishment of the microflora occurs in concert with maturing intestinal anatomy and physiology, and birth and weaning represent key stages at which bacteria colonize and species are established. The fetal gut is sterile and in contact only with amniotic fluid. Bacterial colonization of the gut is driven by contact between the infant and its environment, and is also influenced by the mode of delivery, hygiene levels and medication [5]. Enterobacteria species, such as Escherichia coli, and bifidobacteria are detectable in infant faeces a few days after birth [6] when it is then soon influenced by feeding habits. The establishing flora is then under the nutritional influence of milk, with breast milk producing a flora rich in bifidobacteria and low in Clostridia and Bacteroides, whereas formula-fed babies have a wider range of anaerobes and fewer bifidobacteria [7]. Differences in composition of gut microflora and incidence of infection occur between breast-fed and formula-fed infants [5], as many different components within human milk, such as antimicrobial factors, may greatly influence which species predominate.

Early colonization may also depend on genetic influences. Research suggests that early bacterial colonizers may be able to modulate gene expression in the host and generate a suitable environment for themselves. Thus B. thetaiotaomicron induces alterations in the intestinal epithelial expression of glycoconjugates in germ-free mice, which facilitates the nutritional requirements of the bacterium [8]. This microbial–epithelial interaction also involves alterations in the expression of genes in B. thetaiotaomicron [9]. Other studies have shown that the intestinal microbial flora is influenced by host-derived factors. For example, following antibiotic treatment, the faecal bacterial population reverts to the original composition in mice exposed to identical environments and diets, but with significant differences between individual animals which appear to be genetically regulated [10].

Enteric bacteria are influential over the structural and functional integrity of the gut, and are important for the development of a competent immune system [11]. Germ-free-raised mice possess architectural abnormalities with crypt hyperplasia and lack of lymphoid follicle development. However, introduction of segmental filamentous bacteria into the intestine of germ-free mice raises the numbers of lymphoid cells in the lamina propria and increases the numbers of IgA-secreting cells with elevated IgA secretion [12].

The resident intestinal microflora of the adult may alter with ingestion of pathogenic and non-pathogenic microorganisms, lifestyle and diet [13]. Although there appears to be stability in the composition of bacterial species of the normal intestinal flora, studies suggest that this may not be the case for bacterial strains [14,15]. Changes in the microflora with age have also been described [16,17]. They include reduction in bifidobacteria in the older age group compared with younger adults and children, and increased diversity of the species Bacteroides [18]. Such alterations in the resident microflora may affect resistance to colonization by pathogens such as Clostridium difficile.

Mucosal responses to luminal bacteria

The host mucosa is exposed to vast numbers of metabolically active microbial cells and cell wall components, such as LPS (lipopolysaccharide) and peptidoglycan. A unique feature of host–microbial interactions in the intestine is the lack of pro-inflammatory responses in the mucosa exposed to the resident luminal microflora, whilst retaining the capability to respond to luminal pathogenic bacteria via the recruitment of acute inflammatory cells from the systemic circulation. There is increasing appreciation of the probable mechanisms by which host responses to pathogens are elicited, which is facilitating current research aimed at understanding the reasons for the lack of host mucosal pro-inflammatory responses to the resident luminal bacteria. In general, host responses to pathogenic micro-organisms are mediated by innate and adaptive immune responses. In the intestine (Figure 1), components of innate immunity are either pre-existing or are rapidly activated and, in addition to mediating antimicrobial effects, also regulate the highly specific adaptive immune responses.

