

Quarterly Review

Probiotic-induced changes in the intestinal epithelium: Implications in gastrointestinal disease

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ABSTRACT

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There is resurgent interest in the use of probiotics to maintain gastrointestinal and systemic health, driven by recent advances in knowledge of bacterial interactions with the epithelium and innate immune system of the intestine. The effects of probiotic bacteria on the intestinal epithelium and their downstream consequences are reviewed. Probiotics prevent pathogen adherence and invasion of the epithelium, partly by blocking adherence sites but also by upregulating gene expression of *MUC2* and of antimicrobial peptides. Metabolic effects of probiotics on the intestinal epithelium include production of short chain fatty acids which influence epithelial cell metabolism, turnover and apoptosis. Bacterial metabolism of unabsorbed dietary constituents with production of free radicals and phenolic metabolites can lead to DNA damage and cancer; probiotics restore eubiosis and potentially prevent this. Probiotics alter expression and redistribution of tight junction proteins and reduce intestinal permeability limiting absorption of noxious molecules from the gut lumen. Most studied are the effects of probiotics on epithelial cells which are the first line of innate immune-capable cells that encounter luminal flora. Probiotics, through secreted molecules, influence the innate inflammatory response of epithelial cells to stimuli from the gut lumen, and reduce mucosal inflammation. Through effects on dendritic, and possibly epithelial, cells they influence naïve T cells in the lamina propria of the gut and thus influence adaptive immunity. These varied effects of probiotics have implications for the treatment of several gastrointestinal diseases including antibiotic-associated colitis, acute gastroenteritis, inflammatory bowel disease, colon cancer, and irritable bowel syndrome.

KEYWORDS: probiotics, inflammatory bowel disease, antibiotic-associated colitis, irritable bowel syndrome.

Introduction

Probiotics (probiosis = for life) are defined as living micro-organisms which, when administered in adequate amounts, confer health benefits on the host.^{1,2} Although fermented foods containing live micro-organisms have been used for centuries, the recent surge of interest in probiotic preparations is attributed to our increased understanding of the innate immune system and how it distinguishes and reacts to harmless or beneficial bacteria as opposed to harmful bacteria. Probiotics are ingested; they survive gastric acid and duodenal bile and reach the small and large bowels to exert their effects. Their first prolonged contact with the human host is with the epithelium of the gastrointestinal tract, which thus acquires much significance when trying to unravel the physiological effects of probiotics.

Probiotics must be ingested regularly for any health promoting activity to persist. It is possible to manipulate (at least temporarily) the composition of the intestinal microflora through dietary supplementation with probiotics. The wide marketing of probiotics throughout the world confirms the popularity of this concept. Most probiotics in current use

comprise bacteria, either *Lactobacilli* or *Bifidobacteria*, although some yeast species are also used. *Lactobacilli* are Gram-positive, non-spore forming rods or coccobacilli.³ They are found in a variety of habitats where rich, carbohydrate-containing substrates are available, such as human and animal mucosal membranes, on plants or material of plant origin, sewage and fermenting or spoilt food. *Lactobacilli* are normally found in the intestine of infants in high numbers, but decline rapidly after infancy.⁴ *Bifidobacteria* are non-motile, non-sporulating Gram-positive rods with varying appearance. Most strains are strictly anaerobic. They constitute a major part of the normal intestinal microflora in humans,⁵ appearing in the stools a few days after birth and increasing in number thereafter. The number of *bifidobacteria* in the colon of young children is high, but this number decreases rapidly with age.⁴

Probiotics may influence gastrointestinal health in a variety of ways (**Table I**). This review outlines the scientific evidence which suggests that interactions of probiotic microorganisms with the intestinal epithelium leads to health benefits in the gastrointestinal tract.

Table 1: Intestinal effects of probiotics that may have an impact on gastrointestinal health

