Probiotics and Inflammatory Bowel Disease

Gordon S. Howarth[1,2,3,4,5,6]

[1]Centre for Paediatric and Adolescent Gastroenterology, Children, Youth and Women's Health Service; Disciplines of [2]Physiology, [3]Paediatrics, and [4]Agricultural and Animal Science, University of Adelaide; [5]School of Pharmacy and Biomedical Sciences, University of South Australia; [6]School of Biological Sciences, Flinders University, Adelaide, South Australia.

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Correspondence: Associate Professor Gordon S. Howarth

Centre for Paediatric and Adolescent Gastroenterology

Children, Youth and Women's Health Service

72 King William Road

North Adelaide, South Australia, 5006, Australia

Email: gordon.howarth@adelaide.edu.au

Tel: 61-8-8161-6872

Fax: 61-8-8161-6088

Abstract

The aetiology of inflammatory bowel disease (IBD) remains obscure, currently thought to be associated with a genetic predisposition, dysregulation of the mucosal immune system, and a loss of antigen tolerance to enteric microflora, further influenced by a range of other environmental factors. In many cases, disease activity can be unremitting and refractory to treatment, with an unpredictable response to conventional therapy. To this end, new treatment strategies are being pursued on the basis of our understanding of IBD pathogenesis, and there is increasing evidence that at least some components of the enteric flora are primary contributors. Restoring the balance of the colonic microbiota to a less-pathogenic state is therefore a desirable strategy. Probiotics are currently defined as live non-pathogenic micro-organisms that, when ingested, exert a positive influence on host health beyond basic nutrition. On this basis, probiotics hold the potential to restore normal intestinal homeostasis. Despite more than a century of anecdotal reports of probiotic efficacy in gastrointestinal disease, only relatively recently have well-controlled, scientific studies and clinical trials, been conducted. Whilst reliable *in vitro* predictors of potential *in vivo* efficacy of putative probiotics await development, well-characterised animal model systems are proving valuable for the methodical, pre-clinical development of probiotics. Although early probiotic applications focussed largely on lactic acid bacteria (*lactobacilli*) and *bifidobacteria*, the range of candidate probiotics has now expanded significantly. Successful clinical application of the probiotic formulation, VSL#3, for treatment of the pouchitis variant of IBD, has instilled new excitement into the applicability of probiotics to IBD treatment, and the potential importance of probiotic combinations. The availability of new recombinant methodologies to develop 'designer' probiotics, capable of synthesizing and secreting specific factors, ranging from vitamins through to antibodies, further broadens the scope for probiotic application in IBD. Indeed, there are encouraging reports that probiotics may not need to be viable, or even intact, to exert their beneficial effects, with reports of therapeutic benefit from bacterial components such as DNA. In addition to the development of rigorous predictive systems to ascertain probiotic efficacy, challenges for the future will include determining the

optimal probiotic, or probiotic combination, and its timing of administration during phases of IBD relapse and remission. At present, our understanding of the intestinal microflora, and the importance of its composition and variability between individuals, is limited. However, once this understanding has been attained, strategically-designed probiotic formulations could ultimately be ‘tailored’ to suit individual IBD patients.

Introduction

Inflammatory bowel disease (IBD) is the collective term for a group of chronic, idiopathic inflammatory disorders that affect the gastrointestinal system. Crohn's disease and ulcerative colitis (UC) are the most common and serious variants of IBD, together imposing significant patient morbidity, economic burden, and long-term healthcare dependence. The aetiology of inflammatory bowel disease (IBD) remains obscure, currently thought to be associated with a genetic predisposition, dysregulation of the mucosal immune system, and a range of other environmental factors (1). In many cases, disease activity can be unremitting, with an unpredictable response to conventional therapy.

Considerable progress has been made in studies of IBD genetics over the last decade, and the complementary strategies of genome-wide scanning and candidate gene-directed studies have led to the identification of a number of genetic markers that appear to predict disease susceptibility and behaviour (2). Identification of genetic markers that predispose to inflammation (alleles DR2, DRB1*0103 and DRB1*12) and a Crohn’s disease susceptibility gene on chromosome 16 [NOD2/CARD 15: Nucleotide-binding oligomerization domain protein 2] has paved the way for exciting new developments in therapeutic approaches for IBD (3,4). NOD2/CARD 15 is a cytosolic protein involved in intracellular recognition of microbes by sensing peptidoglycan fragments, and there is compelling evidence that it serves as an intracellular pattern recognition receptor to enhance host defence by inducing the production of antimicrobial peptides such as human beta-defensin-2 (5).

