Probiotics and inflammatory bowel disease: from fads and fantasy to facts and future

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Probiotic therapy is attracting the renewed interest of clinicians and basic investigators from a variety of traditional research disciplines. While the theoretical rationale for modifying the commensal flora of the gastrointestinal tract in specific circumstances appears sound and requires scientific pursuit, the field of probiotics has been clouded by exaggerated claims from some quarters. In general, many of the claims for therapeutic efficacy have not been well substantiated, but the field is now poised for evaluation within the realm of evidence-based medicine. Alterations in commensal bacterial flora within the gastrointestinal tract are associated with susceptibility to pathogens such as *Clostridium difficile* and there is persuasive evidence that the normal flora may participate in the pathogenesis of inflammatory bowel disease and other chronic diseases in genetically susceptible individuals. This has prompted various strategies to fortify or otherwise modify the enteric flora by dietary supplements containing probiotic formulations. Detailed comparisons of probiotic performance amongst different bacterial strains have not been performed *in vivo* in man or under clinical trial conditions, and the level of scientific characterisation of individual organisms has been variable. In addition, it cannot be assumed that the same probiotic is equally suitable for all individuals. Moreover, the heterogeneity of clinical disorders such as Crohn's disease and ulcerative colitis implies that strain-specific properties may be required for subset-specific categories of patients. While cocktails of probiotics offer convenience, therapeutic progress may require clarification of the mechanism of probiotic action and may be delayed until individual bacterial components have been rigorously studied. More importantly, the full potential of therapeutic manipulation of the enteric flora with probiotics or other strategies may not be optimally realised until the composition and metabolic activities of the normal flora are better understood.

Inflammatory bowel disease: Intestinal microflora: Mucosal immunity

Introduction

Therapeutic manipulation of the gastrointestinal commensal flora with probiotics is both conceptually appealing and biologically plausible for several common clinical disorders. The concept is not new and was given prominent endorsement early in the last century by the Russian Nobel laureate, Elie Metchnikoff, who is said to have credited his well-being and longevity to the consumption of fermented food products containing organisms that are now referred to as probiotics. Since then, the field has met with controversy and scepticism, much of which has been generated because of inappropriate or poorly supported claims for probiotic efficacy in a range of conditions. While probiotics promise much, the debate surrounding their role in modern medicine has been associated with dubious epithets ranging from 'conbiotics' (Berg, 1998) or 'snake oil' (Atlas, 1999) to the more optimistic '...bugs for the new millennium' (Konings *et al.* 2000).

Notwithstanding legitimate doubts regarding their precise clinical role, probiotics are just one aspect of the emerging field of functional foods (Diplock *et al.* 1999; Shanahan & McCarthy, 2000) and are beginning to attract renewed enthusiasm and more rigorous scientific pursuit. The rationale for therapeutic modification of the intestinal flora is on firmer ground than hithertofore, and the field is likely to benefit from critical scrutiny in the modern era of evidence-based medicine (Shanahan, 2000; McNaught & MacFie, 2001). Modification of the enteric flora with probiotics may have a contributory role in the management of several disorders with different underlying mechanisms including allergic (Murch, 2001), neoplastic (Dugas *et al.* 1999) and infectious pathophysiology (Zubillaga *et al.* 2001). Comprehensive review of each of these conditions is beyond our scope here. In this commentary,

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the inflammatory bowel diseases, Crohn's and ulcerative colitis, will be focused upon to illustrate the scope, rationale and evidence for efficacy in modifying the intestinal microflora with probiotics.

Gastrointestinal commensal flora in health and disease

The relationship between the host and the commensal bacteria within the lumen of the gastrointestinal tract is complex and appears to be regulated by reciprocal signalling mechanisms that are incompletely understood. The gut flora comprise over 400 different species, over half of which are still unculturable by conventional methods but can be studied by molecular techniques (Akkermans *et al.* 2000; Vaughan *et al.* 2000). After weaning, the composition of the flora is relatively stable throughout life but is distinct in different individuals. With more bacterial cells in the gut than eukaryotic cells in the human body and an average mass of 1–2 kg, the collective metabolic activity of the normal flora represents a virtual hidden organ that would rival the activity of the liver (Bocci, 1992; Berg, 1996).

Beneficial metabolic activities of this 'neglected organ' include the synthesis of B and K vitamins, production of epithelial nutrients such as short-chain fatty acids, metabolism of dietary carcinogens to inactive compounds and the conversion of pro-drugs to active drugs. Direct involvement of the flora in host defence is best illustrated when disturbed by broad-spectrum antibiotic therapy that is occasionally complicated by overgrowth of *Clostridium difficile*. The indigenous flora also promotes host defence indirectly by influencing the development and function of the mucosal immune response. Exposure to commensal flora is critical for oral tolerance and fine tuning of T-cell receptor function and mucosal cytokine profiles (Rook & Stanford, 1998). Comparative experiments using germ-free and colonised animals several decades ago revealed the conditioning effects of the bacterial flora on mucosal integrity, structure and function. Thus, the flora have a controlling effect on epithelial turnover, mucosal vascularity, lymphoid tissue mass, and peristalsis (Berg, 1996; Midtvedt, 1999). The application of modern technology including gene array analysis, real-time polymerase chain reaction and laser microdissection is now being used to identify the molecular basis of the bacterial signalling that regulates mucosal integrity (Hooper *et al.* 2001).