Highly conserved structures of pathogenic microorganisms, designated PAMPs (pathogen-associated molecular patterns), are recognized by pattern-recognition receptors [19]. Studies have demonstrated the importance of the TLR (Toll-like receptor) family of molecules in host recognition of PAMPs. At least ten members of the TLR family have been described in mammals which recognize specific conserved constituents of micro-organisms [20–22]. Thus ligands recognized by TLR2 include lipoproteins/lipoproteins and peptidoglycan. TLR4 recognizes LPS (a component of the outer membrane of
NF-κB (nuclear factor-κB) is a transcription factor that is activated by various intracellular and extracellular signals. It plays a crucial role in the innate immune response by regulating the expression of inflammatory genes. NF-κB is a dimer of p50 and p65 subunits that is normally sequestered in the cytoplasm by inhibitory proteins. Activation of NF-κB pathways (NF-κB and MAPK pathways) occurs in response to various stimuli, including pathogen-induced activation and signaling pathways.

NF-κB dimer translocates to the nucleus to induce the transcription of pro-inflammatory genes, leading to the production of cytokines such as tumor necrosis factor (TNF-α) and interleukins (IL-1, IL-6). Activation of NF-κB pathways is essential for the host defense against pathogens, but it can also be detrimental if not tightly controlled, leading to chronic inflammation.

In the context of innate immunity, NF-κB activation can be triggered by the binding of pathogen-associated molecular patterns (PAMPs) to specific pattern-recognition receptors (PRRs). One such PRR is the Toll-like receptor (TLR) family, which includes TLR1, TLR2, TLR4, TLR5, TLR6, TLR7, TLR8, and TLR9.

TLR5 binds flagellin and bacterial CpG DNA, activating NF-κB and MAPK pathways. The latter includes NF-κB and MAPK pathways. Pathogen-induced activation of NF-κB has been shown to be important in intestinal epithelial cells.

Recent studies have shown that, in addition to transmembrane receptors for PAMPs, there are also intracellular sensors of bacterial products, such as Nod1 and Nod2. These cytoplasmic sensors activate NF-κB and MAPK pathways.

Thus, pathogenic bacteria may elicit recruitment of inflammatory cells, via chemokine secretion by epithelial cells, either following enterocyte invasion (e.g. by S. flexneri) or secretion of toxins (e.g. by C. difficile) [32,33]. Studies over the past few years have investigated potential mechanisms by which such activation of pro-inflammatory genes is avoided in epithelial cells in response to commensal bacteria and their products. LPS is a well-characterized PAMP and its interaction with the host systemic innate immune system is believed to be of major importance in Gram-negative sepsis. Epithelial cell responses to LPS present in the intestinal lumen may normally be avoided by lack of expression on the cell surface of the relevant pattern-recognition receptors TLR4, TLR2 and the co-receptor MD-2 [34,35]. Up-regulation of TLR4 expression in epithelial cells of patients with IBD may enable these cells to express pro-inflammatory cytokines in response to LPS [36]. Studies in an epithelial cell line have also demonstrated expression of TLR4 in the cytoplasmic compartment and it was postulated by these investigators that tolerance to LPS occurs by inhibition of the TLR4 signalling pathway [37]. Responses to bacterial flagellin may be avoided by expression of its receptor TLR5 on the basolateral, but not apical, surface of the epithelial cells [38]. In vitro studies have also demonstrated the ability of non-pathogenic bacteria to induce anti-inflammatory effects in epithelial cells by inhibition of NF-κB activation [39] and induction of TGF-β (transforming growth factor-β) [40].

Commensal bacteria or their products may be denied access to the epithelial surface by a barrier created by epithelial secretion of mucin glycoproteins, intestinal trefoil factor [41,42], antimicrobial peptides and sIgA (secretory IgA). Antimicrobial peptides are increasingly recognized as an important arm of secreted innate immunity in the gastrointestinal tract. Detailed discussion of this topic is beyond the scope of this article, but reviews on this subject have been published recently [43–45]. Epithelial secretion of water and electrolytes may also flush bacteria and their products from the mucosal surface, and this secretion can be mediated by enteric secretory reflex in response to luminal toxins, for example from E. coli [46].