The characterisation of newly-identified NOD2/CARD-15 mutations is providing new information on the likely contribution of a defective host anti-microbial defence system to IBD aetiology and pathogenesis. This could have exciting implications for the use of candidate micro-organisms as a novel treatment strategy for IBD. Prospectively, this information would be particularly valuable when combined with a concomitant strategy to address the genetic basis of responsiveness to IBD therapy. Indeed, the spectrum of new treatment modalities for IBD has expanded exponentially in recent years (reviewed in 6). These genetic and genomic strategies could form the basis for better predicting the likelihood of responsiveness to newly-developed IBD therapies associated with the utilisation of micro-organisms, or factors derived from such organisms.

Probiotics

Although under continual review, the generally agreed definition of probiotics encompasses "live micro-organisms which, when consumed in adequate amounts, confer a health benefit on the host beyond basic nutrition" (7). This definition, however, may prove to be too limited and the term ‘alimentary pharmabiotics’ has been coined to further encompass dead organisms, or bacterial constituents which may be genetically or otherwise modified, and may not necessarily be restricted to those of human origin. *Lactobacilli* (8,9) and *bifidobacteria*, (10) species are generally referred to as archetypical probiotics, with increasing reports of probiotic properties attributed to non-pathogenic *Escherichia coli* (11,12) and non-bacterial organisms, such as *Saccharomyces boulardii* (13). Probiotics are thought to function through a number of different actions including: the production of antimicrobial agents such as bacteriocins, hydrogen peroxide and organic acids; blocking adhesion of pathogens or toxins to epithelial cells; providing antioxidant agents; and modulation of the immune system (14,15). Although the current review focuses on probiotic effects in the context of IBD, the complex interplay of mechanisms associated with specific organisms, or combinations of probiotic organisms, extends to applications for a broader range of medical disorders.

Probiotics and IBD

Perhaps not surprisingly, the idiopathic nature of IBD, combined with its profound inflammatory characteristics, has resulted in current therapeutic strategies being targeted, almost exclusively, at disease immunomodulation (16). Notwithstanding this historical approach, new treatment modalities are now being pursued on the basis of our increased understanding of IBD pathogenesis. To this end, there is accumulating evidence that at least some components of the enteric flora are primary contributors to the unrelenting intestinal inflammation so characteristic of IBD. In general terms, restoring the balance of the colonic microbiota to a 'less-pathogenic' state would appear a desirable preventative, or therapeutic, strategy. However, before we consider probiotics as a potential therapeutic option in IBD, we should consider the resident enteric microflora and the potential impact of probiotic organisms in the context of IBD aetiology.

The intestinal microbiota and IBD aetiology

Although the intestinal flora is essential for host defence, some of its constituents may, in genetically susceptible hosts, become a risk factor for IBD, and it has been proposed that resident bacterial flora play a pivotal role in IBD pathogenesis (17). Mechanisms underlying the influence of the intestinal flora on mucosal homeostasis, mucosal protection, development and function of immune responses, and metabolism of fecal residue are undergoing increased scientific scrutiny. Strategies to enhance the beneficial properties of endogenous microflora, or alternatively, to minimise deleterious effects, represent a logical therapeutic approach, forming the basis for manipulation of the intestinal flora in IBD treatment (18).

Nevertheless, it is important to understand that an inappropriate reaction to infection by a specific persistent pathogen has not yet been eliminated as a possible aetiological component in IBD. Indeed, Crohn's disease and UC share histopathological similarities with defined intestinal infections, and occur in areas with highest luminal bacterial concentrations (17,18)**.** Strengthened by the causative link between *Helicobacter pylori* and gastric ulceration (19), many microbial pathogens have been promoted as initiators and perpetuators of IBD. Perhaps

Mycobacterium avium, subspecies *paratuberculosis* (MAP) provides the most persuasive evidence as a causative agent in IBD since it manifests a Crohn's-like phenotype in livestock in the condition known as Jonne's disease (20). Other potential pathogenic causes have included *M. kansaii*, *Escherichia coli*, Diplostreptococcus species, viral vectors (measles, RNA viruses), *Listeria monocytogenes*, *Fusobacterium necrophorum*, *Chlamydia* species, *Pseudomona maltophila* and *Helicobacter hepaticus* **(**21**,**22,23). Taken together, identified probiotics capable of altering the intestinal environment such that colonization by the aforementioned species is inhibited, would therefore appear to be a logical strategy in IBD.

