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Understanding Probiotic Treatment Strategies in Inflammatory Bowel Disease, with a Special Emphasis on the Intestinal Barrier

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Abstract

The incidence of inflammatory bowel disease (IBD) is rising and, despite several decades of scientific work, the pathogenesis is still patchy. This has hampered the development of targeted therapeutics and many patients have turned to alternative treatment options, such as probiotics. Recent interest in host–microbe interactions has helped to unravel underlying mechanisms of probiotic effects. Early animal studies and anecdotal reports were promising and clinical trials were initiated. Despite the obvious versatility of probiotic bacteria with regard to effects on intestinal inflammation, results from clinical trials were by and large disappointing. This article provides an overview of probiotic effects in relation to current concepts of IBD pathomechanisms.

Keywords

Probiotic bacteria, inflammatory bowel disease, ulcerative colitis, Crohn’s disease, pouchitis, lactobacilli, bifidobacteria, Escherichia coli, intestinal microflora

Inflammatory bowel diseases (IBD), such as Crohn’s disease (CD), ulcerative colitis (UC) and pouchitis, are chronic idiopathic inflammatory disorders affecting mainly young people in their productive years. These diseases are characterised by abdominal pain and bloody diarrhoea. UC is limited to the large intestine, while CD can affect the whole gastrointestinal tract, from the mouth to the anus. Pouchitis is an unfortunate inflammatory complication that can occur following colectomy in patients with UC.

A sharp rise in the incidence of IBD has occurred in northern Europe, the UK and North America since the early 1950s, but recently this has levelled out. The incidence in eastern Europe, South America, Asia and the Pacific region is markedly lower, although it has increased in the last two decades. The reason for this phenomenon is not clear, but since IBD is thought to be the result of an altered microbiota coupled with an overly aggressive immune response in a genetically susceptible host, westernisation of these regions with changes in hygiene standards and diet may be responsible. Due to the multifactorial pathogenesis of IBD the development of targeted treatment strategies is difficult. Current treatment is not curative and is also prone to side effects. As a result, up to 70% of IBD patients use complementary alternative medicines alone or in addition to standard medical therapy. Recently, probiotics have gained wider acceptance due to an increased understanding of the benefits associated with the symbiotic relationship between commensal bacteria and the human host. Consequently, increased scientific effort has identified numerous beneficial effects of probiotics, and this has stimulated a number of clinical trials.

This article is aimed at critically analysing recent literature regarding the rationale for probiotic treatment strategies and their clinical application. This is preceded by a brief overview of current pathophysiological concepts in IBD.

Pathophysiological Concepts in Inflammatory Bowel Disease

IBD is a multifaceted disease, involving environmental, genetic, immunological, microbial and other disturbances, none of which alone is sufficient or essential to cause disease. Consequently, IBD is broadly defined as an overly aggressive immune response to ubiquitous antigens (e.g. commensal bacteria) in a genetically susceptible host.

The Role of a Genetic Predisposition in Inflammatory Bowel Disease

Family and twin studies have provided compelling evidence that genetic factors play a significant role in determining susceptibility to both CD and UC. These studies have demonstrated that the risk of IBD is higher for first-degree relatives than for other family members, and is greatest for twins of a CD proband. In cohorts from northern Europe, the combined concordance rate was 36% for CD and 17% for UC in monozygotic twins, but only 4% for both clinical phenotypes in dizygotic twins. A recent follow-up of the Danish and Swedish twin cohorts reported a high degree of concordance with regard to age at diagnosis, disease location and disease behaviour in monozygotic twins concordant for CD but less consistent for UC. However, as the concordance rates in
monzygotic twin pairs do not approach 100%, these studies also underscore the importance of environment in the development and progression of IBD.4–5