A major player in mucosal adaptive immunity to luminal micro-organisms is sIgA, which has been studied over many years [47,48]. The largest number of Ig-producing cells in the body are present in the gastrointestinal tract and they synthesize > 4 g of IgA/day. Lamina propria cell-derived dimers of IgA are transported into the lumen via epithelial cells through polymeric immunoglobulin receptor to provide sIgA, which is believed to inhibit interactions between bacteria (and their products) and epithelial cells. Recent studies have shown that commensal bacteria persist in intestinal dendritic cells, which are restricted to mesenteric lymph nodes to induce the production of local protective IgA [49].
The potential importance of IgA in the regulation of the commensal bacterial flora has also been reported recently [50]. Moreover, dimeric IgA in the intracellular compartment of epithelial cells has been shown to be capable of neutralizing LPS [51].

A large number of macrophages are present in the intestinal lamina propria close to the epithelial monolayer [52]. They are capable of responding to LPS by expressing the pro-inflammatory genes IL-1β [53] and TNF-α (tumour necrosis factor-α) [54]. However, in vivo there is either absent or negligible expression of these cytokines in mucosal samples [53,55–57], implying lack of exposure to bacteria or their products (such as LPS) due to an effective overlying epithelial barrier created by constituents such as those described above.

Metabolic products of commensal bacteria may mediate beneficial effects to the mucosa. The best characterized products are the short-chain fatty acids acetate, butyrate and propionate released by fermentation of undigested carbohydrates [58]. Butyrate has been shown to be a source of energy for epithelial cells, especially in the distal colon [59].

**ROLE OF BACTERIA IN THE PATHOGENESIS OF IBD**

There is a wealth of clinical and experimental data discriminating bacteria in the pathogenesis of IBD. These studies suggest that, in susceptible individuals, IBD occurs as a result of loss of tolerance to resident intestinal luminal bacteria. The development of immunological responses to these micro-organisms and their products is believed to lead to chronic intestinal inflammation.

**Evidence from animal models**

Studies in animal models have made a major contribution to our understanding of the probable importance of dysregulation of interactions between host mucosal cells and the resident luminal bacteria in the aetiology of IBD. Early studies demonstrated the immunological basis of chronic intestinal inflammation [60] and implicated the role of the intestinal microflora [61], including the contribution of an immune response to specific strains of a resident bacterium (*B. vulgatus*) in the severity of the mucosal inflammation [62]. A number of rodent models of IBD have been described over the last decade in which chronic intestinal inflammation occurs spontaneously in wild-type mice with a specific genetic background or genetically manipulated (transgenic, knockout) mice. The disease can also be induced by exogenous agents or by transfer of T-cells into immunologically compromised animals. For an in-depth consideration of these models, the reader is referred to some of the relevant review articles that have been published [63–65]. These models have shown, with remarkable consistency, the requirement of the resident microflora in the development of chronic inflammation, with some studies showing that there are some strains of bacteria that have ‘pro-inflammatory’ effects and others that may be protective. Moreover, antibiotics are beneficial in a number of these models. The host mucosal cell types that have been shown to be important in disease pathogenesis are T-cells, dendritic cells, macrophages and epithelial cells. Although models with Th2-type responses have been described, in the majority, the disease is mediated by Th1-type cells, via the cytokines IFN-γ (interferon-γ) and IL-12. TGF-β and IL-10 are important ‘anti-inflammatory’ cytokines in these models expressed by regulatory T-cells [66].

**Evidence from patient-related studies**

Although over the years various pathogenic micro-organisms have been proposed, to date there is no convincing evidence of an aetiological role for any of these agents in ulcerative colitis or Crohn’s disease. Studies in the 1980s implicated the role of the resident luminal bacteria in disease pathogenesis [67], and these have remained the main focus of patient-related research in the field of host–microbial interactions.

Immunohistochemical studies have demonstrated the presence of antigens of *E. coli* and *Streptococci* in lamina propria macrophages under ulcers and fissures in patients with Crohn’s disease [68,69], implying penetration by these luminal agents following the loss of the epithelial barrier. Pro-inflammatory cytokine expression by lamina propria macrophages in response to these bacterial products would be expected to perpetuate chronic intestinal inflammation.

