Practical Insights:

Probiotics and Safety

Probiotics are generally accepted as live microorganisms which when administered in adequate amounts confer a health benefit on the host. There is considerable interest in probiotics for a variety of medical conditions, and millions of people around the world consume probiotics daily for perceived health benefits.

Many of the organisms to which we ascribe probiotic effects have their origins in dairy products and fermented foods. They have been consumed as constituents of these foods without apparent ill effect for centuries. For example, Persian tradition has it that Abraham owed his fertility and longevity to the regular ingestion of yogurt. In the early 20th century, the Russian immunologist Elie Metchnikoff proposed that lactic acid bacilli may have beneficial health effects and attributed his own longevity to regular probiotic ingestion.

Defining Probiotics

According to the FAO/WHO guidelines for the evaluation of probiotics in food, probiotics must contain live microorganisms and in adequate dose in order to exert desirable effects on the host. To confer a significant health benefit, the ideal probiotic should remain viable at the level of the intestine and should adhere to the intestinal epithelium. It must also be resistant to gastric acid digestion and to bile salts to reach the intestine intact. Most of all, it should be non-pathogenic.

Most of these probiotics belong to the genera lactobacilli and bifidobacteria and are part of the normal intestinal flora.

Safety of Probiotics

Most probiotics are marketed as dietary supplements. Consideration of the safety of probiotics is therefore of utmost importance. The most important area of concern with probiotic use is the risk of sepsis. Probiotics have been widely used in food processing for many years, and overall have an excellent safety record. Many small studies also support the safety of particular probiotic strains in particular high-risk populations. For example, different Lactobacillus strains have been fed to adults and children infected with HIV, to term infants, and to premature infants with no significant adverse effects.

Probiotics are generally safe when consumed by healthy individuals. Cases of probiotics bacteraemia or fungaemia have been reported in patients with underlying immune compromise, chronic disease, or debilitation. Most cases of probiotic sepsis resolved with appropriate antimicrobial therapy, but in some cases patients have developed septic shock. In other cases the outcome has been fatal, but these fatalities were usually related to underlying disease rather than directly to probiotic sepsis.

Many case reports of probiotic sepsis describe persons with pre-existing intestinal pathology, including diarrhoea and short intestine. These may be common indications for probiotic use, but would also be expected to increase the risk of probiotic translocation through the intestinal mucosa. Some cases have occurred after probiotic strains were given via jejunostomy tube, bypassing gastric acid, and this would be expected to increase the numbers of viable probiotic bacteria that reach the intestine. The presence of a central venous catheter is also a common finding in cases of probiotic sepsis and has been shown to be a possible source of sepsis.

Lactobacillus species are generally recognized as safe but have been identified as a well-recognized (although rare) cause of sepsis in immunocompromised individuals or those with serious underlying disease. It has been suggested that *Lactobacillus rhamnosus* in particular warrants surveillance because it is associated with more cases of bacteraemia than other lactobacilli. However, it is worthwhile to note that *L. rhamnosus* is among the most common Lactobacillus species in the human intestine so this may be relative to its extensive presence in the intestine.

Two clinical cases have been reported in which a lactobacillus indistinguishable from an ingested probiotic strain has been identified in association with infection. One case was of a 74-year-old diabetic woman who developed *L. rhamnosus* GG (LGG) liver abscess and pneumonia 4 months after commencing daily LGG supplementation. The second one was of a 67-year-old man with mitral regurgitation who took probiotic capsules daily and developed *L. rhamnosus* endocarditis after a dental extraction. These reports are highly suggestive of probiotic supplement-related sepsis, but it should be noted that LGG and other strains of *L. rhamnosus* can sometimes be found in the intestinal microbiota of healthy humans, so the source of infection in these cases is not conclusively proven.

Besides the risk of pathogenicity and infectivity, other concerns regarding the safety of probiotics are toxic or metabolic effects on the gastrointestinal tract, and the transfer of antibiotic resistance in the gastrointestinal flora.

