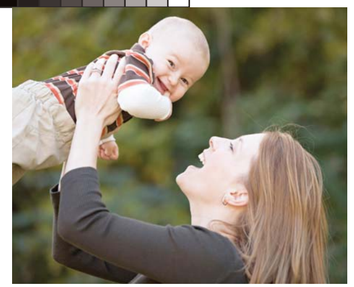

Practical Insights:
Probiotics and Immunity

For many years, probiotic bacteria have been known to confer health benefits to the consumer. While this beneficial effect was originally thought to stem from improvements in the intestinal microbial balance, there is now substantial evidence that probiotics can also provide benefits by modulating immune functions.

Intestinal Flora: Effect on the Gut Defense Mechanisms

Microbial colonization begins after birth, but the development of the intestinal microflora and the gut barrier is a gradual process. The maternal intestinal flora is a source of bacteria colonizing the newborn's intestine. Colonization is also determined by contact with surroundings. Initially, facultative anaerobic strains dominate. Thereafter, differences exist in the composition of species, mainly because of the type of diet. Breastfeeding encourages the growth of bifidobacteria, whereas formula-fed infants have a more complex microflora made up of bifidobacteria, bacteroides, clostridia, and streptococci. After weaning, the composition of the microflora resembles that of the adult flora.

The gut microflora is an important constituent in the intestine's defense barrier, as shown by increased antigen transport across the gut mucosa in the absence of an intestinal microflora. This notion is further supported by a demonstration that the gut microflora elicit specific immune responses at a local and a systemic level. Moreover, the gut flora is shown to induce and maintain oral tolerance in experimental animal models. The intestinal flora allows for persistence of systemic hyporesponsiveness to an antigen and shortens the abrogation of hyporesponsiveness mediated by *Escherichia coli* toxin.

In addition to participation in tolerance induction, intestinal colonization acts as an important antigenic stimulus for the maturation of the gut-associated lymphoid tissue. The capacity to generate IgA-producing cells progressively increases in response to intestinal antigenic stimulation, particularly the establishment of the gut microflora.

Studies of microflora development in vaginally born and cesarean delivery infants whose mothers received prophylactic antibiotics, indicate major differences in the culturable microflora. Differences were still observed at age 6 months when a substantial proportion of children born by cesarean delivery were not colonized with *Bacteroides fragilis*. Colonization appeared to be associated with the maturation of humoral immune mechanisms. Interestingly, *B. fragilis* and, to a lesser extent, bifidobacteria, were important in this respect because infants harbouring these organisms had more circulating IgA- and IgM-secreting cells. These results suggest that intestinal microflora is important in human individuals and that qualitative differences in the composition of the microflora might affect immunologic homeostasis. The effect of gut microflora on the maturation of the gut immune defense culminates in early infancy when the mode of immune responsiveness to antigens is consolidated.

Bacteriotherapy: Probiotics in Modulating Immune Responses

The demonstration that the gut microflora is an important constituent in the intestine's mucosal defense barrier, has introduced the concept of probiotic therapy. **In addition to the effects of probiotics on non-immunologic gut defense, which is characterized by stabilization of the gut microflora, probiotic bacteria have been shown to enhance humoral immune responses and thereby promote the intestine's immunologic barrier.** The stimulation of the host's non-specific and specific humoral immune responses to potentially harmful antigens has been documented for, among others, *Bifidobacterium bifidum*, *Bifidobacterium breve*, and *Lactobacillus rhamnosus* GG. The specific IgA response could contribute to the preventive potential of probiotics. This was clinically documented in a reduction of diarrhoeal episodes in infants who were administered *Lactobacillus helveticus*- and *Streptococcus thermophilus*-fermented formula, *Lactobacillus acidophilus*- and *Lactobacillus casei*-fermented milk, or a formula supplemented with *B. bifidum* and *S. thermophilus*.

Moreover, probiotic bacteria have been shown to stimulate non-specific host resistance to microbial pathogens, and thereby aid in immune elimination. Several strains of live lactic acid bacteria have been shown to induce in vitro the release of pro-inflammatory cytokines, tumour necrosis factor α, and interleukin 6. Enhanced phagocytosis was also reported in humans by *L. acidophilus* La1 and *L. rhamnosus* GG.