Probiotic effect	Outcome	Implication for disease
Production of SCFA	Proliferation of epithelium	Helps mucosal repair
	Maturation of epithelium	Anti-carcinogenic
	Apoptosis of damaged cells	Anti-carcinogenic
	Increases vascular supply	Helps mucosal repair
	Innate immune effects	Decreased inflammation
Production of lactate and other metabolites	Antagonism of enteric pathogens	Prevents gastroenteritis Prevents <i>C. difficile</i> overgrowth
	Antagonism of enteric pathogens	Prevents gastroenteritis Prevents <i>C. difficile</i> overgrowth
Secretion of protein factors	Prevents pathogen adherence to epithelium	Prevents gastroenteritis
Altered luminal metabolism	Xenobiotic metabolism	Reduces production of carcinogens Increases breakdown of carcinogens
	Diverts nitrogen breakdown to ammonia through alternate pathways	Ameliorates hepatic encephalopathy
	Reduces sulphide production	Prevents premature epithelial cell death
	Reduces free radical production	Prevents epithelial DNA damage
	Prevent pathogen adherence to epithelium	Prevention of gastroenteritis
Upregulation of <i>MUC</i> gene expression in epithelial cells	Strengthen intestinal barrier	Prevents or ameliorates inflammation
Altered tight junction production expression	Strengthens intestinal barrier	Prevents translocation from gut and cytokine release in liver disease
Immune conditioning in neonatal period (pathways unclear)	Oral tolerance	Prevents gut inflammation
	Maintain Th1-Th2 balance	Prevents allergic diseases
TLR9 stimulation	Maintains Th1-Th2 balance	Prevents gut inflammation
Increased defensin production	Antimicrobial effect	Prevents gut inflammation
Increased secretory IgA	Increased clearance of luminal antigens	Prevents gut inflammation

Probiotics and effects on intestinal luminal metabolism and epithelial cell function

The human gastrointestinal tract contains over 500 species of bacteria. Some of these are harmless to the host, some are beneficial and yet others are harmful to the host. In health there is an optimum gut flora balance; the beneficial bacteria, such as *Lactobacilli* and *Bifidobacteria*, predominate. It is suggested that the balance should be such that at least 85% of the intestinal microflora in a healthy person should be beneficial bacteria.⁶ Under certain conditions this balance, or

eubiosis as it has been eloquently termed, is lost leading to dysbiosis. A reduction in beneficial bacteria such as *Lactobacilli* and *Faecalibacterium prausnitzii* have been implicated in the pathogenesis of inflammatory bowel disease and to a lesser extent in colon cancer.⁷⁻¹¹ Such an altered bacterial balance may potentially change fluxes through the metabolic pathways in epithelial cells, or it may alter immune balance through altered innate immune activation involving epithelial and subepithelial cells. The regular ingestion of probiotics is one possible way of restoring balance in the intestinal microbial ecosystem.

Luminal antagonism of enteric pathogens

Probiotics can provide protection against a broad range of pathogens, including certain forms of *Clostridia*, *Escherichia coli*, *Salmonella*, *Shigella*, *Pseudomonas*, and yeasts such as *Candida albicans*. They may inhibit the growth of intestinal pathogens by competing for nutrients consumed by pathogenic bacteria or by producing substances that directly affect growth of pathogens. These inhibitory substances include metabolites such as lactic acid, short chain fatty acids and hydrogen peroxide, that are inhibitory to both gram-positive and gram-negative bacteria.¹² They also include bacteriocins and other soluble factors that inhibit the growth of pathogenic bacteria. *Lactobacilli* produce bacteriocins such as sakacin, lactocin, amylovorin and acidophilin.¹³ They exert antimicrobial activity in vitro against pathogenic *E. coli*, *Salmonella*, *Bacillus cereus*, *Campylobacter jejuni* and *H. pylori*. *Bifidobacteria* are also known to produce bacteriocins (eg. bifidin and bifidocin) and have antimicrobial activity against several pathogens including *E. coli*, *B. cereus*, and *S. aureus*.¹³⁻¹⁵

Blockage of epithelial adherence and invasion by pathogens

Probiotic *Lactobacilli* and *Bifidobacteria* can adhere to intestinal epithelial cells through surface-expressed proteins.^{16,17} In the case of *Lactobacillus casei* it has been shown that the bacteria bind to extracellular matrix components such as collagen, fibronectin or fibrinogen.¹⁸ It is hypothesised that some secreted factors of probiotics may inhibit binding of pathogenic bacteria to the appropriate receptor on the epithelial surface in addition to inhibiting epithelial cell invasion by the pathogen.^{16,17,19-21} The surface of the intestine is covered by mucus, which contains mucins secreted by intestinal epithelial cells as a major component. It has been shown that probiotic *Lactobacilli* upregulate the MUC2 mucin protein in epithelial cell lines^{22,23} and inhibit attachment of enterohemorrhagic *E. coli*.²³ These findings have correspondingly also been detected in vivo in rats, where a probiotic mixture of *Lactobacilli* and *bifidobacteria* increased the secretion of mucin and stimulated MUC2 gene expression (with minor effects on MUC1 and MUC3 gene expression) in the colon.²⁴ The *Lactobacilli* present in the mixture were the most potent in stimulating colonic mucin secretion. A major stimulatory effect of *Lactobacilli* on MUC3 mucin mRNA expression and extracellular MUC3 secretion has been shown in the HT29 colonic epithelial cell line.²⁵