The intestinal microbiota plays an important role in many metabolic activities, including complex carbohydrate digestion, lipid metabolism, and glucose homeostasis. There is therefore a theoretical risk of adverse metabolic effects from manipulation of the microbiota with the use of probiotics, even if such manipulation is only temporary. This is especially undesirable for patients with short bowel syndrome. For these patients, it is possible that the conjugated bile acid metabolites might accumulate and lead to malabsorption, which might lead to the risk of lactate accumulation and a theoretical risk of colon cancer. However, the likelihood of significant adverse effects seems low, and probiotic studies to date have not shown significant adverse effects on growth or nutrition.

There is potential for viable probiotics to colonize the intestinal tract and transfer genetic material. In most circumstances, the available data suggest that probiotics colonize the human intestine transiently. Nevertheless, concern exists regarding the possible transfer of antimicrobial resistance from probiotic strains to more pathogenic bacteria in the intestinal microbiota. Many Lactobacillus strains are naturally resistant to vancomycin, which raises concerns regarding the possible transfer of such resistance to more pathogenic organisms, particularly enterococci and *Staphylococcus aureus*. However, the vancomycin-resistant genes of Lactobacillus spp. are chromosomal and therefore, not readily transferable to other species. Conjugation studies have not found the vancomycin-resistant genes of lactobacilli to be transferable to other genera.

Conclusion

On the basis of published findings, probiotics are generally regarded as safe for use in otherwise healthy persons. The use of probiotics must be carefully considered when they are used therapeutically in patients at high risk for opportunistic infections like the immunocompromised or those having concomitant serious illnesses. In view of the increasing use of probiotics as health supplements and therapeutic agents, clinicians need to be aware of the risks and benefits of these treatments.

A Note of Appreciation to Dr Charles Vu

We would like to express our heartfelt gratitude to Dr Charles Vu, who has decided to step down as our medical advisor due to his work commitment. Dr Vu has been a great help who never fails to give his most valuable advice in every issue of The Probiotics News. From all of us of the editorial team, thank you Dr Vu for journeying with us thus far!

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For enquiries, comments, suggestions or article contribution, please write to:

The Editor (The Probiotics News)
MD Pharmaceuticals Pte Ltd
896 Dunearn Road #02-01A
Sime Darby Centre Singapore 589472

Tel: (65) 6465 4321
Fax: (65) 6469 8979

Website: http://www.mdpharm.com
Email: liching.nah@mdpharm.com or waisin.leong@mdpharm.com

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Greetings from the Editor

Renowned Conrad Taff Professor of Nutrition and Paediatrics at Harvard Medical School and Professor of Nutrition at the Harvard School of Public Health, Prof W. Allan Walker is our honoured guest writer on **Probiotics and Paediatrics** in this 8th issue of MD Pharmaceuticals's Probiotics Newsletter.

Another two informative topics regarding Probiotics that may be relevant are their association with regards to influenza vaccination and safety aspects.

Your views, feedbacks and suggestions will certainly enhance the educational quality of our newsletter.

Wishing all our readers a joyous, successful and healthy 2012.

God Bless!

Melvin Wong
Editor-in-chief

Probiotics and Paediatrics

W. Allan Walker, MD
Conrad Taff Professor of Nutrition and Paediatrics, Harvard Medical School Director, the Mucosal Immunology Laboratory, Massachusetts General Hospital for Children, Boston, MA

Initial Bacterial Colonization

The initial bacterial colonization of the intestinal tract is important to health. In utero, the epithelium is very immature and turns over slowly, with a lack of lymphoid elements. In contrast, once an infant has entered the extra-uterine environment, there is a mature, actively turning over epithelium with all the subclasses of epithelial cells and an abundance of lymphoid elements. This underscores the importance of the initial colonization of the development of the gastrointestinal tract in the infant.

W. Allan Walker, MD is the Conrad Taff Professor of Nutrition and Paediatrics at Harvard Medical School (HMS) and Professor of Nutrition at the Harvard School of Public Health. He is the Chairman of the Division of Nutrition, Principal Investigator of an NIH-funded Clinical Nutrition Research Center at HMS and Director of the Mucosal Immunology Laboratory at Massachusetts General Hospital for Children (MGHfC). His research interests include defining the role of initial bacterial colonization in the development of intestinal host defense and determining the protective effects of breastfeeding in the prevention of disease in neonates.