Recently, probiotics were shown to modulate the host's immune responses to foreign antigens with a potential to down-regulate hypersensitivity. In a study conducted to determine cytokine production by anti-CD3-induced peripheral blood mononuclear cells in atopic infants with cow allergy, it was found that unhydrolyzed casein increased the production of interleukin 4, whereas *L. rhamnosus* GG-hydrolyzed casein reduced it. This indicates that probiotics modify the structure of potentially harmful antigens and reduce their immunogenicity.

The Role of Probiotic

It seems that the probiotic approach, that is, therapeutically consuming beneficial microorganisms, holds great promise for the prevention and treatment of clinical conditions associated with impaired gut mucosal barrier functions and sustained inflammations. Being a well-tolerated and effective nutrient, probiotic may currently be among the best candidate to play this critical role in future epidemics of metabolic and inflammatory conditions. It may now be timely to redefine THE ROLE OF PROBIOTIC!

Quick Facts

The combination of microorganisms present in the gut is unique for every individual - almost like fingerprints!

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The Probiotics news

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Think BIG, Think micro

An educational project by MD Pharmaceuticals Pte Ltd

Dear readers...

As MD embarks on the 4th edition of The Probiotics News, we are privileged to feature Dr Ling Khoon Lin's article on "Gut Microbiota in Health and Disease" which you will inevitably find it informative and beneficial to the understanding and use of probiotics in your practice.

Another two must read educational topics touch on how probiotics help reduce cold and flu-like symptoms in children, and how probiotics modulate the immune system.

Besides US, Europe and Japan, your medical colleagues in Asia have also initiated researched base and clinically proven probiotics in their prescriptions over the last three years. There are just mounting evidence of the benefits probiotics have in human health and disease.

From all of us of the Editorial Team, we wish all our readers Love, Joy, Peace, Success and Good Health in 2010.

God bless!

Melvin Wong
Editor-in-Chief

Gut Microbiota in Health and Disease

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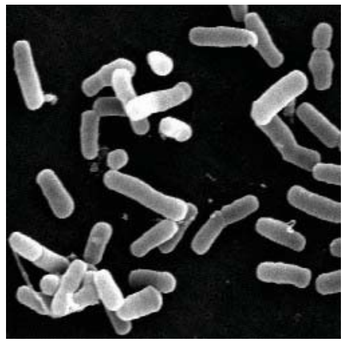
The human gastrointestinal tract is responsible for the digestion and absorption of nutrients. A vast array of micro-organisms is found in the gut, the majority of which are bacteria. The majority of these bacteria cannot be cultured in the laboratory. The development of molecular profiling methods e.g. 16S rRNA gene sequence analysis, has led to a revolution in the understanding of the intestinal microbiota by allowing culture-independent analyses of microbial community composition. They are beginning to reveal the complexity of intestinal microbial communities and indicate that the variability between individuals at the bacterial species level is quite high. However, common patterns emerge when microbial communities are compared at the phylum level. In all vertebrates, the Firmicutes and Bacteroidetes are the most common intestinal phyla. Few of these bacteria are found in the upper gastrointestinal tract, but the numbers increase as one moves down the jejunum and into the colon. It is estimated that in the colon, there are about 10^{12} bacteria per gram of intestinal contents.

Gut Microbiota Contribution to Host Physiology

These bacteria play a role in the normal biology of the host. Gut bacteria contribute to the host by transforming complex nutrients, such as dietary fiber or intestinal mucins that otherwise would be lost to the human host, into simple sugars, short-chain fatty acids and other nutrients that can be absorbed. They produce essential micronutrients e.g. vitamin K and vitamin B12, contribute to intestinal bile acid metabolism and recirculation, and transform potential carcinogens such as N-nitroso compounds (NOCs) and heterocyclic amines (HCAs). Gut microbiota also contribute to host physiology in ways beyond gastrointestinal nutrient processing, including



modulation of gut-derived endocrine, neuronal and inflammatory signals. **Differences in environmental factors, diet, and hosts genetics are thought to contribute to microbiota diversity.**

Gut Mucosal Immune System

In addition to the microbiota, the gut is also home to the largest collection of lymphocytes in the body. They are found in the epithelial layer and in the lamina propria as individual lymphocytes. They are also found as organized lymphoid tissue collectively known as the gut associated lymphoid tissue (GALT). This consists of isolated lymphoid follicles and Peyer's patches. **Gut microbiota play an important role in the development of the GALT.** Animals reared in a germ-free environment have poorly developed GALT.

