To enhance antibacterial and anti-inflammatory activities of the intestinal epithelium, probiotics stimulate cytoprotective protective protein synthesis and secretion, including heat shock protein, defensin, angiogenin, and mucin by intestinal epithelial cells. Recent studies provide significant evidence that probiotics and probiotic-derived soluble factor prevent cytokine and chemical-induced epithelial apoptosis and disruption of barrier function. *Bifidobacterium infantis*-conditioned medium enhances intestinal epithelial barrier function in experimental colitis and prevents cytokine-induced disruption of tight junctions through regulating MAPK activation and tight junction protein expression.

NF-κB signalling is a critical mediator of intestinal epithelial cell crosstalk with immune cells. Optimal NF-κB activity plays a significant role in maintaining normal intestinal homeostasis and injury repair responses. However, hyperactivation of NF-κB results in chronic intestinal inflammatory disorders. One mechanism of probiotic effects is through the suppression of NF-κB signalling to limit excessive inflammation. Soluble factors released by *Bifidobacterium breve* C50 in the culture supernatant reduce TNF-induced cytokine production through inhibition of NF-κB and activator protein 1-dependent transcription in intestinal epithelial cells. These soluble factors and soluble factors-conditioned dendritic cells prevent trinitrobenzene sulfonic acid-induced colitis in mice. However, these effects were not found in response to *B. breve* ATCC 15698, *L. rhamnosus* ATCC 10863, and *Eubacterium rectale* L15. Yet, factors present in conditioned media of *Lactobacillus plantarum*, but not *L. acidophilus, L. paracasei, B. fragilis, B. breve, E. coli* F18 or enteropathogenic *E. coli,* inhibit TNF-induced NF-κB-binding capacity, IκB degradation, and proteasome activity in intestinal epithelial cells, macrophages, and dendritic cells. These studies indicate probiotic strain-specific secretion of factors regulate NF-κB activation.

Probiotic immunoregulatory effects independent of NF-κB have also been reported. *E. coli* Nissle 1917 expresses a direct anti-inflammatory activity on human epithelial cells via a secreted factor, which suppresses TNF-induced interleukin (IL)-8 transactivation, which occurs in the absence of NF-κB inhibition.

Quick Facts

Probiotics are not the same thing as **prebiotics** - non-digestible food ingredients that selectively stimulate the growth and/or activity of beneficial microorganisms already in people's colons. When probiotics and prebiotics are mixed together, they form a **synbiotic.**

Intestinal Immune Responses

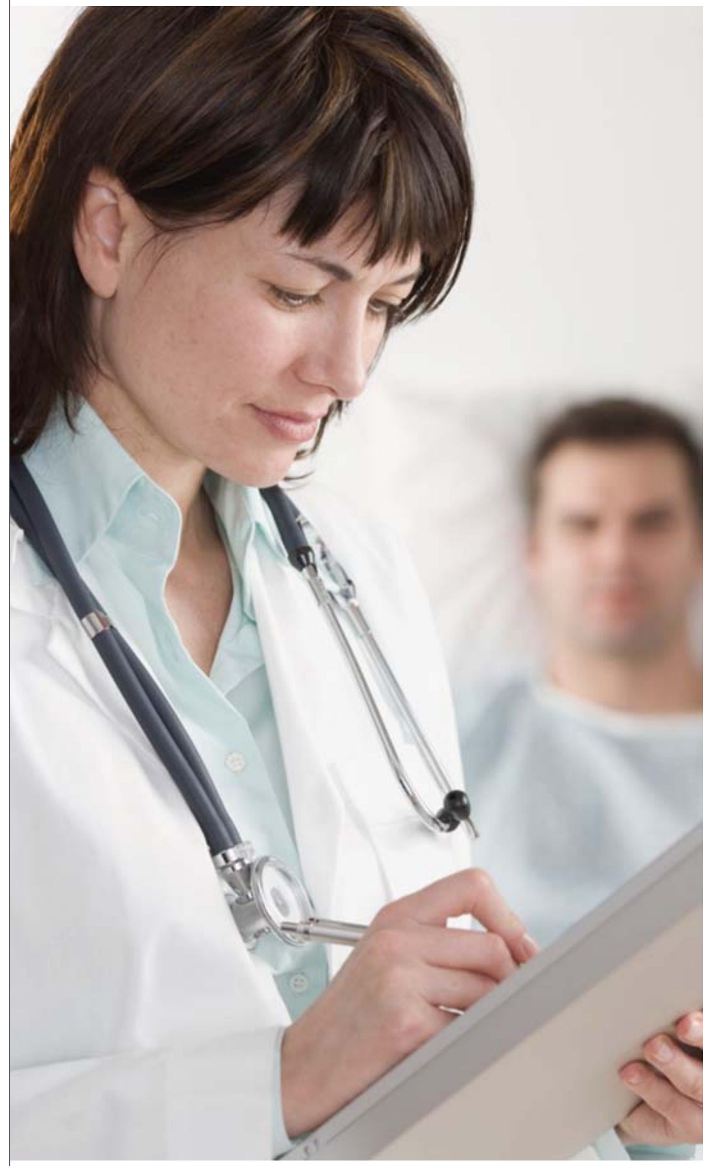
Both in-vitro and in-vivo studies have shown effects of probiotics on defining and maintaining the delicate balance between necessary and excessive defense mechanisms, including innate and adaptive immune responses; upregulation of immune function may improve the ability to fight infections; downregulation may prevent the onset of intestinal inflammation and autoimmunity. Previous studies have demonstrated that probiotics perform these immunoregulatory effects through enhancing innate immunity, promoting anti-inflammatory, and inhibiting pro-inflammatory cytokine production. Activation of Toll-like receptor-regulated signalling is one of the known mechanisms for probiotic regulation of immune functions.