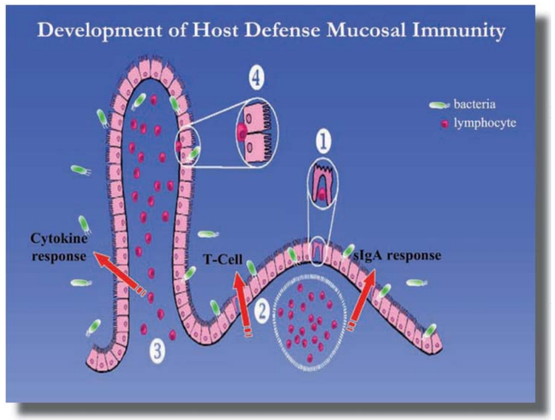
Under normal circumstances, the foetus leaves a germ-free environment and naturally delivers through the birth canal where it picks up a healthy bolus of bacteria from the mother's colonic and vaginal cavities. With the introduction of feeding, these bacteria are stimulated and proliferate. They then change slightly with weaning when breast or formula feeding is transitioned to solid foods, so that by 18 months to 2 years of age, the intestine is completely colonized and remains with the infant for life.

The number of bacteria that have been identified within the intestine ranges between 400 and 1000 different strains. This is 10-fold greater than the total number of cells in the body, which is why the intestine is sometimes considered a separate organ of the body. Under normal colonized conditions, the large diverse intestinal flora exists in a balanced ecosystem where health-promoting bacteria balance potential pathogens and there is no expression of disease.

Another factor in the development of host defense is oral tolerance. Oral tolerance is defined as the down regulation of systemic immunity after oral exposure to "harmless" bacteria and antigens. It is one of the most important mature mechanisms of the intestinal tract because it discerns between potential hazardous antigens and organisms, which require an acute inflammatory response to contain and innocuous antigens and bacteria, which should not cause an immunologic response.

Clinical Consequences of Inadequate Colonization

In 2002, the *New England Journal of Medicine* published a review article that compared the disease burden over the last half century with regard to infectious versus immune disorders. The epidemiological evidence showed that in developed countries there has been a striking decrease in infectious diseases, such as hepatitis, tuberculosis, measles, and mumps. Unfortunately, this has been substituted by a shifting paradigm in disease, where there is an increase in immune mediated diseases, such as allergy and autoimmune diseases like Type I Diabetes and Crohn's Disease.



Development of Host Defense

As a function of gestation, the various immune components of the intestine develop differently. For example, a specific form of epithelium, the microfold cell is formed, which facilitates antigens and bacteria from crossing into aggregates of lymphoid elements and Peyer's patches. Interstitial and interepithelial lymphocytes are also formed. These cells all developed at birth, but they do not function until they are stimulated initially by colonizing bacteria. The colonizing bacteria stimulate the lymphoid elements, so that they mature, produce both protective and regulatory cytokines and secretory IgA.

IgA is a protective immunoglobulin that coats mucosal surfaces and balances T-helper cell response. Healthy full-term newborns are deficient at birth and it takes a finite amount of time for protective levels to be produced, which requires that the first two stages of bacterial colonization occur.

This observation has been explained by the "Hygiene Hypothesis", which states that due to a decreased exposure to microorganisms during infancy, the mucosal immune system fails to develop properly resulting in aberrant responses to allergens and autoantigens leading to an increase in disease burden.

One large study looked at infants who were born in a sterile, urban environment without pets or siblings and who had very little exposure to microorganisms compared to infants who were born in a rural farm setting, with animals and lots of exposure to microorganisms. Symptoms of allergy, including wheezing, hay fever, and positive responses to outdoor and indoor antigens were considerably higher in those who were born in an urban setting, strongly suggesting that lack of exposure to organisms in infancy is a factor in the development of disease.

In the past decades, additional factors have contributed to inadequate initial colonization. These include premature delivery, caesaran section, and an inappropriate use of perinatal antibiotics. One study in animals, which provided the broad-spectrum antibiotic, kanamycin, during the perinatal period, showed that there is a much higher development of IgE antibodies, the basis for allergic disease, than those not given antibiotics.