Early attempts to identify susceptibility loci for IBD focused on functional candidates and, in particular, polymorphic genes located within the major histocompatibility complex on the short arm of chromosome 6. Many of these studies yielded inconclusive results and others were common to both (e.g. IBDD), suggesting a lending support to the notion that these diseases are polygenic disorders that share some, but not all, susceptibility genes.3 In 2001 the linkage between CD and IBD1 was pinpointed to a single gene encoding the nucleotide-binding oligomerisation domain protein 2 (NOD2, also known as CARD15).19,20 NOD2 is a cytosolic pattern recognition receptor that activates nuclear factor-kappa B (NF-κB), which in turn triggers a pro-inflammatory cytokine cascade when NOD2 detects the muramyl-dipeptide component of bacterial cell walls. Three single nucleotide polymorphisms (SNPs; R702W, G908R, 1007fs) have been demonstrated to be independent risk factors for CD, presumably by impairing activation of NF-κB. Discovery of these NOD2 SNPs provided the first genetic evidence that host-microbe interactions are central to the pathogenesis of IBD.

The next major advance in the hunt for IBD genes resulted from the International HapMap Project’s cataloguing of nearly four million common SNPs.21 The convergence of HapMap, high-density SNP array technology and statistics paved the way for genome-wide association studies (GWAS).22 These global scans of the human genome and the ensuing meta-analyses have led to the identification and confirmation of 32 new genetic susceptibility factors for CD and 17 risk loci for UC.22–26 Many of these, such as NOD2, play pivotal roles in mechanisms of innate immunity or in the induction of adaptive immunity. Foremost examples include autophagy-like 16L1 (ATG16L1), immunity-related GTPase family M (IRGM) and genes in the interleukin (IL)-12/23 pathway, including IL receptor 23 (IL23R), IL-12B (IL12B), Janus kinase (JAK2) and signal transducer and activator of transcription 3 (STAT3).27 ATG16L1 and IRGM are highly expressed in intestinal epithelial cells, antigen-presenting cells and subsets of T cells. Small interfering RNA (siRNA) knock-down studies have demonstrated that these genes are crucial in the handling, processing and elimination or ‘autophagy’ of intracellular pathogens, such as Salmonella typhimurium and Mycobacterium tuberculosis.28,29 SNPs in ATG16L1 and IRGM have been consistently and strongly associated with CD in multiple independent cohorts.30 However, there is little evidence to suggest that these genes, and thus defects in autophagy, are important predisposing factors for the development of UC.

By contrast, the IL-12/IL-23 pathway has been implicated in both CD and UC.31 This pathway is activated when IL-23, a heterodimer composed of IL-23A and β1 subunits, binds to its heterodimeric receptor, comprising IL-23R and IL-12Rβ1. The ensuing signalling cascade leads to the expression of pro-inflammatory cytokines and the differentiation of naïve CD4+ T cells into TH17 cells, which produce the large amounts of IL-17 necessary to activate and recruit neutrophils to sites of infection.32 Central to this cascade is the interaction between IL-23R and JAK2, which results in autophosphorylation of JAK2 and subsequent activation and translocation of STAT3 to the cell nucleus.33,34 The CD GWAS by Duerr et al.34 provided the first genetic evidence to support the long-held belief that the adaptive immune system and the IL-12/IL-23 pathway, in particular, are pivotal to IBD pathogenesis. This GWAS identified a highly conserved non-synonymous SNP (Arg381Gln) within IL-23R, which was significantly under-represented in CD patients compared with controls.35 Subsequent genotyping in replication cohorts found that heterozygous carriage of the minor (glutamine) allele of this SNP also conferred a protective effect against development of UC.36 Although no functional studies have been conducted on Arg381Gln, its location within the binding domain of JAK2 suggests the glutamine allele influences naïve CD4+ T-cell differentiation by altering autophosphorylation of JAK2.37 Subsequent GWAS and meta-analyses have identified significant association of IL-12B, STAT3 and JAK2 with IBD,38 and these genes and IL-23R with psoriasis and ankylosing spondylitis,39,40 thereby providing overwhelming genetic evidence that the IL-12/IL-23 pathway is central not just to IBD but to the pathologies of inflammatory diseases in general.