Alterations in mucosa-associated flora in patients with IBD, in response to different types of treatment, have been investigated previously [70,71]. Studies in which bacteria were cultured from colonoscopic biopsies have either shown no difference when compared with controls [72] or variation in species of bacteria isolated [73]. In more recent studies in which molecular techniques (especially *in situ* hybridization for bacterial ribosomal RNA) were used, bacteria were found in the mucus layer of IBD mucosal samples, but these micro-organisms were either absent or present in very small numbers in tissue samples of healthy controls [74–76]. *Bacteroides* and *Enterobacteriaceae* (mainly *E. coli*) were the main anaerobes and aerobes (respectively) present [76]. Both bacterial culture [72] and *in situ* hybridization studies [75] showed that there were similar numbers of bacteria in biopsies from different regions of the colon, including the rectum. In two studies by *in situ* hybridization [75,76], bacteria were not seen in the lamina propria. This difference from immunohistochemical studies (above) could be due to the fact that bacteria taken up by macrophages have been killed and the microbial rRNA degraded.
In a recently published study in which mucosa-associated bacteria were investigated using 16S rDNA based single-strand conformation polymorphism and real-time PCR, reduction in bacterial diversity was reported in both Crohn’s disease and ulcerative colitis [77]. This reduction in diversity was due to loss of anaerobic bacteria. No association between CARD15/NOD2 status and bacterial diversity was seen in patients with Crohn’s disease.

T-cells from normal human intestinal mucosa are tolerant to bacteria on their surface, as demonstrated by lack of a proliferative response to bacterial sonicates [78,79]. However, these mucosal T-cells proliferate in response to luminal bacteria derived from other individuals [79]. It is of interest that T-cells isolated from intestinal samples affected by active IBD proliferate in response to sonicates of bacteria cultured from their mucosal surface [79]. These studies suggest that, in patients with active IBD, there is loss of immunological tolerance to their ‘own’ resident bacteria present on the mucosal surface. Mechanisms by which mucosal tolerance to luminal antigens may occur, with involvement of regulatory T-cells and dendritic cells, is of considerable current interest [66,82].

The recent identification by numerous groups of NOD2 (CARD15) as a susceptibility gene for Crohn’s disease [29,30] provides further evidence for the important role of host-microbial interactions in the aetiology of Crohn’s disease, because (as outlined above) Nod2 protein has been shown to be capable of detecting peptidoglycan, which is a cell wall constituent of Gram-positive and Gram-negative bacteria [24,25]. Nod2 protein has been shown to be expressed in a number of cell types [81–86], but the mechanisms by which alterations in the NOD2 gene lead to the development of Crohn’s disease remain to be fully characterized.

MANIPULATION OF INTESTINAL BACTERIA FOR THERAPEUTIC PURPOSES

The rationale for the manipulation of luminal bacteria in IBD is the proposal that some microbial strains in the colon exert a ‘protective’ effect against other bacteria that may have a ‘pro-inflammatory’ effect in a susceptible individual. Some studies suggest that there is a reduction in putative ‘protective’ bacteria (such as bifidobacteria) and an increase in bacteria with a potential for ‘pro-inflammatory’ effects (such as Enterobacteriaceae and Bacteroides) in faecal and mucosal flora of patients with IBD [87,88].

In vitro studies suggest that some strains of resistant bacteria or their products may have the ability in isolation to induce pro-inflammatory effects. For example, enterotoxigenic strains of B. fragilis can be present in the lumen of healthy adults, but the purified toxin is capable of inducing loss of epithelial barrier function [89] and pro-inflammatory cytokine barrier function by human intestinal epithelial cells [90].

Probiotics, prebiotics and synbiotics

The term probiotic is often used for orally ingested (usually with food) live micro-organisms that provide health benefits for the host. The definition of probiotic has changed over the years and was recently proposed as: “A preparation of or a product containing viable, defined micro-organisms in sufficient numbers, which alter the microflora (by implantation or colonization) in a compartment of the host and by that exert beneficial health effects in this host” [91]. Bacterial species that have been used as probiotics include Lactobacillus (e.g. L. acidophilus, L. plantarum), Bifidobacterium (e.g. B. longum) and Streptococcus (e.g. Strept. lactis) [92].