Effects of probiotics on luminal metabolism

Bacteria produce a variety of metabolites, some of which are useful and others which are harmful to the human host. The

colonic bacteria derive metabolic energy from unabsorbed carbohydrates in the lumen of the gut, fermenting them to various products the most important of which are short chain fatty acids. Short chain fatty acids such as acetate and butyrate influence colonic epithelial function in many ways; they are the major metabolic fuel for the epithelial cells, they influence gene expression in the epithelium, and they influence epithelial proliferation and barrier function.²⁶⁻²⁹ Reduction in short chain fatty acid production may play a role in the pathogenesis of a variety of gastrointestinal illnesses including antibiotic-associated colitis, inflammatory bowel disease, colon cancer and hepatic encephalopathy.²⁶ *Eubacterium rectale* and *Faecalibacterium prausnitzii*, two of the major short chain fatty acid-producing bacteria in the colon, are reduced in number in patients with colon cancer.²² Short chain fatty acids help in differentiation of the colonic epithelium as well as in inducing apoptosis in damaged epithelial cells, an action that is likely to be useful in prevention of colon cancer. Probiotics stimulate the production of short chain fatty acids by bacteria in the colon,³⁰ and may potentially be useful in disease conditions where deficiency of short chain fatty acids are postulated to be involved in pathogenesis.

Bacteria in the lumen of the intestine also produce molecules that may be toxic to the intestinal epithelium. For instance bacterial metabolites, such as hydrogen sulphide produced by sulphate-reducing bacteria and extracellular superoxide produced by bacteria such as *Enterococcus faecalis*, can damage DNA in the colonic epithelium.³¹⁻³³ These changes may increase the rate of occurrence of somatic mutations, and may result in development of colon cancer in predisposed individuals. Sulphide interferes with metabolism of short chain fatty acids and thus may cause premature death of epithelial cells.³⁴ Epithelial cells in the colon are important sites of detoxification of bacterial metabolites, especially phenolic metabolites resulting from protein breakdown, produced in the lumen of the colon.^{35,36} Alterations in the balance of bacteria in the colon may lead to the generation of more metabolites than the epithelium can cope with. For instance, damage of DNA induced by bacterial superoxide may result in somatic mutations in epithelial cells that predispose to the development of colon cancer. The administration of probiotics can potentially reduce the levels of sulphide and superoxide produced by luminal bacteria and protect epithelial cell metabolic and repair processes.

Hepatic encephalopathy is another condition in which bacterial production of ammonia and other metabolites from nitrogenous substrates in the intestinal lumen contributes to pathogenesis. Traditionally the treatment involves administration of an antibiotic or a prebiotic (eg. Lactulose) to alter bacterial metabolism. There is now experimental evidence that luminal bacterial metabolism may be directed away from ammonia into other pathways, leading to reduced systemic blood levels of ammonia and related metabolites.^{37,38}

Effects of probiotics on intestinal epithelial tight junctions

The intestinal mucosal barrier is composed of epithelial cells, the tight junctions between the cells, and the mucus layer overlying the epithelium. The tight junctions between epithelial cells are a significant component of the intestinal barrier. They are made up of a number of proteins including the claudins, occludin, zona occludens (ZO) proteins, and junctional adhesion molecules, which together determine tight junction

structure and function. Tight junction structure and function is affected by a number of nutrients and bacterial molecules which affect the expression of the tight junction proteins. Tight junction protein expression may also be influenced by genetic factors, resulting in barrier defects in celiac disease and ulcerative colitis. A variety of probiotic bacteria, including *Lactobacillus rhamnosus* GG, *Bifidobacterium infantis*, *Bifidobacterium lactis* and *Escherichia coli* Nissle 1917, appear to increase tight junction integrity and prevent tight junction disruption by noxious chemical influences or secondary to microbial pathogens.³⁹⁻⁴⁴ There is evidence that the effects of probiotic bacteria are mediated by soluble peptides which are secreted into the medium. The biochemical pathways mediating the probiotic effect on tight junction function include protein kinase C and MAP kinase pathways, and involve both redistribution and altered expression of the tight junction proteins occludin, ZO-1 and ZO-2 and claudins 1, 2, 3 and 4.^{39,42,43}