Despite the success of recent GWAS, our knowledge of the genetic factors that contribute to IBD is still woefully incomplete. It is estimated that the loci identified by GWAS explain only approximately 20% of the total genetic risk of IBD. Whether re-sequencing of known risk loci and the study of other classes of genetic variants, such as copy number polymorphisms, may account for this missing heritability remains to be seen. Either way, a more complete understanding of IBD risk loci and their functional consequences on the immune system is arguably an important prerequisite not just for more rational design of drug therapies but also for the optimisation of clinical probiotics.

The Role of the Intestinal Barrier

The intestinal barrier is a functional unit consisting of the underlying epithelium, mucus and secreted components, such as defensins, lysozymes and immunoglobulins. It is not a completely impervious barrier because, in addition to excluding pathogens, antigens and commensal bacteria, it must allow the selective absorption of essential nutrients, salts and water. As a result there is a constant flux of material across the intestinal barrier, and changes in the permeability, or ‘leakiness’, of the intestinal barrier are implicated in IBD. Support for this comes from a recent GWAS that reported Sp13.1, a Crohn’s disease locus contained within a 1.25Mb gene desert, is associated with disease susceptibility. The associated alleles correlated with quantitative expression of the prostaglandin receptor EP4 (also known as PTGER4), which is expressed in intestinal epithelial cells and regulates epithelial barrier function. Significantly, EP4-knockout mice are more susceptible to dextran sodium sulphate (DSS) colitis than wild-type controls.41

The outer layer of the intestinal barrier is the mucus, which is secreted by goblet cells throughout the gastrointestinal tract and functions as a lubricant, facilitating the passage of luminal contents.42 However, particles, viruses and bacteria are also trapped in this layer and are expelled by the peristalsis, thus preventing pathogens and antigens gaining access to the underlying epithelial layer. Mucins also concentrate the antimicrobial agents that are secreted by the intestinal epithelium at the epithelial surface;43 these include immunoglobulin A (IgA) and defensins. IgA binds bacteria and viruses, preventing epithelial attachment, while defensins are cationic antimicrobial peptides that have antimicrobial activity against bacteria.44–46 The 10 human defensins are divided into two groups, α and β defensins, and their expression profile varies along the intestinal tract. In the small bowel, human α defensins 5 and 6 are...
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synthesised by Paneth cells, while α-defensins 1–4 are produced by granulocytes.44 In the colon, epithelial cells and cells within the lamina propria express human β-defensins (HBD). HBD1 is constitutively expressed, while HBD2, 3 and 4 expression is induced by inflammatory and bacterial stimuli.45

The mucus and secreted antimicrobial agents function to prevent access of bacteria to the epithelial surface, and normal intestinal epithelium is relatively sterile, with little evidence of adherent or invasive bacteria.46 However, both adherent and invasive bacteria are present in the mucosa of IBD patients,78 and there is evidence of altered mucus secretion66–68 and a reduction in the secretion of defensins in IBD.69–73

Underlying the mucus layer is the intestinal epithelium, which forms a physical barrier and is the primary determinant of intestinal permeability. This physical barrier consists of the transcellular and the paracellular pathways. Both pathways contribute to the flux of material across the epithelium and thus the permeability of the epithelium, but in general the increased permeability associated with IBD is thought to be a consequence of changes in the paracellular pathway.74–76