The gut immune system is in a constant state of activation, and the gut is said to be in a state of 'physiological inflammation'. In the normal gastrointestinal tract, antigen presenting cells (APC) e.g. dendritic cells send processes out into the gut lumen to sample the contents of the gut lumen. These APCs may also take gut microflora into the mucosa and transport it to the Peyer's patches or to the mesenteric lymph nodes. This process is important for the gut immune system to react appropriately to defend the host against pathogenic bacteria e.g. Shigella and Salmonella. The same process is important to 'educate' the immune system such that the mucosal immune system will not respond to commensals in a pro-inflammatory manner while they are in the gut lumen but to respond after they have breached the mucosa. The gut mucosa is usually populated with a group of immunosuppressive cells: the regulatory T cells. They are thought to help maintain the gut in 'physiological inflammation' without spilling over into overt clinical inflammation when the gut immune system is presented with antigens from commensals. Patients with a deficiency in these cells spontaneously develop intestinal inflammation and present with inflammatory bowel disease-like symptoms.

In addition to its role in promoting the development of the mucosal immune system, intestinal bacteria direct class switching in human intestinal B cells and they govern the development of intestinal effector T cells. In addition, intestinal bacteria impact the outcome of systemic immune responses by determining the ratio of Th1 and Th2 effector cells. Various commensal bacteria have been found to modulate the immune system by inducing the secretion of cytokines e.g. IL10 and TGFβ1. The short chain fatty acids e.g. butyrates and acetates produced by some gut commensals have been found to be important in the treatment of colitis in murine models. It has also been suggested recently that they are important in modulating inflammation in a murine model of asthma.

Intestinal microbes also play an important role in protecting their hosts against invasion by pathogenic bacteria. Two distinct factors contribute to this protective effect. First, intestinal pathogens, such as Salmonella and Shigella species, have a limited repertoire of saccharolytic enzymes in comparison to commensals. These pathogens are therefore poorly adapted to compete with commensals for nutrients from the host diet, restricting their luminal colonization. Symbiotic intestinal microbes also stimulate immune responses that are cross-protective against pathogens. For example, invasion and dissemination of *Salmonella typhimurium* are limited by stimulation of epithelial Toll-like receptors (TLRs) by symbiotic bacteria. This stimulation triggers expression of a variety of antimicrobial proteins, which likely play a role in limiting Salmonella penetration of the epithelial barrier. It has been shown that some bacteria usually promote an anti-inflammatory response in the gut while others promote a more pro-inflammatory response.

Gut Microbiota in Disease

Over the last decade, there has been an increasing amount of experimental data which suggest that the gut microbiota play an important role in the pathophysiology of some diseases. The diseases which have been most intensely studied are probably the inflammatory bowel diseases (IBD). **IBD is thought to arise in the genetically predisposed host who develop an inappropriate immune response to an environmental trigger. An altered gut microbial composition and defective clearance of bacteria by the gut immune system is thought to contribute to this environmental trigger. The stool microbial composition of IBD patients has also been found to be different from that of normal controls.** In particular, members of the Firmicutes and Bacteroidetes phyla are reduced in IBD patients. In addition, specific candidate microbial agents have been implicated in IBD pathogenesis including *Mycobacterium avium* subspecies *paratuberculosis* (MAP) and adhesive-invasive *Escherichia coli* (AIEC).

The importance of the intestinal microbiota in metabolic diseases is highlighted by alterations in stool microbial composition in metabolic diseases, e.g. diabetes mellitus and obesity. In obese individuals, the microbiota is dominated by members of the Firmicutes, whereas lean people harbour a higher number of Bacteroidetes. Murine data suggest that this difference is probably due mainly to the diet of the obese individual with a smaller contribution by the genes of the individual. The weight of the individual mouse per se did not seem to contribute to alterations in the microbiota. The effect of modifying gut microbiota are also observed in mouse models of type 1 diabetes (T1D), and specific bacteria belonging to phyla normally found in healthy animals confer protection against the onset of T1D in genetically susceptible mice.

Probiotics

Probiotics are microorganisms that have beneficial properties for the host. Most have been derived from food sources, especially cultured milk products. With mounting murine and human evidence of perturbations in the gut microbiota in disease states, there have been an increasing number of studies examining the utility of probiotics in the treatment of human disease. **To date, they have been most useful in the treatment of pouchitis, acute diarrhoea and antibiotic induced diarrhoea.** While there is evidence for the utility of probiotics in the treatment of other diseases e.g. irritable bowel syndrome, ulcerative colitis and liver disease, it is not as robust. There are ongoing studies of probiotics in these diseases.