Studies focusing on immunoregulatory effects of probiotics on colitis show that the mixture of *L. acidophilus* and *Bifidobacterium longum* prevents experimental colitis through expansion of intestinal intraepithelial γσT cells and regulatory T cells (Treg), as well as downregulation of pro-inflammatory cytokines, TNF, and monocyte chemotactic protein 1 and upregulation of the anti-inflammatory cytokine IL-10. In another in-vivo study, *B. infantis* drives the generation and function of Treg cells to suppress lipopolysaccharide (LPS)-induced NF-κB activation and *Salmonella typhimurium* infection.

Furthermore, several probiotic-derived factors mediate probiotic function in immunity. For example, *Lactobacillus reuteri* secreted factors promote TNF-induced apoptosis and antiapoptotic protein production (Bcl-2 and Bcl-xL) in human myeloid cells by inhibiting NF-κB activation and enhancing MAPK signalling. Two identified factors of probiotics have been reported. S layer protein A of *L. acidophilus* NCFM promotes dendritic cell maturation and function to stimulate T helper cell 2 T-cell polarization. Polysaccharide A, made by *B. fragilis,* protects against experimental colitis through inducing IL-10 production.

Conclusion

Understanding probiotic action may permit modulation of the immune system, both locally and systemically. Knowledge of probiotics on the host immune system has entered a new and fascinating phase of research and progression in this field is likely to offer novel and useful means to modulate host immunity for protection from, or treatment of, a wide variety of human disorders, like inflammatory bowel disease.



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The Probiotics news

Think BIG, Think micro

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Message from the Editor

In this 7th issue, we would like to thank Dr Mary Ellen Sanders for her educational article on probiotic microbiology. Dr Sanders is the Executive Director of the International Scientific Association for Probiotics and Prebiotics (ISAPP) and had also served on the World Gastroenterology Organization Guidelines Committee preparing guidelines for the use of probiotics and prebiotics for gastroenterologists.

Our readers will also benefit from an article on childhood constipation as this complaint is quite frequently brought up by many young couples to their respective family doctors.

Understanding the mechanisms of action of probiotic should be informative to many physicians as they look towards evidence based probiotic products.

God Bless!

Melvin Wong
Editor-in-chief

Perspectives on a Quality Probiotic

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Introduction

Probiotics are live microorganisms, which when administered in adequate amount confer a health benefit on the host. The range of probiotic products is increasing globally. However, not all products labelled "probiotic" meet the criteria for a quality product. Although it is difficult for consumers to differentiate among the commercial products available, some key attributes to look for in probiotics may guide this effort (Sanders, 2009).

Key Attributes of Probiotics

A probiotic is a microbe. Bacteria and yeast have been studied as probiotics. Bacteria used for probiotics include strains of *Lactobacillus* species, *Bifidobacterium* species, *Streptococcus thermophilus, Bacillus* species and *Escherichia coli.* A variant of *Saccharomyces cerevisiae,* S. boulardii, has also been used as a probiotic. Live viruses used for vaccines are considered outside the realm of probiotics.

A probiotic must be alive when administered. However, a probiotic may die after administration to the host. Viability at the site of action is presumed to be important, but some effects may be mediated by cell components. Recovery of the fed strain in faeces is suggestive that the microbe is alive at active sites within the alimentary canal.

A probiotic must be taxonomically defined and named according to currently accepted nomenclature. The microbe must be identified to the genus, species and strain level, must be named according to current nomenclature, and must be taxonomically assigned using best methods, generally DNA-based.

A probiotic must be safe for its intended use. Different categories of products have different safety standards. In general, foods must be safe for the generally healthy population. In contrast, drugs can balance risk with benefit.