The permeability of the paracellular pathway is determined by the apical junctional complex (AIC),72–75 which consists of the outer tight junction and the underlying adherens junction.76 The outer tight junction forms a continuous, circumferential, belt-like structure at the luminal end of the intercellular space. It is a multiprotein complex consisting of structural proteins (e.g. claudins, occludin) that span the junctional space and determine the permeability of the paracellular pathway. These proteins are in turn attached to cytoskeletal and cellular regulatory proteins. The adherens junction also forms a continuous belt around the epithelial cells and, as its name suggests, provides adhesion between adjacent epithelial cells. It consists of three classes of proteins, adhesion molecules (e.g. cadherins and nectin) that span the junctional complex between cells, a cytoskeletal network (e.g. actin) and cytoskeletal/membrane plaque proteins (catenins and afadin) that link the adhesion molecules to the cytoskeleton.77

Alterations in intestinal permeability that occur with IBD have been associated with structural changes in both the adherens junction and the tight junction. In the adherens junction, expression of the E-cadherin and alpha catenin is altered in the inflamed intestine of CD and UC patients.78–80 Similarly, ultrastructural studies suggest that the tight junctions are abnormal in IBD patients, with increased distance between the enterocytes and reduced complexity of the tight junctional strands in both CD79 and UC.80 Immunohistochimical studies show loss of occludin from the tight junction of the colonic mucosa of patients with CD and UC.79 More recent studies indicate that there are variations in pattern of claudin expression.81 Many of these changes are mediated by inflammatory cytokines secreted in IBD, and it is now recognised that the AIC is a dynamic structure,75,82 subject to regulation not only by physiological stimuli83–85 but also by pathological stimuli, such as bacterial toxins86 and cytokines.87

The Role of the Intestinal Immune System

The intestinal immune system is the largest immune-competent organ in the body. It is in constant contact with ~10–100 trillion organisms consisting of at least 500–1,000 species, which make up the intestinal microbiota.88 Consequently, the ability of the intestinal immune system to differentiate between the beneficial, neutral and harmful components of the microbiota is essential for immunohomeostasis. Therefore, it is not surprising that disruption of this system contributes to IBD, and Dachmann has convincingly demonstrated that patients with IBD have a loss of tolerance to their own bacterial flora.89 It is generally agreed that IBD is the result of an over-aggressive immune response to ubiquitous bacterial antigens in the intestinal lumen. A leaky intestinal barrier allows luminal antigens uncontrolled access to the innate and adaptive immune systems, which elicit an aberrant immune response. In IBD it seems that the immune response is disturbed at several levels.

The functionality of the immune system is genetically determined and, as discussed above, many of the IBD susceptibility loci are associated with innate immunity, autophagy and phagocytosis. The innate immune system constantly monitors the intestinal microbiome via 10–15 membrane-associated Toll-like receptors (TLRs), cytosolic NOD1 and 2 and other pattern-recognition receptors. NOD2 is expressed in various intestinal cells (epithelial cells, Paneth cells, macrophages and dendritic cells) and functions as a pattern-recognition receptor for peptidoglycan from Gram-positive and Gram-negative bacteria. Activation of NOD2 stimulates an inflammatory response via the NF-κB pathway. Polymorphisms in NOD2 expression lead to an altered host-microbe interaction, which contributes to CD.

Further monitoring of the microbiome occurs through contact of microorganisms with TLRs. These are specialised to recognise different gut bacteria. TLRs are expressed on a variety of immune cells and are necessary for maintaining tolerance and the elimination of pathogens. Several polymorphisms in TLRs have been associated with the susceptibility to IBD (e.g. TLR-5 in Ashkenazi Jews) when TLRs, upon interaction with the specific ligand, elicit aberrant immune responses.90

Dendritic cells are central to control the immune response. Depending on location, stimulus and degree of maturation, dendritic cells initiate and control intestinal immune responses and maintain tolerance by constantly sampling the luminal contents. To do this, dendritic cells project finger-like processes through gaps between epithelial cells to sample intestinal contents.91 It appears that in IBD the response of the dendritic cells may be dysregulated, leading to an overly aggressive response to commensal organisms and contributing to the development of IBD. Essentially, it is felt that in IBD dendritic cells incorrectly identify commensal bacteria, LPS and CpG-DNA via TLR4, and contribute to the Th1-mediated inflammatory response and IL-17 release via the IL-23 pathway.92–94 IL-23 in turn mediates intestinal inflammation as it drives the development of IL-17, IL-6, IL-8 tumour necrosis factor-alpha (TNF-α)-producing T cells, an effect that can be abrogated in rodents by anti-IL-23 antibody treatment.95