Probiotic effects on immune conditioning via the epithelium and sub-epithelial cells

The intestine is sterile at birth. Colonisation by bacteria begins immediately after birth and is influenced by the route of delivery, hygiene of the neonatal environment, and maternal bacterial flora in the diet. The initial exposure to intestinal bacteria is very important in immune conditioning of the host. The commensal bacteria in the gastrointestinal tract are the primary stimulus for the intestinal mucosal immune system and are necessary for normal immune development. For instance, bacteria-free animals grew to develop a Th2 predominant immune system with excessive production of IL-4.⁴⁵ In these animals intestinal exposure to the single bacterium *Bacteroides fragilis* returned the immune balance to normal by inducing the production of interferon- γ .⁴⁵ This cytokine, which is produced by CD4+, CD8+ and natural killer cells, is essential for successful clearance of intracellular pathogens such as viruses, and for host defense against malignant transformation. Using genetically modified *Bacteroides fragilis* in germfree animals, it has been shown that polysaccharides produced by the bacteria were important in this neonatal immune conditioning. In the absence of the relevant polysaccharide, the animals had smaller spleens and lymphoid tissue and their immune system responded with a Th2 type of response. There is evidence from clinical studies that administration of probiotics to adults may also result in production of Th1 responses and interferon- γ . For example, administration of probiotics regularly increased resistance to viral infections in several settings.⁴⁶⁻⁴⁸ Whilst it is not known how these effects are mediated, increase of interferon- γ is a likely mechanism, given its role in containing viral infections. The intestinal epithelium and intestinal dendritic cells are both likely to play roles in the genesis of this effect of probiotics. Another implication of the immune effects of probiotics is the possibility that altered immune conditioning in the intestine in the neonatal period may predispose individuals to develop atopic diseases in later life, by skewing their immune responses to a Th2 nature. Probiotic exposure at an early age may potentially prevent expression of such diseases.

The mucosal immune system of the gastrointestinal tract is regulated in such a manner that it causes controlled inflammation. Unlimited immune activation in response to

commensal bacteria could lead to the risk of inflammation. The intestinal mucosal immune system has developed specialised regulatory, anti-inflammatory mechanisms for eliminating or tolerating non-antigenic food substances and commensal micro-organisms (oral tolerance).⁴⁹ At the same time the mucosal immune system must provide local defence mechanisms against environmental threats such as invading pathogens.

This important feature is coordinated by strongly developed innate defense mechanisms ensuring appropriate functioning of the mucosal barrier,⁵⁰ existence of unique types of lymphocytes and their products, transport of polymeric immunoglobulins through epithelial cells into secretions⁵¹ and migration and homing of cells originating from the organised tissues in the mucosa and exocrine glands.

Oral tolerance is the phenomenon where the oral administration of an antigen (to which the body is already sensitised) produces a gradual reduction in the immune or inflammatory response to it. This phenomenon is deficient in germ-free mice due to a failure to generate suppressor cells. Oral tolerance can be induced in these animals by colonisation of the bowel with *Bifidobacterium infantis*.⁵² The supernatant of *Bifidobacterium breve* has been shown to induce dendritic cell activation and maturation and to prolong dendritic cell survival through a toll-like receptor 2 (TLR2)-dependent pathway.⁵³ This is characterised by increased production of IL-10, the regulatory cytokine, which may be responsible for amelioration of inflammation.

Effects of probiotics on innate immune and inflammatory pathways in epithelial cells

The most prominent effects of probiotics on the intestinal epithelium relate to innate immunity. The immune system has two components – the innate immune system which is conserved across species⁵⁴ and provides basic but non-specific recognition of harmful motifs in the environment, and the adaptive immune system which recognises specific antigens and reacts to them. The gastrointestinal mucosa, through its folds, villi and microvilli, provides the largest surface area in the body, in excess of 100 m², and contains a mucosal immune system that protects it from the external environment.