While the flora is generally an asset to the health of the host, it may become a liability under certain circumstances. These include syndromes of bacterial overgrowth and/or translocation and the expression of metabolic pathways for the conversion of procarcinogens to carcinogens (Berg, 1996). In addition, several lines of evidence discussed later indicate that, depending on the genetic susceptibility of the host, an abnormal interaction between the enteric bacteria and the local immune response may lead to chronic inflammatory bowel disease.

Importance of the enteric flora in inflammatory bowel disease

The interacting triad of genetic predisposition, environmental factors and immune (dys)regulation is a common unifying pathophysiological theme that appears to underlie many of the 'autoimmune' chronic inflammatory conditions in modern medicine (Erman & Fathman, 2001). In some instances, the environmental trigger may be a transmissible agent, but infectious agents may also have a more indirect role by influencing immune development, tissue cytokine profiles and immune activation. Childhood mucosal infections contribute to the education and fine tuning of the mucosal immune response (Rook & Stanford, 1998). So too, the commensal flora conditions the level of activation of the mucosal immune response and appears to be a key factor in driving mucosal inflammation in genetically susceptible individuals.

Several lines of observational and experimental evidence implicate the normal flora in the pathogenesis of Crohn's disease and ulcerative colitis (Shanahan, 2000). Firstly, the distribution of the lesions in these conditions is greatest in areas of highest numbers of luminal bacteria. Secondly, the continuity of the faecal stream has been linked with disease activity; interruption of the stream is associated with clinical improvement but relapse is predictable following surgical restoration. Thirdly, lesions of Crohn's disease may be induced by direct instillation of faecal contents into apparently unaffected loops of bowel in susceptible individuals (Harper *et al.* 1985; D'Haens *et al.* 1998). Fourthly, there is persuasive evidence for loss of immunologic tolerance to components of the commensal flora in patients with inflammatory bowel disease and this is reflected in serologic and cellular immune reactivity to enteric microbes that has formed the basis of putative diagnostic tests (MacPherson *et al.* 1996; Shanahan, 2001). Finally, the most compelling evidence for the interactive roles of genes, bacteria and immunity has been derived from experimental animal models of both Crohn's-like and colitis-like disease (Elson *et al.* 1995; Fuss & Strober, 1998; Blumberg *et al.* 1999). While a diversity of sporadic and engineered genetic defects have been described which predispose to chronic inflammatory bowel disease in rodents, with varying immunologic mechanisms mediating tissue damage, colonisation with normal enteric flora is required for full expression of the disease. Thus, the normal flora is a common factor driving the inflammatory process irrespective of the underlying genetic predisposition and immunologic effector mechanisms.

Animal models have been particularly useful in providing insights into the fundamental cellular and molecular pathways triggering and regulating the inflammatory process. In some models, the inflammatory disease has been adoptively transferred by T-cells that are reactive against the flora but not against dietary or mucosal antigens (Cong *et al.* 1998). In addition, animal models have enabled investigators to separately study the balance of effector and regulatory T-cells in these inflammatory processes (Powrie, 1995; Kronenberg & Cheroutre, 2000). Indeed, if results of experiments demonstrating the existence of regulatory T-cells that control mucosal immune reactivity to the enteric flora can be extrapolated to man, it may provide new therapeutic strategies and might even account for the apparent anti-inflammatory effects of probiotics in animal models. Thus, one of the potential

mechanisms of action of probiotics may be at the level of regulatory T-cells.

Therapeutic modification of gut flora in inflammatory bowel disease

Conventional drug therapy for inflammatory bowel disease primarily involves suppression or modulation of the host immunoinflammatory response with little attention to the contribution of the intestinal microenvironment (bacterial flora) to the pathogenesis. Although antibiotics seem an obvious method of altering gut flora, and are commonly used in patients with pouchitis and perianal Crohn's disease, their role in uncomplicated inflammatory bowel disease is not based on strong evidence of benefit (Feagan, 1997; Present, 1998). Chronic antibiotic usage is also associated with negative side-effects and risk of bacterial resistance. It is also noteworthy that elemental and polymeric dietary therapies for Crohn's disease may exert their effects by altering the enteric flora and barrier function (Shanahan, 2000).

Probiotics have been tested by several investigators in murine models of inflammatory bowel disease (Madsen *et al.* 1999; O'Mahony *et al.* 2001). In general the counter-inflammatory efficacy is modest but consistent. In addition, a strain of *Lactobacillus salivarius* (subsp. *salivarius* UCC118) appears to reduce the progression from inflammation through dysplasia to colon cancer in interleukin-10 deficient mice, when compared with non-probiotic-fed animals (O'Mahony *et al.* 2001). This may have particular relevance to the human condition where longstanding inflammation predisposes to colon cancer. Few well-designed trials of probiotic therapy have been conducted in human subjects with either Crohn's disease or ulcerative colitis (Hamilton-Miller, 2001). A non-pathogenic strain of *Escherichia coli* has been reported to have therapeutic efficacy equivalent to that of mesalazine in patients with ulcerative colitis (Kruis *et al.* 1997; Rembacken *et al.* 1999). By far the most impressive evidence for efficacy of probiotics has been with a cocktail of eight strains that were highly effective in maintaining remission in patients with pouchitis (Gionchietti *et al.* 2000). If this is replicated by other investigators, it will radically change current clinical practice in relation to maintenance therapy for patients with a surgically constructed ileo-anal pouch for ulcerative colitis.