The Role of the Intestinal Microbiota

We are born with a sterile intestine and colonisation occurs through close and intimate contact with our mother and immediate surroundings. In these early years, the microbiota is responsive to outside influences,96,97 but in adults the composition is relatively stable and changes are only transient. Normally, humans live in a stable and symbiotic relationship with their intestinal bacterial communities, and these communities play an important role in nutrient absorption.98–100 However, it is clear that the intestinal microbiota contributes to IBD, as IBD occurs preferentially in areas of high bacterial concentrations, such
as the large intestine,46 and broad-spectrum antibiotic therapy or faecal stream diversion (bowel rest) can influence the course of the disease.47-50 Furthermore, in IBD numerous bacteria (mainly bacteroides and enterobacteria) inhabit the mucus layer, whereas in the healthy intestine it is normally sterile.51-53 As noted above, the effect of the intestinal bacteria could result from a compromised intestinal barrier. However, there is also evidence of dysbiosis in IBD.50-56 Patients with quiescent colonic CD have increased numbers of enterobacteria, whereas in active disease there is a trend towards reduced bifidobacteria counts111 and many of the under-represented bacterial genera are short-chain fatty acid (SCFA)-producing, commensal bacteria.112 SCFAs, such as butyrate, serve as an important source of energy for intestinal cells, and can regulate gene expression.113

Although dysbiosis is evident in IBD, no single causative agent has been identified.54 Histopathological similarities between CD and Johne’s disease and clinical studies suggested that Mycobacterium avium subspecies paratuberculosis (MAP) may be a causative agent.115 However, it was not possible to show an association between MAP and NOD2 mutations in CD, a link that would be expected given that MAP is an intracellular organism,116 and the clinical application of triple antimeycobacterial therapy aimed at MAP in CD patients did not have any sustained benefit.117

By contrast, adherent/invasive Escherichia coli (AIEC) is found in isolates of patients with CD, but less so in UC, while it is almost absent in normal controls.1 Furthermore, the expression of the intestinal AIEC receptor CAECAM1 is increased in the ileum of patients with active CD.118

Probiotic Properties

Probiotics are defined as “live organisms which, when consumed in adequate amounts as part of food, confer a health benefit to the host”.119 Probiotic bacteria, mainly different lactobacilli, bifidobacteria, E. coli and yeasts, have been used to promote human and animal health for a long time, and are most widely used in gastrointestinal disorders and post-antibiotic treatment. However, results of probiotic treatment interventions in IBD have been disappointing,120 and research to further understand their action and improve their clinical efficacy has so far been hampered by the inconsistent use of various strains, unsubstantiated health claims by manufacturers and poorly designed trials.

Probiotics clearly have potential for the treatment of IBD. First, they modulate the intestinal microflora by suppressing pathogenic effects. Second, they directly affect the intestinal immune system and can stimulate anti-inflammatory cytokine secretion, while suppressing pro-inflammatory mediators. Lastly, some probiotic strains directly reinforce the intestinal barrier through stimulation of mucus and defensin secretion and interaction with the AJC.