In another study, mothers without a family or self history of allergy, who delivered naturally, were compared to, 1) mothers who had allergy or a family history of allergy and delivered naturally, and 2) mothers who had allergy or a family history of allergy and delivered by caesaran section. The mothers in the first group were found to have two and a half times the risk of having allergy, while the mothers in the second group had almost an 8-fold increase in risk of allergy. This illustrates the importance of initial colonization in the development of disease.

Probiotics as "Surrogates" for Colonization

Under circumstances, such as incomplete colonization or excessive use of antibiotics, there can be a shift in the ecologic balance of bacteria in the intestine, which makes one much more susceptible to disease. When incomplete colonization occurs, however, it has been suggested that probiotics can act as a surrogate for colonization. With the addition of probiotics, a balanced system can be restored as long as the probiotics are continuously used as their effects are transient. The bacteria that have been used most commonly as probiotics in paediatrics and studied the most are *Bifidobacterium lactis* Bb12 and *Lactobacillus rhamnosus* GG.

All babies are born with an imbalanced immune response to protect them from rejection in utero, so they have an imbalanced Th2-helper cell system, which favours the development of allergy. With colonization and stimulus of other immune cells, there is a balance and no expression of disease. A number of studies have shown that when there is a persistent Th2 response, using probiotics can stimulate Th1 response and cause a balance, helping to prevent allergy.

Probiotics in Treatment and Prevention of Paediatric Disease

Oral probiotics have also been shown to reduce the incidence and severity of necrotizing enterocolitis (NEC) in very low birth weight infants. NEC is a condition in which there is a very high inflammatory response with a low ability to break down inflammatory cytokines with appropriate enzymes. A number of studies have looked at the effectiveness of probiotics in helping to ameliorate and reduce this condition. A study published in *Paediatrics* showed that when *Bifidobacteria* and *Lactobacillus* were used in 1500 gram or smaller infants, they had decreased mortality and morbidity compared to the control group. In addition, when exposed to human foetal enterocytes in culture, these same probiotics modulated the inflammatory response, making it more similar to a mature intestine.

In one study the introduction of *Lactobacillus* GG in the latter stages of pregnancy, continued through lactation, produced a striking reduction in expression of atopic dermatitis even at two and four years. This suggests when probiotics are introduced early, in a situation where there might be inadequate colonization, the process may be reversed. Additional studies suggest that probiotics may be effective in the treatment of allergy.

Many studies have also shown that probiotics are effective in reducing the duration and the severity of acute diarrhoea. The use of antibiotics can produce symptoms of diarrhoea in many children and adults causing a condition called antibiotic-associated diarrhoea (AAD). When *Saccharomyces boulardii* was provided to children, there was a significant decrease in the side effects of antibiotic therapy.

Conclusions

The field of probiotics is an evolving field so we do not yet have all of the answers. Probiotics have been shown to be very effective in the field of paediatrics. It is important, however, to be aware of the specific strain of probiotic, the dose of probiotic, and the condition for which the probiotic has been shown to be clinically effective. When these factors are taken into consideration, probiotics may be helpful in the prevention and treatment of different conditions, including allergy and NEC.

Quick Facts

There are substances in the diet that can affect the stimulus of endogenous bacteria, but there is no evidence of a nutritional way of maintaining or enhancing a probiotic. The only way to maintain a probiotic, is to continually provide it.

What's new?

Probiotics increase adaptive immune response to vaccination

A randomized, double-blind, placebo-controlled study showed that supplementation with probiotics enhanced antibody responses in healthy individuals after an influenza vaccination.

This is a four-arm, parallel group study conducted in 211 subjects (56% females, mean age 33.2 (SD 13.1) years). The subjects consumed either a capsule containing the probiotic strain *Bifidobacterium animalis* ssp. *lactis* (BB-12®), a placebo capsule, an acidified dairy drink (110 ml) containing the probiotic strain *Lactobacillus paracasei* ssp. *paracasei* (L. casei 431®), or a placebo acidified dairy drink (110 ml). The probiotic products contained a minimum of 10^8 colony-forming units/dose, and subjects consumed one capsule or one drink once daily for 6 weeks. After 2 weeks, a seasonal influenza vaccination was given. Plasma and saliva samples were collected at baseline and after 6 weeks for the analysis of antibodies, cytokines and innate immune parameters.