Selection criteria and mechanisms of action

Much has been debated and written about selection criteria for probiotic organisms (Diplock *et al.* 1999; Dunne *et al.* 2001), but it seems intuitive that definitive criteria for selection of probiotic strains will depend on the intended clinical indication in addition to safety or biological considerations such as ability to survive gastrointestinal transit and bile/acid tolerance. It is also naïve to assume that a single probiotic will suit all individuals or even the same individual in different phases of a disease. In the context of inflammatory bowel disease, it appears the commensal flora vary in their pro-inflammatory capacity depending on the genetic predisposition of the host; some of the

flora, such as bifidobacteria, lactobacilli, non-pathogenic *E. coli* and other organisms, appear to lack this activity and have been studied as candidates for probiotic therapy in inflammatory bowel disease (Bengmark, 1998; Dunne *et al.* 1999; Shanahan, 2000). Rigorous comparisons of probiotic performance *in vivo* have not yet been performed and some investigators have used a cocktail of up to eight different probiotic organisms (Gionchietti *et al.* 2000). However, as with all combination medications, it is preferable that the properties and behaviour of the individual components of probiotic cocktails be fully determined *in vivo*, with synergistic or antagonistic activities identified, before they are adopted for widespread routine use.

Multiple different mechanisms have been proposed to account for probiotic action in different clinical circumstances. In the context of host defence against infection, probiotic mechanisms may include competitive metabolic interactions, the production of antimicrobials and inhibition of adherence or translocation of pathogens. In the context of inflammatory bowel disease, anti-inflammatory effects may involve signalling with the gastrointestinal epithelium and perhaps with mucosal regulatory T-cells (Shanahan, 2000). Probiotic effects on epithelial and barrier function have been demonstrated *in vitro* and *in vivo* (Isolauri *et al.* 1993). Just as the commensal flora exchanges regulatory signals with the epithelial and subepithelial components of the mucosa, the same is also likely with consumed probiotics. Indeed, some non-pathogenic organisms that have probiotic potential have been shown to counterbalance epithelial responses to invasive bacteria by regulating cytokine transcription factors (Neish *et al.* 2000). Finally, an anti-cancer effect has been proposed for probiotics, particularly in relation to colon cancer, and multiple mechanisms seem possible (Dugas *et al.* 1999).

Future scope and potential for probiotics

Genetically engineered probiotic organisms can radically redefine and extend the scope of probiotic action to include delivery of anti-inflammatory molecules or other biologically relevant molecules to the inflamed mucosa. Proof of this principle has already been accomplished with the food-grade *Lacotcoccus lactis* which has been engineered to secrete interleukin-10 (Steidler *et al.* 2000). When administered intragastrically to two murine experimental models of inflammatory bowel disease, its therapeutic efficacy was comparable with that of conventional steroids. While the acceptability of genetically modified food-grade organisms for many individuals seems dubious, and several safety concerns still need to be addressed, this approach has obvious advantages. These include convenience, cost effectiveness, and organ-specific delivery to the site of mucosal inflammation.

An alternative approach to the delivery of biotherapeutic molecules to the gut mucosa by probiotic organisms is the use of cell surface proteins to anchor therapeutically relevant molecules for display on the bacterial surface. Thus, surface protein modification with the creation of hybrid proteins circumvents the need for genetically modified organisms in food and the attendant negative connotations

genetically modified food has for much of the lay public (Leenhouts *et al.* 1999).

Conclusion

The promise of probiotics has often been overstated; without genetic engineering or surface protein modification, their clinical efficacy in inflammatory conditions is likely to be modest, albeit important. However, over the next decade, the field of probiotics can progress with rigorously designed, controlled clinical trials of efficacy in well-categorised patients. This should be supported by an improved understanding of mechanisms of probiotic action underpinned by elucidation of the genomics and proteomics of different probiotic strains. As with the interface between the host and the commensal flora, there are unacceptable gaps in our understanding of host–probiotic interactions that require basic investigation.

Notwithstanding the scientific hurdles ahead, the idea that what one ingests is likely to influence the health of the gut is conceptually appealing to many patients. Dietary adjustments for conditions such as inflammatory bowel disease consist largely of nutritional replenishment and correction of specific deficits with little evidence for a primary therapeutic benefit. Therapeutic modification of the gut flora with functional foods such as probiotics may empower patients and enable them to achieve an enhanced sense of control in the management of their illness. In this respect, functional nutrients such as probiotics promise to become a useful adjunct to conventional drug therapy.

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