Antibiotics have a defined role in the management of IBD and its complications, although their long-term usage is undesirable due to the risk of toxicity, bacterial resistance and overgrowth. Nevertheless, it would be fair to state that the current weight of scientific literature identifying a single organism as causative of IBD, although highly desirable, is not particularly compelling. Although several authorities, including the Joint Food and Agricultural Organization of the United Nations and the World Health Organization (24,25), have described selection criteria for probiotic organisms, to date, no reliable *in vitro* predictors of *in vivo* efficacy of putative probiotics have been identified. This has been compounded by the realization that individual probiotics do not appear to act through a single mechanism, further complicated by the likelihood that probiotic combinations, as opposed to specific candidates, may be indicated for certain disorders. Indeed, the mechanism of action of probiotics is likely to vary with different strains and may also be dependent on the clinical condition for which it is applied. Although well-characterised animal models are gaining momentum as predictive pre-clinical efficacy systems, a discussion of likely probiotic mechanisms is indicated.

Mechanisms of probiotic action in IBD

Although perhaps a little simplistic, the ability for probiotics to prevent or combat infections may be partially- or entirely dependent upon mutual competitive metabolic interactions with

potential pathogens, production of anti-microbial peptides such as bacteriocins (25) or inhibition of epithelial adherence and translocation by pathogens (26,27). Interestingly, Collado et al (28) reported that in general, bifidobacterial strains of animal origin demonstrated an improved capacity to inhibit and displace pathogens from human mucus than were human strains, further reporting that bifidobacteria were capable of producing antimicrobial peptides directed against *Helicobacter pylori* (29). Cross-species utilization of probiotics may therefore provide another important level of complexity for probiotic utility in clinical disorders such as IBD. In certain allergic disorders, including atopic eczema, probiotic influences on mucosal barrier function may be operative (30), whilst multiple mechanisms may account for anti-neoplastic effects (31).

Probiotics, tolerance and the immune response

Since control of bowel inflammation has generally been recognised as paramount in IBD, it is perhaps not surprising that down-regulation of mucosal inflammation has been identified as the primary focus of both conventional and probiotic-targeted therapy, fuelled by the cumulative clinical experience with anti-TNF based treatment successes (32).

Rodents and humans are normally tolerant to autologous microbiota, and an association between breakdown of this tolerance and the development of chronic intestinal inflammation has been demonstrated (32). Potentially, pathological responses to components of the intestinal flora may occur under normal physiological conditions, but these may be suppressed by immunoregulatory mechanisms. In a recent review by Thompson-Chagoyán et al (33), 11 models of IBD have been described, in which inflammation was found to be dependent on the presence of normal flora; the absence of normal flora being associated with non-appearance of the condition (34,35,36,37). This phenomenon has been reported across species (mice, rats and guinea pigs), and to occur in manipulated organisms such as transgenic mice with targeted deletion of the T-cell receptor (TCRα), that spontaneously develop colitis in response to the gut microbiota (38). Mucosal inflammation in rats and mice with induced IBD has also been reported to respond to treatment with broad-spectrum

antibiotics (39). Moreover; in some animal model systems, colonisation with normal flora results in the rapid development of T-cell-mediated gut inflammation which can be transferred to other animals using activated T cells directed against enteric bacteria (40). Nevertheless, it appears that not all commensal bacteria have an equivalent ability to induce mucosal inflammation, which is also influenced by the host genetic background.

Following cell surface recognition via Toll-like receptors (41), the anti-inflammatory effects of probiotics require signalling with the epithelium and hence, the mucosal immune system (42). Although transduction of bacterial signals into host immune responses presumably involves more than one pathway, NF-kappaB has been established as a central regulator of epithelial responses to invasive pathogens (43,44). Non-pathogenic components of the flora may attenuate pro-inflammatory responses by delaying degradation of IkappaB, the counter-regulatory factor to NF-kappaB (44). Indeed, it may be that probiotic bifidobacteria and lactobacilli may not use the same mechanism to achieve their anti-inflammatory effects as other signal transduction pathways begin to emerge.