A probiotic must be studied in humans, and shown to have a health benefit. Since demonstration of a health benefit is part of the definition of "probiotic," products calling themselves "probiotic" must have studies that demonstrate a health benefit.

A probiotic may be sold in different regulatory categories. It is conceivable for probiotics to be foods, dietary supplements, drugs or animal feeds. Each category will have specific requirements.

A probiotic may function outside the GI tract. Although most probiotic benefits are associated with GI effects, probiotics have been shown to impact the oral cavity, vaginal tract, skin and on systemic organ function.

The term probiotic is not synonymous with native putatively beneficial microbes. Candidate probiotics are commonly isolated from the pool of native, putatively beneficial bacteria found in humans, but it is not correct to equate probiotic and native commensal microbes.

The term probiotic is not synonymous with "live active cultures." Live cultures are microbes associated with foods, often as food fermentation agents. Many of these have not been directly tested for health benefits.

Probiotic effects must be considered to be strain-specific, until proven otherwise. Different strains of even the same genus and species may have different physiological effects. However, the observation that several strains have been shown to be effective for one particular indication should not be interpreted to mean that any possible probiotic strain will be effective.

Effective doses of probiotics differ among different products. Although it is tempting to stipulate a "minimum dose" that applies to all probiotics, different probiotics are effective at different levels. Some products have been shown to be effective at doses of 50 million CFU (colony-forming units)/day, others require more than 1 trillion CFU/day. It is essential that recommended probiotic usage levels are based on levels that were tested in human studies and shown to be effective, not on some arbitrary dose.

Labels for probiotic products. A probiotic product label should disclose the following:

- Genus, species and strain designation of each probiotic strain contained in the product.
- The number of live microorganisms (CFU) that are delivered in each serving or dose, through the expiration date. Levels indicated "at time of manufacture" are not adequately informative.
- The suggested serving size or dose.
- What substantiated health benefits the product has been shown to deliver.
- Proper storage conditions.
- Corporate contact information (including a web site or consumer hotline number where additional information can be obtained).

Conclusion

Not all probiotics are the same. Effects are strain-, dose-, and possibly matrix-specific. Consumers and healthcare professionals must do some ground work on products prior to using or recommending them. Products should be from a reputable manufacturer and should be labelled in a truthful and not misleading fashion.

Suggested Reading

See the homepage of the International Scientific Association for Probiotics and Prebiotics website (www.isapp.net) for Consumer Guidelines for Probiotic Products.

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About the author

Mary Ellen Sanders, Ph.D. is a consultant in the area of probiotic microbiology. Her focus is in the areas of efficacy substantiation, regulatory issues and communication to healthcare professionals and consumers on the science behind probiotics. She has collaborated on clinical studies to validate probiotic efficacy, served on GRAS determination panels, participated in a working group convened by the FAO/WHO to establish guidelines for use of probiotics, and served on the World Gastroenterology Organization Guidelines Committee preparing guidelines for the use of probiotics and prebiotics for gastroenterologists. She is Executive Director of the International Scientific Association for Probiotics and Prebiotics (ISAPP; www.isapp.net) and hosts a website along with the California Dairy Research Foundation to provide objective, evidence-based information on probiotics (www.usprobiotics.org).

Probiotic shows child constipation potential

Results from a recent pilot study suggested that probiotics may be effective in the treatment of constipation in children.

Functional constipation is a common and frustrating problem in childhood. This chronic condition is characterized by infrequent defaecation less than three times per week, more than two episodes of faecal incontinence per week, the passage of large and painful stools which clog the toilet and retentive posturing. This often causes distress to the child and family, and results in severe emotional disturbance.

To date, patients are treated with a combination of education, toilet training and oral laxatives. Disappointingly, only 50% of all children followed for 6 to 12 months recovered and were successfully taken off laxatives. Despite intensive medical and behavioural therapy, study found that 25% of patients developing constipation before the age of 5 years continued to have severe complaints of constipation beyond puberty. Furthermore, in 50% of the patients using these compounds, adverse side effects were registered such as abdominal pain, bloating, flatulence, diarrhoea, nausea and bad taste.

Previous studies in constipated adults with Bifidus yogurt, containing *Bifidobacterium breve, Bifidobacterium bifidum* and *Lactobacillus acidophilus,* have shown significant increase in defaecation frequency without any side effects. Based on the positive data in constipated adults, the researchers performed a pilot study to determine if *Bifidobacterium breve* is effective in the treatment of children with constipation.

Twenty children, aged 3 to 16 years old with functional constipation according to the Rome III criteria for the last 2 months were recruited in the study. During the 4-week study, the children received one sachet of powder containing $10^{8\text{-}10}$ CFU *Bifidobacterium breve* daily. They were instructed to try to defaecate on the toilet for 5-10 minutes after each meal and to complete a standardized bowel diary daily. The primary outcome measure was change in defaecation frequency at the end of the study period compared to baseline. Secondary outcome measures were stool consistency, pain during defaecation, frequency of abdominal pain, frequency of adverse effects (nausea, diarrhoea and bad taste), and frequency of intake of bisacodyl (rescue medication).