Modulation of the intestinal Microbiota

Probiotic bacteria, like any other micro-organisms, employ a number of strategies to improve their survival in the hostile environment of the intestine.121-126 These so-called fitness factors include specialised energy-generating systems (e.g. siderophores), secretion of antimicrobial substances (bacteriocins),127 competition for space128 and blockade of enterotoxin.129 As an example of the fierce competition that exists in the intestine is the successful application of probiotic therapy in infections with Clostridium difficile (e.g. Saccharomyces boulardii) and rotavirus (LGG).130-132 Therefore, given that dysbiosis occurs in IBD, therapeutic manipulation of the intestinal microflora appears an obvious concept in probiotic treatment of IBD. However, the adult intestinal microflora is very stable and resistant to change, and, with the possible exception of strains administered very early in life, probiotic bacteria are by and large unable to persistently colonise the human gut.133-136

Immune Modulation

In animal models for IBD, immune modulation by probiotics has been demonstrated. In general, probiotic benefits are more pronounced in preventive than therapeutic treatment protocols. Madsen and others have demonstrated a preventive effect of different lactobacilli in IL-10-/- mice.137-140 This has been confirmed several times in different animal models and with different strains of probiotics.141-145 This appears to be a result of modulation of the immune system, since several probiotic preparations induce the production of protective cytokines, including IL-10 and transforming growth factor-beta (TGF-β), and suppressed pro-inflammatory cytokines, such as TNF-α in IL-10-/- mice.143,144,146 Similar results were apparent in the mucosa of pouchitis patients and CD patients following treatment.147-149 Further evidence of a direct effect of probiotics on the immune system comes from studies of dendritic cell function. In IBD, dendritic cells are dysfunctional and initiate an overaggressive immune response.40 Probiotics have been shown to ameliorate this response in dendritic cells as well as in peripheral mononuclear cells, resulting in a more anti-inflammatory, regulatory cytokine profile rather than a pro-inflammatory cytokine profile.143,147-149

Cell culture studies provided some mechanistic data for this. Viable LGG and L. casei strain Shirota or LGG culture supernatant block TNF-α production induced by bacterial cell wall components (lipopolysaccharide)144,146 by stimulating increased granulocyte colony-stimulating factor secretion.144 Furthermore, a differential effect on distinct T-cell populations by E. coli Nissle 1917 was observed that might be the basis for immunoregulatory properties allowing a potent, but limited, inflammatory response on the mucosal level leading to a reduced secretion of pro-inflammatory cytokines (TNF-α, interferon-γ [IFN-γ], IL-2) and upregulation of regulatory cytokines (IL-10). These effects seem to be mediated by Toll-like receptor-2 (TLR-2) and TLR-4 regulated pathways, as Ecn is ineffective in knockout mice.150,151

Interestingly, non-viable components of probiotic bacteria seem sufficient to induce host responses.152-154 CpG motifs of bacterial DNA elicit pro-inflammatory Th-1-mediated responses that led to aggravated disease in an animal model of colitis152 and contributed to perpetuation of chronic intestinal inflammation.155 If given prior to the initiation of colitis, a prophylactic effect was observed.156 For VSL#3 it was demonstrated that the beneficial effects are mediated by their own DNA via TLR-9 signalling157 and not, as is the case for Ecn, via TLR-2 and TLR-4.158

The Physical Barrier

Clearly, modification of the properties of the intestinal barrier is an important component of IBD pathogenesis. Increased permeability will result in increased exposure of the immune system to bacterial antigens and production of cytokines. There are numerous examples of probiotic bacteria modulating each of the components of the intestinal barrier and in general ‘strengthening’ the barrier. These actions most likely contribute to effects of probiotics in IBD. In vitro,
inulin-oligofructose) was promising with a reduction in inflammatory effect, with a higher rate of patients remaining in remission.

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In conclusion, despite an increased understanding of host-microbe interactions and the properties of probiotic bacteria, there are few reports of effective treatment of IBD with probiotics and the overall effects are modest. However, before a decision either for or against the use of probiotics can be made, there is a need for larger, well-designed trials that take into consideration strain-specific effects of probiotics. The likely outcome is that tailor-made probiotic formulations targeting well-characterised disease subgroups will be an effective treatment option in the future.


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