Clearly, a better understanding of the interplay between prokaryotes and eukaryotes, and subsequent effects on metabolic activity, will facilitate our knowledge of probiotic mechanisms. Bacterial adjuvants, including peptidoglycan, lipopolysaccharide, and DNA (CpG) bind to membrane-bound Toll-like receptors (TLR-2, 4, and 9. respectively), or cytoplasmic (NOD1 and NOD2) receptors (pattern recognition receptors), that activate nuclear factor-kappaB and transcription of many proinflammatory cytokines (45). Prokaryotic DNA perhaps represents the first description of a growing number of bacteria-sourced factors with the potential to alter host epithelial and mucosal immune responses. Un-methylated cytosine–guanine (CpG)-containing DNA, the ligand for Toll-like receptor 9 (TLR9), is a recently recognized microbial product with immuno-stimulatory and immuno-regulatory effects (46). TLR9 is expressed by many cell types located in the intestine, including epithelial cells and dendritic cells, and subcutaneous administration of immuno-stimulatory

DNA has been reported to reduce the severity of experimental and spontaneous colitis in murine models of IBD (47) via a mechanism attributed to TLR9 signalling (48).

A decrease in the secretion of pro-inflammatory cytokines, IFN-gamma, TNF-alpha and IL-12, and interference with bacterial adherence to the epithelium has been demonstrated following probiotic administration, associated with NF-kappaB inhibition, heat-shock protein induction and proteasome inhibition, although NF-kappaB induction has also been demonstrated (49,50). Unexpectedly, many of these beneficial effects have been achieved not only by live bacteria, but also by gamma-irradiated non-viable bacteria, bacterial DNA components and probiotic-cultured media (49,51). Investigations into probiotic supernatants and their therapeutic potential in IBD are therefore forming the basis for new directions in IBD research. In summary, although mechanisms of probiotic action may vary, depending on the experimental or clinical context, and depending on differences in the host and in the bacterial strain, the engagement with host immunity is pivotal to probiotic action in IBD.

Probiotics as therapeutic agents in IBD

The human colon is a densely populated microbial ecosystem with several hundred bacterial species usually present with a total weight estimated to be several hundred grams (52). There are up to 10^{13}–10^{14} total bacteria in the human intestinal tract, representing 10- to 20-fold more than the total number of tissue cells in the entire body, with most bacteria being obligate anaerobes, including clostridia, eubacteria, bacteroides groups and the genus *bifidobacterium*, such as *Bifidobacterium bifidum* and *Bifidobacterium infantis* (53).

Probiotic efficacy has been confirmed in animal model studies by several investigators with different probiotic strains (37,53), although the use of probiotic therapy for IBD has only recently attracted serious interest from clinicians. Clinical trials in Crohn's disease with organisms, including lactobacillus (54) yeast (13), and coliforms (56), have been confounded by small patient numbers, differences in disease activity and variations in disease distribution (56). These deficiencies have been exacerbated by a growing, but poorly regulated,

commercial market for probiotics, often linked with somewhat tenuous, or exaggerated, claims for health benefits. Historically, the stability, optimal dose-range, frequency of administration and vehicle for delivery, have rarely been determined. However, at present in Europe, well-designed and appropriately controlled trials of individual probiotic preparations are underway in both Crohn's disease and ulcerative colitis, to ascertain their efficacy in both active disease and in prevention of relapse.

Individual probiotics and IBD treatment

The predominant, potentially health-enhancing, bacteria in IBD are the bifidobacteria and lactobacilli, both of which belong to the lactic acid bacteria (LAB) group (57). These two genera do not include any significant pathogenic species and their dominance in the faeces of breast-fed babies is thought to impart protection against infection (58,59). Most commercially available probiotics meet minimum selection criteria including acid and bile resistance and survival during gastrointestinal transit, but an ideal individual probiotic strain for any given indication has still yet to be defined.