Primary efficacy variables were vaccine-specific plasma IgG and subclasses IgG1 and IgG3, and vaccine-specific salivary IgA, IgG and IgM. Secondary variables were adaptive and innate immune responses assessed by total plasma IgA, IgM, IgG and subclasses IgG1 and IgG3; total salivary IgG, IgA and IgM; plasma concentrations of interferon-ϒ, IL-2 and IL-10; natural killer cell activity; CD4 + T-lymphocytes and phagocytosis.

Influenza-like illness was rated by the subjects in a diary, while infection status was evaluated at each study visit by a physician. As safety variables, measurement of tetanus-specific IgG in plasma was included before and after the vaccination, and vital signs were measured at each study visit. Information on adverse events was collected from screening to the end of study.

Changes from baseline in vaccine-specific plasma IgG, IgG1 and IgG3 were significantly greater in both probiotic groups compared with the corresponding placebo group (L. casei 431®, P=0.01 for IgG; P<0.001 for IgG1 and IgG3). The number of subjects obtaining a substantial increase in specific IgG (defined as ≥2-fold above baseline) was significantly greater in both probiotic groups compared to placebo (BB-12®, P<0.001 for IgG, IgG1 and IgG3; L. casei 431®, P<0.001 for IgG1 and IgG3). Significantly greater mean fold increases for vaccine-specific secretory IgA in saliva were observed in both probiotic groups compared to placebo (BB-12®, P=0.017; L. casei 431®, P=0.035). Similar results were observed for total antibody concentrations. No differences were found for plasma cytokines or innate immune parameters.

The incidence of influenza-like illness was very low in all groups and no infections were diagnosed during the study in any of the intervention groups. Tetanus-specific IgG were not modified across the groups from baseline to day 42, and no difference between the groups was observed, demonstrating that supplementation of the probiotics is not associated with non-specific stimulation of the immune system. Vital signs raised no safety concerns. No serious adverse events occurred during the study.

These data suggest that dietary supplementation with BB-12® or L. casei 431® leads to an increased adaptive immune response to vaccination, which is in line with results on other probiotic strains tested in healthy subjects. This is an important finding because protection against infection with pathogens that penetrate through the mucosa, such as the influenza virus, requires responses from both the mucosal and the systemic part of the adaptive immune system.

The fact that both IgG1 and IgG3 were significantly increased after the probiotic supplementation suggests that the activities of both T-helper (Th)1 and Th2 lymphocytes are promoted, as IgG1 and IgG3 are considered to be more indicative of Th2 and Th1 functionality, respectively. Notably, besides being preferentially correlated with Th1 and Th2 T-cell subsets, IgG1 and IgG3 are also associated with the optimal activation of complement and phagocytosis by macrophages, respectively. Because complement and phagocytosis work synergistically to eliminate pathogens, the fact that supplementation with these probiotics enhances production of both types of antibody subclasses further supports the potential beneficial effects of BB-12® and L. casei 431®.

The augmentation of all the subclasses of antibodies - with the exception of IgM - indicates that the immune response enhanced by supplementation of these probiotics is a secondary response, which is the type of immune response expected upon an influenza vaccination. This confirms that the dietary supplementation stimulates antigen-specific responses directed towards the antigen to which the immune system has been exposed. Influenza-specific memory B-cells alone were restimulated by the vaccine; the effect of the restimulation on these cells was specifically strengthened by the probiotics. Additionally, analysis of tetanus-specific IgG concentrations was included as a safety parameter, which confirmed that supplementation with BB-12® and L. casei 431® only elicited antigen-specific responses without resulting in unspecific, generalized immune activation.

The study concluded that consumption of either of the probiotic strains BB-12® or L. casei 431® significantly increases antigen-specific immune responses in healthy individuals receiving an influenza vaccination. Dietary supplementation with BB-12® or L. casei 431® may thus be a safe and effective means to improve immune function by augmenting the response to challenges.