The adaptive immune system in the gut, also known as the gastrointestinal-associated lymphoid tissue, has inductive and effector sites.⁴⁹ The inductive sites include the Peyer's patches in the small intestine, isolated lymphoid follicles scattered in the lamina propria, and mesenteric lymph nodes, which together make the gut the largest lymphoid organ in the body. The effector sites include lymphocytes in the lamina propria surrounding Peyer's patches and lymphocytes scattered throughout the epithelium. The epithelial cells that line the gut separate the lumen from the underlying lymphoid tissue. Specialised areas of epithelium, such as the follicle-associated epithelium overlying the Peyer's patches and lymphoid follicles that contain microfold M cells, are particularly the site of endocytosis of foreign antigens or particles. Antigens may also gain entry to the basolateral surface of intestinal epithelial cells by disrupting the tight junction structure.

The intestine is rich also in cells that represent the innate immune system.⁵⁰ The epithelial and dendritic cells in the intestinal mucosa possess pattern recognition receptors (PRR) which recognise basic microbe-associated molecular patterns that distinguish broad classes of harmful and

harmless bacteria. The Toll-like receptors (TLRs) are the best known of the PRRs.⁵⁵ In addition to recognising and initiating signaling cascades for taking action against harmful bacteria, these receptors also recognise some beneficial bacteria. The innate immune cells, exemplified here by the intestinal epithelial cells, secrete effector molecules including cytokines and chemokines or microbicides, in response to microbes that are perceived as pathogenic. Cytokines or chemokines recruit inflammatory and immune cells to the intestine. They may also influence the development of adaptive immunity. Antigens, taken up by the microfold cells covering lymphoid nodules, are processed through antigen presenting cells to influence T cell differentiation into Th1 or Th2 cells, or T regulatory cells. Th1 activation is characterised by overproduction of interferon- γ and tumor necrosis factor- α that, when excessive, leads to inflammation. Th2 activation on the other hand is characterised by the cytokines interleukin-4 and interleukin-5 and when excessive leads to allergic diseases. T regulatory cells maintain the balance between Th1 and Th2 polarisation. It is now considered that probiotic bacteria, through their effect on the innate immune system, play significant ongoing roles in modulating T cell differentiation and polarisation of the immune response. The epithelial cells of the intestine participate in the innate immune responses to luminal bacteria and provide communication between luminal stimulants and underlying lymphoid elements in the lamina propria. In response to interaction with microbes in the lumen, the epithelium regulates the transcription of inflammatory cytokines for excretion into the lumen and interstitium and upregulates surface molecules, such as class-II antigens and the polymeric immunoglobulin-A (poly-IgA) receptor, for control in handling foreign antigens.⁵¹ This interaction with luminal pathogens and commensal flora with the gut is termed microbial-epithelial "cross talk" and represents an important contributor to intestinal barrier function.

The cytokine and chemokine response of epithelial cells has been examined by observing their interaction with bacterial pathogens. A variety of bacterial and viral pathogens up-regulate the expression of chemokines in intestinal epithelial cells.⁵⁶⁻⁶⁰ Most prominent among these are interleukin 8 and CXCL-5. This ability of epithelial cells to produce various factors promoting the infiltration of neutrophils and lymphocytes to the site of infection serves as an 'early warning system', whereby the epithelium signals to the underlying immune and inflammatory cells. Probiotics block the release of chemokines such as IL-8 and of inflammatory cytokines from epithelial cells in response to pathogenic bacteria or other inflammatory stimuli,^{59,61} and diminish pathogen-induced epithelial cell death.⁶² Pre-incubation with *Lactobacillus casei* protected intestinal epithelial cells against *Shigella flexneri* infection.⁶³ Blockade by probiotics of the pro-inflammatory response of the intestinal epithelium to both *Shigella* and *Vibrio cholerae* appears to be mediated through blockage of inhibitory κ B degradation leading to suppression of NF κ B signaling.^{59,63} Secreted factors may also be responsible for the effect of probiotics in preventing pathogen infection of epithelial cells, and these have been shown to act through ERK and p38 pathways to reduce interleukin-8 secretion.⁶⁴ Probiotic *Lactobacilli* and *bifidobacteria* reduced IL-8 secretion and increased IL-10 secretion in response to *Listeria monocytogenes* in an epithelial cell line.⁶⁵ LPS-induced NF κ B activation and inflammatory gene expression in HT-29 cells were attenuated by probiotic *bifidobacteria*.⁶⁶ *B. infantis* and *L.*

salivarius attenuated IL-8 secretion at baseline and in response to *S. typhimurium* and flagellin.⁶⁷ Upregulation of *MUC2* gene expression in epithelial cell lines also constitutes a part of the defense system activation by probiotics.⁶⁸