A probiotic is defined as “a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon” [92]. Prebiotics are generally non-digestible oligosaccharides such as inulin and lactulose. A synbiotic contains a mixture of probiotic and prebiotic.

Probiotics, and to a lesser extent prebiotics and synbiotics, are being investigated for their potential beneficial effects in a number of gastrointestinal and non-gastrointestinal diseases. Possible mechanisms of action of probiotics are also being studied. Those mechanisms of action that may be of relevance in IBD will be briefly considered, followed by a review of studies of efficacy in animal models and in patients.

Potential mechanisms of action of probiotics

This is an area of considerable current interest as the cellular and molecular mechanisms of action of probiotics have only recently begun to be investigated in earnest (Figure 2). Probiotics may impair the ‘pro-inflammatory’ activity of other luminal bacteria via secretion of antimicrobial products [93] or competition for binding sites on epithelial cells [94]. They have been shown to enhance epithelial barrier function [95–98] and to inhibit apoptosis in epithelial cells [99], which would be expected to facilitate maintenance of barrier against luminal microorganisms and their products. L. plantarum has recently been reported to increase the secretion of the anti-inflammatory cytokine IL-10 by lamina propria mononuclear cells [100].

Some studies suggest that probiotics are capable of modulating systemic and mucosal immune responses (reviewed by Ouwehand et al. [101]) and in a double-blind randomized placebo-controlled trial Lactobacillus GG has been shown to be effective in prevention of early atopic disease in children at high risk [102]. It is of
Studies in animal models

Since animal models of IBD have provided the strongest evidence for the role of resident luminal bacteria in disease pathogenesis, it is appropriate that they are used to test the therapeutic efficacy of probiotics. Results of studies to date are summarized in Table 1. A number of different rodent models and different probiotics (single or in combination, and in different doses) have been used to date. In some studies, probiotics exerted a beneficial effect in terms of protection against the severity of intestinal inflammation induced or enhancement of disease resolution. Protection against recurrence of the chronic inflammatory disease has also been reported. Although in some studies the probiotic preparations did not have any beneficial effects, they do not appear to be harmful to these models.