The results showed that the mean defaecation frequency per week significantly increased from 0.9 at baseline to 4.9 at week 4 ($p < 0.01$). In addition, the mean stool consistency score significantly increased from 2.6 at baseline to 3.5 at week 4 ($p = 0.03$). The mean faecal incontinence episodes per week significantly decreased from 9.0 at baseline to 1.5 at week 4 ($p < 0.01$). Pain during defaecation decreased from 71% at baseline to 33% at week 4 ($p = 0.08$). Abdominal pain episodes per week significantly decreased from 4.2 at baseline to 1.9 at week 4 ($p = 0.01$). The usage of bisacodyl dropped from 45% at week 1 to 20% during week 4. No side effects were reported.

This pilot study showed that intake of *Bifidobacterium breve* is effective in the treatment of childhood constipation. Based on these encouraging results and their safety profile, adding probiotics to the standard treatment of functional constipation in otherwise healthy children is promising. A large randomized placebo controlled trial is needed to confirm these data.

Practical Insights:

Mechanisms of Action of Probiotics

The intestinal microbiota plays a fundamental role in maintaining immune homeostasis. In controlled clinical trials, probiotic bacteria have demonstrated a benefit in treating gastrointestinal diseases, including infectious diarrhoea in children, recurrent *Clostridium difficile*-induced infection, and some inflammatory bowel diseases. This evidence has led to the proof of principle that probiotic bacteria can be used as a therapeutic strategy to ameliorate human diseases. The precise mechanisms influencing the crosstalk between the microbe and the host remain unclear but there is growing evidence to suggest that the functioning of the immune system at both a systemic and a mucosal level can be modulated by bacteria in the gut. Recent compelling evidence has demonstrated that manipulating the microbiota can influence the host.

Mechanisms of Probiotics and Probiotic-derived Factors Regulating Host Homeostasis

Several new mechanisms by which probiotics exert their beneficial effects have been identified and it is now clear that significant differences exist between different probiotic bacteria species and strains. These mechanisms include increasing enzyme production, enhancing digestion and nutrient uptake, maintaining the host microbial balance in the intestinal tract through producing bactericidal substances that compete with pathogens and toxins for adherence to the intestinal epithelium, promoting intestinal epithelial cell survival, barrier function, and protective responses, and regulating immune responses by enhancing the innate immunity and preventing pathogen-induced inflammation. These responses are mediated via regulation of signalling pathways, including nuclear factor kappa B (NF-κB), phosphatidylinositol-3'-kinase (PI3K)/Akt, and mitogen-activated protein kinase (MAPK) in intestinal epithelial and immune cells to facilitate probiotic action. Interestingly, some of these mechanisms of action appear to be mediated by probiotic-derived soluble factors.

Here we focus on the most recent probiotic advances in their mechanisms of action.

Intestinal Development

In the absence of microbes, there are profound deficiencies in intestinal epithelial and mucosal immunological development and function, including the inability to generate proper immune responses to protect against infection and inflammation. Recent findings show that probiotics may exert protective effects for developing the healthy intestinal system. The administration of a probiotic bacterium, *Lactobacillus casei* DN-114001 in fermented milk to nursing mice or their offspring improves the gut immune response in mothers and their offspring through the stimulation of immunoglobulin A^+ cells, macrophages, and dendritic cells.

Nutrition

Studies have repeatedly demonstrated that the intestinal microbiota promotes human and other host nutritional status, including promotion of polysaccharide digestion and uptake of nutrients by intestinal cells. As such, probiotic bacteria are widely used as nutritional supplements to improve the digestibility and uptake of dietary nutrients by host intestinal cells. For example, bacterial lactase is a well known enzyme produced by probiotic bacteria that degrades lactose in the intestine and stomach and prevents symptoms of lactose intolerance. A study focusing on the role of probiotics in the function of intestinal electrolyte absorption reveals that *Lactobacillus acidophilus* secretes soluble molecule(s) that stimulate apical chloride/hydroxyl exchange activity via a PI3K-dependent mechanism.

Intestinal Epithelial Homeostasis

In addition to nutritional functions, the intestinal epithelial cells are critical for maintaining normal intestinal homeostasis through several protective defense mechanisms, including barrier function formed by tight junctions, antibacterial substances synthesis, and active involvement in innate and adaptive immunity. Probiotics facilitate intestinal epithelial homeostasis through a number of biological responses, including promoting proliferation, migration, survival, barrier integrity, antimicrobial substance secretion, and competition for pathogen interaction with epithelial cells.