The anti-inflammatory effects of probiotics on the intestinal epithelium have also been shown in other experimental situations. *Bifidobacteria* modulated inflammatory cytokine secretion in biopsies from ulcerative colitis patients.⁶⁹ Similarly, probiotic bacteria down-regulated secretion of tumour necrosis factor- α in explant organ cultures of colonic mucosa from patients with inflammatory bowel disease or animals with experimental colitis.^{70,71}

On the other hand, probiotic bacteria may also induce production of pro-inflammatory cytokines by the intestinal epithelium. It has been reported that the probiotic micro-organism *Lactobacillus reuteri* transiently induced interleukin IL-1, IL-6, interferon-gamma-inducible protein 10, and macrophage inflammatory protein 2, while transiently decreasing the regulatory proteins A20 and Toll-interacting protein, in intestinal epithelial cells in primary culture.⁷² It has been hypothesised that this transient activation in the presence of established microflora may aid mucosal homeostasis.

The Paneth cells in the intestinal epithelium secrete antimicrobial peptides such as the defensins and cathelicidins. α -defensins secreted by Paneth cells are processed by matrilysin or matrix metalloprotease 7 to form microbicidal peptides. These are regulated through the NOD2 pathway and probiotic bacteria may be important in maintaining Paneth cell secretion of these antimicrobial peptides.^{73,74}

Probiotic microorganisms reduce mucosal inflammation in a variety of animal models of inflammatory bowel disease.^{71,75–80} There are several genetically determined models of colitis, where mice spontaneously or after appropriate stimulation develop colitis, and where probiotics attenuate or prevent colitis. It has been shown that the anti-inflammatory effect of probiotics in experimental colitis may be mediated by stimulation of toll-like receptor 9 by unmethylated CpG DNA of the probiotic bacteria.^{81,82}

Although this review primarily deals with the intestinal epithelium, it must be recognised that probiotics have effects on sub-epithelial immune cells, including the dendritic cells and T-cells,^{83,84} and the effects of probiotics on intestinal immune and other responses must ideally be considered a composite response of the epithelium and sub-epithelial cells. Dendritic cells are antigen-presenting cells found throughout the gut that continually sample enteric antigens and have the ability to discriminate the different microbial strains using the pathogen associated molecular patterns. They then influence naive T cells in the intestine, resulting in T-cell activation and differentiation to Th1 helper, Th2 helper or regulatory Th3 cells.⁸⁵ The interaction of probiotic bacteria with dendritic cells results in the upregulation of IL-10 production and decreased production of co-stimulatory molecules and Th1 proinflammatory cytokines. The increase in IL-10 production may have anti-inflammatory effects mediated in part by enhanced numbers of T-regulatory cells.⁸⁶ Some studies suggest that probiotic bacteria also upregulate expression of non-epithelial cell molecules involved in host anti-bacterial defense. Probiotic supplementation with strains of *Lactobacillus* and *Bifidobacterium* increased the numbers of IgA secreting cells in milk-fed infants.^{87,88} Secretory IgA levels have been shown to increase following chronic ingestion of probiotics.^{89,90}

Implications of probiotic effects on the epithelium for gastrointestinal disease

Antibiotic-associated diarrhoea and *Clostridium difficile* diarrhoea:

The normal commensal flora in the lumen of the colon remains in a state of balance, as harmful or pathogenic bacteria are not allowed to flourish in significant numbers in health. The administration of antibiotics (notably ampicillin, amoxicillin, cephalosporins and clindamycin) in the host diminishes or alters the normal colonic flora and leads to antibiotic-associated diarrhoea. The pathophysiology of this condition is not fully understood; it has been postulated that the altered fecal flora results in changes in colonic carbohydrate digestion, decreased short-chain fatty acid production and diarrhoea secondary to colonocyte energy depletion.⁹¹ In some patients, there is overgrowth of the pathogen *Clostridium difficile*, which produces toxins that cause colonic damage. *Clostridium difficile* is a gram-positive anaerobic bacterium that produces two toxins, an enterotoxin A and a cytotoxin B, which cause colitis.^{92,93} Both bacteria and yeast have been studied in the prevention of antibiotic-associated diarrhoea and *Clostridium difficile*-associated diarrhoea. In a meta-analysis of 10 trials including 1986 children,⁹⁴ the per-protocol analysis of 9 showed statistically significant results favoring probiotics (Relative risk 0.49, 95% CI 0.32–0.74) in the prevention of antibiotic-associated diarrhoea, with the number needed to treat to prevent 1 case of diarrhoea being 10. *Lactobacillus GG*, *Bacillus coagulans*, and *Saccharomyces boulardii* were the most effective. On the other hand, probiotics were not so useful in the treatment of *C. difficile* colitis. Of 4 evaluable studies, only one study showed a statistically significant benefit for probiotics combined with antibiotics while no benefit was seen in the other studies.⁹⁵ Although probiotics were not useful in treatment of *C. difficile* colitis yet their metabolic effects cannot be disregarded. Production of short chain fatty acids may be necessary to regulate colonisation by *C. difficile*. It may also be important via the effects of SCFA on epithelial cell metabolism, blood flow and differentiation. It is also known that probiotics up-regulate *MUC2* and *MUC3* gene expression which may be responsible for the reduced colonisation by *C. difficile*.