<table>
<thead>
<tr>
<th>Model</th>
<th>Probiotic</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trinitrobenzene sulphonic acid</td>
<td><em>Bi. infantis</em></td>
<td>No effect</td>
<td>[110]</td>
</tr>
<tr>
<td>dinitrobenzene sulphonic acid</td>
<td><em>L. acidophilus, L. casei and Bi. animalis</em></td>
<td>Reduced inflammation</td>
<td>[111]</td>
</tr>
<tr>
<td></td>
<td><em>VSL#3</em></td>
<td>No effect</td>
<td>[112]</td>
</tr>
<tr>
<td></td>
<td><em>Lactobacillus GG</em></td>
<td>No effect</td>
<td>[112]</td>
</tr>
<tr>
<td></td>
<td><em>L. plantarum 299</em></td>
<td>No effect</td>
<td>[113]</td>
</tr>
<tr>
<td></td>
<td><em>VSL#3 (DNA, subcutaneously)</em></td>
<td>Reduced inflammation</td>
<td>[105]</td>
</tr>
<tr>
<td>Iodoacetamide</td>
<td><em>VSL#3</em></td>
<td>Reduced inflammation</td>
<td>[112]</td>
</tr>
<tr>
<td></td>
<td><em>Lactobacillus GG</em></td>
<td>Reduced inflammation</td>
<td>[112]</td>
</tr>
<tr>
<td>Acetic acid</td>
<td><em>L. rhamnosus GG</em></td>
<td>No effect</td>
<td>[114]</td>
</tr>
<tr>
<td></td>
<td><em>L. reuteri R2LC</em></td>
<td>Reduced inflammation</td>
<td>[114]</td>
</tr>
<tr>
<td></td>
<td><em>L. reuteri R2LC</em></td>
<td>Reduced inflammation</td>
<td>[115]</td>
</tr>
<tr>
<td>Dextran sodium sulphate</td>
<td><em>VSL#3 (irradiated and DNA</em>)</td>
<td>Reduced inflammation</td>
<td>[105]</td>
</tr>
<tr>
<td>IL-10 knockout mice</td>
<td><em>L. salivarius 118 (subcutaneously)</em></td>
<td>Reduced inflammation</td>
<td>[104]</td>
</tr>
<tr>
<td></td>
<td><em>L. salivarius</em></td>
<td>Reduced inflammation</td>
<td>[116]</td>
</tr>
<tr>
<td></td>
<td><em>Bi. infantis</em></td>
<td>Reduced inflammation</td>
<td>[116]</td>
</tr>
<tr>
<td></td>
<td><em>L. plantarum 299V</em></td>
<td>Reduced inflammation</td>
<td>[117]</td>
</tr>
<tr>
<td></td>
<td><em>VSL#3</em></td>
<td>Reduced inflammation</td>
<td>[96]</td>
</tr>
<tr>
<td></td>
<td><em>L. salivarius</em></td>
<td>Reduced inflammation</td>
<td>[118]</td>
</tr>
<tr>
<td></td>
<td><em>L. reuteri</em></td>
<td>Reduced inflammation</td>
<td>[119]</td>
</tr>
<tr>
<td></td>
<td><em>VSL#3 (DNA, subcutaneously)</em></td>
<td>Reduced inflammation</td>
<td>[105]</td>
</tr>
<tr>
<td>E. coli-induced colitis in IL-2 knockout mice</td>
<td><em>B. vulgatus</em></td>
<td>Reduced inflammation</td>
<td>[120]</td>
</tr>
<tr>
<td></td>
<td><em>Lactobacillus GG</em></td>
<td>Prevented recurrent colitis</td>
<td>[121]</td>
</tr>
<tr>
<td></td>
<td><em>L. plantarum 299V</em></td>
<td>No prevention of recurrent colitis</td>
<td>[121]</td>
</tr>
</tbody>
</table>
Table 2  Effects of prebiotics on intestinal inflammation in rodent models

<table>
<thead>
<tr>
<th>Model</th>
<th>Prebiotic</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trinitrobenzene sulphonic acid</td>
<td>Fructo-oligosaccharide</td>
<td>Reduced inflammation</td>
<td>[111]</td>
</tr>
<tr>
<td></td>
<td>Galacto-oligosaccharide</td>
<td>No effect on inflammation</td>
<td>[122]</td>
</tr>
<tr>
<td>Dextran sodium sulphate</td>
<td>Fructo-oligosaccharide</td>
<td>No effect on inflammation</td>
<td>[123]</td>
</tr>
<tr>
<td></td>
<td>Resistant starch</td>
<td>Reduced inflammation</td>
<td>[123]</td>
</tr>
<tr>
<td></td>
<td>Germinated barley foodstuff</td>
<td>Reduced inflammation</td>
<td>[124]</td>
</tr>
<tr>
<td></td>
<td>Germinated barley foodstuff</td>
<td>Reduced inflammation</td>
<td>[125]</td>
</tr>
<tr>
<td></td>
<td>Inulin</td>
<td>Reduced inflammation</td>
<td>[126]</td>
</tr>
<tr>
<td>IL-10 knockout mice</td>
<td>Germinated barley foodstuff</td>
<td>Reduced inflammation</td>
<td>[127]</td>
</tr>
<tr>
<td></td>
<td>Lactulose</td>
<td>Reduced inflammation</td>
<td>[119]</td>
</tr>
</tbody>
</table>