It must also be noted that probiotics should be used with caution in some groups of patients, e.g. in those with severe acute pancreatitis. Dutch investigators found in a double-blind, placebo-controlled trial that patients with acute severe pancreatitis who were given probiotics had a worse outcome compared with the control group.

Conclusion

The advent of high through-put molecular techniques has enabled researchers to determine the changes in the composition of stool microbiota in patients with a number of inflammatory and metabolic diseases. We shall learn more about the role of commensal microbial agents play in our lives in the coming years. While there is insufficient evidence to routinely recommend probiotics to the majority of patients we look after, we look forward to the day when we can change the course of human disease by changing the gut microbiota in our patients.

Probiotics Reduce Cold and Flu-Like Symptoms in Children

Probiotics have been shown to reduce cold and flu-like symptom incidence and duration in children according to a recent paper published in the August 2009 issue of *Pediatrics*.

The prospective, randomized, double-blind, placebo-controlled, 3-parallel arm study was conducted in a group child care center in Jinhua City, Zhejiang Province, China. The aim of the study was to investigate whether daily consumption of *Lactobacillus acidophilus* NCFM or a combination of *L. acidophilus* NCFM and *Bifidobacterium animalis* subsp *lactis* Bi-07 would affect the incidence and duration of fever, rhinorrhoea, and cough, and the incidence of antibiotic prescriptions among otherwise healthy children.

The researchers recruited 326 healthy children between 3 and 5 years of age and randomly assigned them to receive placebo (n = 104), *L. acidophilus* NCFM at 5 x 10^9 CFU (n = 110), or *L. acidophilus* NCFM in combination with *B. animalis subsp lactis* Bi-07 at 5 x 10^9 CFU (n = 112). The children were treated twice daily for 6 months.

248 of 326 enrolled children completed the 6-month intervention trial. Relative to the placebo group, single and combination probiotics reduced fever incidence by 53% (P = .0085) and 72.7% (P = .0009), coughing incidence by 41.4% (P = .027) and 62.1% (P = .005), and rhinorrhoea incidence by 28.2% (P = .68) and 58.8% (P = .03), respectively. Fever, coughing, and rhinorrhoea duration was decreased significantly, relative to placebo, by 32% (single strain; P = .0023) and 48% (strain combination; P < .001). Antibiotic use incidence was reduced, relative to placebo, by 68.4% (single strain; P = .0002) and 84.2% (strain combination; P < .0001). Subjects receiving probiotic products had significant reductions in days absent from group child care, by 31.8% (single strain; P = .002) and 27.7% (strain combination; P < .001), compared with subjects receiving placebo treatment.

This study showed that probiotic supplementation reduce the incidence and duration of cold and flu-like symptoms, the incidence of antibiotic prescriptions, as well as the number of missed school days attributable to illness, in children aged 3 to 5 years old. Compared with the placebo group, the group treated with the 1-strain product exhibited reduced incidence of fever and cough, whereas the group treated with the 2-strain combination exhibited reduced incidence of fever, cough, rhinorrhoea, and any symptom, revealing the superiority of multiple-strain preparation over single-strain probiotic product. No adverse events were noted during the 6-month study duration.

As the multiple benefits of probiotics are being realized, an important application is in the area of preventing, rather than treating, disease. A limited number of studies have shown that prophylactic administration of probiotics can contribute to reduced incidence or duration of illness in healthy subjects. Even so, this study is the first to show that probiotic (*L. acidophilus* NCFM or a combination of *L. acidophilus* NCFM with *B. animalis subsp lactis* Bi-07) consumption reduced both the incidence and duration of fever, cough, and rhinorrhoea symptoms in children. As well, the study is the first to indicate a trend towards more-significant results with a combination versus single-strain preparation. The researchers attribute the results to the immune-enhancing effect of probiotics.

The potential utility of documented probiotics as a prophylactic therapy against the onset of cold and influenza symptoms may help alleviate the need for medicinal symptom relief. This is especially relevant in light of the flu outbreak (H1N1) which killed many since June 2009. Daily probiotic dietary supplementation may offer a safe and effective way to reduce episodes of influenza symptoms in children by enhancing their body immunity.