Acute gastroenteritis

Probiotics have been used widely in the prevention and treatment of acute gastroenteritis and acute infectious diarrhoea. A meta-analysis of trials involving prevention of diarrhoea evaluated 34 masked, randomised, controlled trials, and concluded that probiotics reduced the incidence of antibiotic-associated diarrhoea by 52% (95% CI 35–65%), reduced the risk of travellers' diarrhoea by 8% (–6 to 21%), and that of acute diarrhoea of diverse causes by 34% (8–53%). Probiotics were more effective in preventing acute diarrhoea in children, reducing the risk among children by 57% (35–71%) compared to 26% (7–49%) among adults. The protective effect did not vary significantly among the probiotic strains used.⁹⁶ A Cochrane meta-analysis evaluated the role of probiotics in treatment of acute diarrhoea and concluded that mean duration of diarrhoea was shortened by 30.48 hours (18.51–42.46) in patients receiving probiotics.⁹⁷ No specific effect of individual probiotic preparations or of diarrhoea

aetiology was noted. The effects of probiotics on epithelial cell responses to bacterial pathogens have already been described above. The clinical effect of probiotics in diarrhoea may be partly related to the epithelial effects of probiotics and to the effects of probiotics on the intestinal barrier including mucus and antimicrobial peptides, which together contribute towards defense against pathogens.

Inflammatory bowel disease

Animal models of inflammatory bowel disease (IBD) clearly demonstrate a role for endogenous and pathogenic intestinal bacteria in driving the abnormal immune responses. Although mice deficient in the immunoregulatory cytokine interleukin IL-10 develop spontaneous colitis, IL-10 deficient germ-free mice remain disease free.⁹⁸ Moreover, feeding specific 'beneficial' bacterial strains to these animals reduces inflammatory lesions and modifies cytokine production. The role of endogenous intestinal flora has also been demonstrated in transgenic HLA-B2 rats with colitis,⁹⁹ and immune-deficient SCID mice reconstituted with immunocompetent CD45+ T cells.¹⁰⁰ Studies indicate that probiotics can prevent or attenuate experimental colitis in a variety of settings and using a variety of probiotics. Administration of *Lactobacillus reuteri* significantly reduced inflammation in acetic acid- and methotrexate-induced colitis in rats.⁷⁵ *Lactobacillus* species prevented the development of spontaneous colitis in IL-10 deficient mice¹⁰¹ while continuous feeding with *Lactobacillus plantarum* was useful in therapy of colitis in the same knockout model.⁷⁶ *Bifidobacterium infantis* and *Lactobacillus salivarius* were able to attenuate inflammation and reduce the ability to produce Th1-type cytokines in the IL-10 knockout model.¹⁰² *Lactobacillus salivarius* reduced the rate of progression from inflammation to dysplasia and colonic cancer in IL-10 deficient mice.¹⁰³ The probiotic mixture VSL#3 resulted in a significant attenuation of inflammation with a decrease of myeloperoxidase and nitric oxide synthase activity of iodoacetamide-induced colitis.⁷⁷