Table 3  Clinical trials of probiotics in patients with IBD

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Treatment</th>
<th>Outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inactive UC</td>
<td>ST + L. acidophilus, Bi. breve and Bi. bifidum (n = 11) compared with controls (n = 10)</td>
<td>Maintained remission</td>
<td>[128]</td>
</tr>
<tr>
<td>Curative resection for CD</td>
<td>Lactobacillus GG (n = 23) compared with placebo (n = 22)</td>
<td>No difference in rates of recurrence or disease severity</td>
<td>[129]</td>
</tr>
<tr>
<td>Inactive UC</td>
<td>E. coli Nissle (n = 57) compared with mesalazine (n = 57)</td>
<td>As effective as mesalazine in maintaining remission</td>
<td>[106]</td>
</tr>
<tr>
<td>Ileal pouch–anal anastomosis in UC</td>
<td>VSL#3 (n = 20) compared with placebo (n = 20)</td>
<td>Reduction in rate of flare-ups of chronic pouchitis</td>
<td>[107]</td>
</tr>
<tr>
<td>Ileal pouch–anal anastomosis in UC</td>
<td>VSL#3 (n = 20) compared with placebo (n = 20)</td>
<td>Reduction in rate of onset of acute pouchitis</td>
<td>[109]</td>
</tr>
</tbody>
</table>

Fewer animal studies have investigated the effects of prebiotics on intestinal inflammation (Table 2). A partially beneficial effect has been reported in some of these studies using fructo-oligosaccharide, inulin, lactulose and germinated barley foodstuff (which contains protein, insoluble fibre and oligosaccharides). Studies comparing one prebiotic preparation with another have not been undertaken. Further investigation of the potential synergistic effects of a prebiotic and a probiotic (synbiotic) will also be of interest.

**Clinical studies**

Table 3 lists the small number of double-blind placebo-controlled trials undertaken to date. Following initial interest with the non-pathogenic *E. coli* strain *Nissle 1917* [106], recent studies have investigated combinations of probiotics. The cocktail VSL#3 [which contains four strains of *Lactobacillus* (*L. casei*, *L. plantarum*, *L. acidophilus* and *L. delbrueckii* subsp. *bulgaricus*), three strains of *Bifidobacterium* (*Bi. longum*, *Bi. breve* and *Bi. infantis*) and one strain of *Streptococcus* (*Strep. salivarius* subsp. *thermophilus*)] has been shown to be effective in the prevention of flare-ups of chronic pouchitis [107]. Total proctocolectomy with formation of an ileal reservoir that empties via the anal sphincter (ileal pouch–anal anastomosis) is the surgical procedure undertaken in many patients with ulcerative colitis. Pouchitis is a complication that occurs in some of these patients and is characterized histologically by acute and chronic inflammation. Although the pouch lumen is normally colonized by bacteria, reduced counts of lactobacilli and bifidobacteria (but an increase in *C. perfringens* and other species) have been reported in stool samples of patients with pouchitis compared with those without pouchitis [108]. In many patients that develop pouchitis, the first attack occurs within the first year after surgery and it is of interest that recent studies have shown effectiveness of VSL#3 in prevention of the onset of acute pouchitis [109].

**CONCLUSIONS**

There is considerable evidence to support the concept that, in susceptible individuals, alterations in responses to the resident luminal bacteria may lead to the development...
and/or perpetuation of IBD. The precise cellular and molecular mechanisms of this susceptibility remain to be determined. However, the recent identification of NOD2 (CARD15) as a susceptibility gene for Crohn’s disease is of major interest, because of the likely importance of the Nod2 protein in innate host responses to bacteria. Some studies support the notion of ‘protective’ and potential ‘pro-inflammatory’ resident luminal bacteria in patients with IBD, but further studies are required. Investigations to determine therapeutic effects of manipulation of the resident flora using probiotics are promising, but further studies need to be undertaken to determine optimal bacterial species and ingredients that promote their growth in the distal gastrointestinal tract. There is also significant interest in investigation of the potential mechanisms of action of probiotics.

REFERENCES


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