Lactobacilli and bifidobacteria have traditionally been the most common candidates for probiotic-based treatments in IBD. Although there is a growing body of scientific literature and a wealth of anecdotal information supporting the utility of lactobacillus strains as therapeutic agents in a range of alimentary disorders, progress has perhaps been hampered by poorly-controlled associations between probiotic administration and clearly-defined clinical end-points. Recently, Hawrelak et al (60) conducted a systematic review of six clinical trials investigating the capacity for *Lactobacillus rhamnosus* GG to prevent the onset of antibiotic-associated diarrhoea. As data sources, these investigators employed computer-based searches of MEDLINE, CINAHL, AMED, the Cochrane Controlled Trials Register and the Cochrane Database of Systematic Reviews and the bibliographies of relevant papers and previous meta-analyses. Four of the six trials found a significant reduction in the risk of antibiotic-associated diarrhoea with co-administration of *Lactobacillus* GG; one trial reported a reduced number of

days with antibiotic-induced diarrhoea, whilst the final trial found no benefit of *Lactobacillus* GG supplementation. These results are not unique in the context of retrospective probiotic studies, highlighting the need for additional research and the development of strictly-controlled predictive systems to clarify the effectiveness of Lactobacillus-based treatments beyond the effects of LGG in prevention of antibiotic-associated diarrhoea.

Bifidobacterium, a member of the dominant microbiota (i.e. $>10^8$–10^9 colony forming unit (CFU)/g, represent up to 25% of the cultivable faecal bacteria in adults and 80% in infants. As probiotic agents, bifidobacteria have been studied for their efficacy in the prevention and treatment of a broad spectrum of animal and/or human gastrointestinal disorders, such as colonic transit disorders, intestinal infections, and colonic adenomas and cancer (10). Indeed, certain strains (eg *Bifidobacterium animalis* strain DN-173 010) have been reported to prevent or alleviate infectious diarrhoea through effects on the immune system and resistance to colonization by pathogens and there is some evidence that certain bifidobacteria may actually protect the host from pro-carcinogenic activity of intestinal flora (10).

Non-pathogenic *Escherichia coli,* especially the Nissle 1917 strain, have generated increasing reports of efficacy in the context of inflammatory disorders. In ulcerative colitis, the non-pathogenic strain of *E. coli* Nissle demonstrates efficacy equivalent to that of mesalazine in ulcerative colitis (61,62). Kamada et al (63) utilising dextran sulphate sodium (DSS)-induced and IL-10 knock-out models of colitis have recently reported the non-pathogenic *E. coli* strain Nissle1917 to prevent both acute and chronic colitis, with its anti-inflammatory properties attributed not only to viable bacteria but also to heat-killed bacteria or its genomic DNA. Obermeier et al (64) demonstrated a pro-inflammatory effect of cytosin-guanosin dinucleotide (CpG)-oligodeoxynucleotide (ODN) treatment in established and chronic DSS-induced colitis, suggesting that DNA derived from luminal bacteria plays a role in the perpetuation of chronic intestinal inflammation. These investigators further speculated that treatment with adenoviral ODN (AV-ODN) could block the known CpG effects in IBD. The apparent discrepancy between bacterial DNA sources and effects on intestinal inflammation highlights

the need for well-controlled comparative studies of bacterial DNA, and, more specifically, CpG motifs, sourced from endogenous or probiotic bacteria.

It should be noted, however, that probiotic properties have not been attributed solely to bacterial sources, since non-bacterial organisms, such as *Saccharomyces boulardii*, or even nematode parasites have been utilised for probiotic purposes (13,65) In summary, it seems unlikely that a single probiotic will be equally suited to all indications; selection of strains for disease-specific indications will be required, and it is in this capacity that well-conducted animal model studies will prove a valuable tool

'Designer' probiotics

Genetic modification of food-grade commensal bacteria will need to be accommodated into our concept of probiotics. To this end, the term "pharmabiotic" has been developed as a more appropriate generic or umbrella term to encompass any form of therapeutic exploitation of the commensal flora (66,67). Food-grade bacteria can be modified, or engineered, to achieve specific functional activity. This can include delivery of anti-inflammatory cytokines or other biologically active molecules and vaccines to the gut. Of relevance to IBD, the food grade organism *Lactococcus lactis*, has been engineered to secrete IL-10 locally within the gut (68). When tested in two animal models of IBD, the magnitude of this effect was equivalent to corticosteroid therapy in its ability to decrease inflammation and disease severity. Another example of the potential applications of engineered commensal organisms is the genetic modification of lactobacilli resident within the female genital tract to express functional two-domain CD4 in order to confer protection against HIV infectivity *in vitro* (69). The future scope for this strategy is limitless, but public health and other safety concerns must be resolved before routine clinical use in humans can be instigated. Other examples of genetically modified (GM) microbes include the delivery of single-chain antibodies for pathogen-specific passive immunity (70,71) and bacterial-derived trefoil factors to promote healing and repair in the inflamed mouse gut (72).