Although there is evidence of benefit from probiotic use in experimental inflammatory bowel disease, it has been hard to translate this to clinical medicine. Numerous clinical trials have been conducted with probiotics in the management of various situations in inflammatory bowel disease. The trials in inflammatory bowel disease have been conducted in different styles, using different probiotic preparations and very variable end-points that do not allow adequate conclusions regarding the utility of probiotics in IBD. Probiotics have been used to induce remission in ulcerative colitis and have been compared to standard therapy or placebo. Two recent reviews of all available studies found no definite evidence that probiotics were effective in inducing remission in ulcerative colitis.^{104,105} Formal meta-analysis was not possible due to heterogeneity in the organisms used, the dosages used, duration of treatment, and comparators. A number of studies have suggested that administration of a probiotic, non-pathogenic *Escherichia coli* (Nissle strain 1917), is as effective as standard therapy with aminosalicylates in the maintenance of remission in ulcerative colitis.¹⁰⁶⁻¹⁰⁹ The data with regard to the use of probiotics in Crohn's disease is even less complete. A recent Cochrane review of the use of probiotics to induce remission in Crohn's disease found only one evaluable study with 11 patients.¹¹⁰ 4 of 5 patients in the probiotic group achieved clinical remission compared to 5 of 6 patients in the

placebo group. There was thus insufficient evidence to confirm or exclude a role for probiotics in induction of remission in Crohn's disease. Three recent reviews, one a Cochrane review, examined the evidence for a role of probiotics in the maintenance of remission in Crohn's disease.¹¹¹⁻¹¹³ All three concluded that there was no evidence to support a role for probiotics in the maintenance of remission in patients with Crohn's disease. The only place in inflammatory bowel disease management where probiotics appear to confer definite benefit is in preventing the onset of acute pouchitis in patients with newly formed ileal pouches, and in maintaining remission following antibacterial treatment of acute pouchitis in patients with a history of refractory or recurrent pouchitis. The benefit is confirmed by a meta-analysis that reviewed 5 trials using the probiotic VSL#3.¹¹⁴

Irritable bowel syndrome

The pathogenesis of irritable bowel syndrome remains unknown, and abnormal neural activation, neural innervations, or abnormal innate immune responses have all been implicated. The therapy of irritable bowel syndrome has remained largely unsatisfactory. Against this background, there have been attempts to evaluate a role for probiotics in the therapy of patients with irritable bowel syndrome.

Several recent meta-analyses have examined the evidence for benefit from probiotics in irritable bowel syndrome.¹¹⁵⁻¹¹⁸ In all analyses, it appeared that probiotics were associated with a modest benefit (approximately 20-24%) compared to placebo. These were short term studies, and since irritable bowel syndrome requires long term therapy whether probiotics will provide sustained clinical benefit over longer periods of time, and whether they are useful in specific sub-groups, needs to be evaluated.

Colon cancer

Probiotics and prebiotics alter metabolic processes within the lumen of the gut and within the colonic epithelium, which impact on carcinogenesis.^{119,120} Such processes include biotransformation of organic compounds to carcinogens, detoxification of carcinogens, DNA damage and repair in the epithelium, and apoptosis of damaged cells. There is evidence from experimental models showing that aberrant crypt foci are reduced and apoptosis of damaged cells increased after probiotic administration.¹²¹⁻¹²³ *Butyrivibrio fibrisolvens*, a butyrate-producing bacterium, has been shown to reduce aberrant crypt foci in experimental colon cancer; this organism is found in the feces of apparently healthy rural residents of south India,¹²⁴ which may explain a lower incidence of colon cancer in this region. However, clinical trials of probiotic use in the prevention and treatment of colon cancer in humans are scarce or non-existent. This may change in the near future with a resurgence of interest in probiotics.

Probiotics in liver disease

In recent years, it has been recognized that several of the manifestations of chronic liver disease including encephalopathy, endotoxemia and bacterial peritonitis have primary origins in the gut through translocation of molecules or bacteria past the intestinal barrier. Probiotics enhance intestinal barrier function; they also influence innate immune

activity and adaptive immunity. Probiotics have therefore been tried in the therapy of chronic liver disease. Small pilot studies or open label studies suggest that probiotics may benefit patients with non-alcoholic steatohepatitis, alcoholic liver disease, and minimal hepatic encephalopathy and lowers endotoxemia in liver cirrhosis.¹²⁵⁻¹²⁹ These findings need to be confirmed in large randomised trials before a place can be established for the use of probiotics in chronic liver disease.

Conclusion

The interaction of bacteria with the intestinal epithelium has been very well characterised and understood in recent years. The role of probiotic micro-organisms in health and several disease conditions of the gastrointestinal tract in experiments has been detailed in this review. However, their use in clinical medicine in the treatment of gastrointestinal disease is considerably less well established, with clear evidence showing benefit only in antibiotic-associated diarrhoea and acute gastroenteritis. Large studies using standardised protocols along with standardised bacteria types and doses are necessary to conclusively establish a place for probiotics in the management of other gastrointestinal diseases.

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