Recently, Inglis et al (73) in a rodent model of intestinal mucositis, utilising the recently-developed non-invasive sucrose breath test, described a decrease in small intestinal inflammation following administration of the folic-acid secreting probiotic, *Streptococcus thermophilus* TH-4. Although not tested directly, these investigators speculated that the 'trickle'-delivery of *S. thermophilus* TH-4 sourced folate to the intestinal enterocytes may have reduced the severity of intestinal damage induced by the folate depletion, and resulting inhibition of DNA synthesis, manifest by methotrexate-based chemotherapy. Similarly, Geier et al (74) have described a partial reduction of colonic inflammation in a rodent model system utilising *Lactobacillus fermentum* BR11 reportedly via its unique capacity to modify the cystine/cysteine equilibrium and hence, reduce oxidative stress.

Public health concerns in relation to the release of genetically-modified organisms into the environment have replaced technological constraints as the major hurdles to be overcome with genetically-modified bacteria, and bio-containment has emerged as an environmental priority (75). Nevertheless, it would appear highly likely that future probiotic studies will be focussed not only on exploiting their existing beneficial properties, but also their capacity to deliver a broad range of specific factors, ranging from vitamins and antibodies through to strategically-developed, genetically-modified agonists and inhibitors.

Probiotic mixtures and formulations

Simple ingestion of a broad spectrum of probiotics would appear to be a pragmatic approach to cover a range of different indications and individual variations in host flora, although this would assume that individual probiotic constituents are not mutually antagonistic. Furthermore, it is a fundamental principle of therapeutic development that the properties and optimal usage of individual components of any mixture or formulation should be comprehensively determined before they can be recommended in combination. Undoubtedly, however, the most compelling evidence for probiotic efficacy in IBD has been reported with a combination of eight bacterial strains in the maintenance of remission of active ulcerative

colitis (76) and prevention of pouchitis (77). This combination, termed VSL#3, comprises four strains of lactobacilli and three strains of bifidobacteria, together with *Streptococcus thermophilus*. Indeed, there is now a sizeable scientific literature on VSL#3 and its application for a growing number of clinical disorders, with a recent report indicating hydrolytic activity against gliadin polypeptides suggesting a possible application for VSL#3 in the control of coeliac disease (78).

Rachmilewitz et al (48) have reported anti-inflammatory effects of VSL#3 in murine experimental colitis, mediated by Toll-like receptor (TLR9) signalling. Importantly, these authors described that intragastric and subcutaneous administration of probiotic DNA ameliorated the severity of experimental colitis, whereas methylated probiotic DNA, calf thymus DNA, and DNase-treated probiotics had no effect. Moreover, colitis severity was attenuated to the same extent by intragastric delivery of non-viable, gamma-irradiated, or viable probiotics, suggesting that the protective effects of probiotics are mediated by their own DNA rather than by their metabolites or ability to colonize the colon. The finding that live micro-organisms were not required to attenuate experimental colitis holds significant implications for the further development of probiotics as prophylactics or therapeutics in IBD. The development of further probiotic combinations for IBD treatment appears highly likely, although the current strategy will remain somewhat empirical until better-defined predictive *in vivo* and *in vitro* systems have been developed.

The emergence of more compelling pre-clinical data from well-controlled animal model studies is therefore proving to be a positive strategy for probiotic efficacy studies in prospective IBD research. Such a strategy, in the absence of a rigorous, rapid throughput *in vitro* screening assay, will become vital given the likely complexity of probiotic formulations comprising mixtures of currently-characterised and newly-developed ‘designer’ probiotics. To test the matrix of probiotic formulations in human clinical trials, even if conducted strategically, would clearly be impossible.

Prebiotics and Synbiotics

Prebiotics are defined as non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth of bacterial species already established in the colon and thus improve host health (79). These are usually of a poly- or oligo-saccharide nature with studies largely confined to fructo-oligosaccharide (FOS), galacto-oligosaccharide (GOS) and maltodextrin (80). The health interest of the *Bifidobacterium* genus is reflected in the commonly-accepted definition of prebiotics: food ingredients that selectively stimulate the growth and activity of bacteria in the gut, usually bifidobacteria (bifidogenic effect) and lactobacilli, thereby producing health benefits.

Although an exhaustive review of prebiotics is beyond the scope of this review, the importance of identified prebiotics in combination with dead or live probiotics (synbiotics), or biologically active bacterial metabolites, should not be under-stated. For example, Kanauchi et al (81) have recently described a synbiotic combination of germinated barley foodstuff (GBF) and *Eubacterium limosum* (*E. limosum*) and its potential as an adjunctive treatment for IBD. Although probiotic approaches for IBD include VSL#3, Nissle1917, *Clostridium butyricum*, and Bifidobacterium-fermented milk, Eubacteria have not been studied to any great extent. *E. limosum* is a commensal micro-organism that is promoted by GBF administration, and its metabolites include butyrate, which can accelerate intestinal epithelial growth and inhibit IL-6 production. GBF is therefore a prebiotic, and its unique characteristics make it highly suitable for applications in IBD. GBF prolongs remission in ulcerative colitis patients and also attenuates clinical activity in non-remissive colitic patients.

The further complexity of incorporating the increasing development and number of prebiotics into the expanding range of newly-developed probiotic formulations described previously, greatly increases the number of synbiotic combinations which await testing in pre-clinical systems. However, although this enormous number of synbiotics and designer synbiotics may be intimidating from a pre-clinical efficacy perspective, there exists the increased potential to identify greater numbers of promising, efficacious candidate treatment formulations for IBD.

Combining this strategy with genetic and genomic approaches to define IBD susceptibility and probiotic responsiveness could ultimately result in individually-tailored probiotic-based treatment approaches in IBD.

Personalised probiotics

Optimal selection of a probiotic may even need to take into account individual variations in host diet and composition of gut flora. In this respect, the apparent influence of human genetic variability on intestinal bacterial composition is particularly intriguing (82). Furthermore, it seems unlikely that a single probiotic will be equally suited to all conditions. As a consequence, selection of strains for disease-specific indications will be required (27). It is beginning to become apparent that our definition of probiotics may be somewhat simplistic since there are indications a probiotic under certain circumstances, may not be a probiotic at all under altered physiological or pathogenic states, or even in different hosts. Different probiotics have distinct properties, and not all models of experimental colitis respond to the same probiotics (83). Almost certainly, there will not be a 'one size fits all' approach to probiotic use in IBD. Personalised, designer probiotics and synbiotics could therefore be a real therapeutic option for IBD sufferers in the future.

Conclusions

Probiotics continue to hold great promise for IBD treatment and prevention, but despite some significant advances, it would be fair to say that this field of research is still in its infancy. Understanding the mechanisms responsible for the beneficial effect of probiotics in inflammatory bowel disease and experimental colitis will aid our understanding of the role of endogenous and exogenous bacteria in IBD aetiology and pathogenesis. The finding that live probiotics may not be essential for therapeutic effects, and that these effects may also be obtained by the systemic route of administration, could have a major impact on the use and manufacture of probiotics. Phenotypic and genomic characterisation of probiotic strains will be required, together with clarification of their mechanisms of action across a broad range of

rigorously-controlled clinical settings. Moreover, results of probiotic studies demonstrating efficacy in a given clinical setting should not be extrapolated to other disease indications without separate controlled assessments.

Over the past decade, there have been quantum advances in probiotic-based research in IBD. Initial, largely anecdotal, reports of lactobacillus efficacy have been replaced by the development of specific 'designer' probiotics, which together with identified prebiotics, could result in a significant repertoire of designer synbiotics for IBD treatment. Such an array of treatment options, combined with genetic advances in IBD susceptibility and probiotic responsiveness, could eventually result in the exciting emergence of individually-tailored probiotic-sourced formulations. The major challenge in the short-term will be the development of rigorous *in vivo* and *in vitro* testing systems to rapidly determine the suitability of any given probiotic-based formulation, not only to a specific condition, but also to the individual.

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