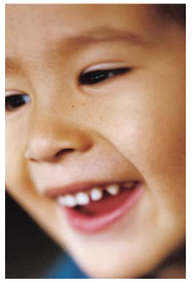
Mechanisms of anti-allergic probiotic action

The precise mechanisms behind the anti-allergic effects of probiotics are not entirely known. Several mechanisms have been observed in vitro and in animal studies. In addition to modulation of the intestinal microbiota, probiotics have been observed to improve the barrier function of the intestinal mucosa, reducing leakage of antigens through the mucosa and thereby exposure to them. Direct modulation of the immune system may be through the induction of anti-inflammatory cytokines or through increased production of secretory IgA. IgA will contribute to an exclusion of antigens from the intestinal mucosa. Further, enzymatic degradation of dietary antigens by enzymes from probiotics will reduce the load of and exposure to antigens.

The Future

Probiotic therapy appears to be a promising approach in the treatment and prevention of allergy. Elucidating the mechanisms of probiotic action on allergy is important for shaping the future directions of probiotics use in allergic diseases. A thorough knowledge of the intestinal microbiota of allergic and healthy individuals presents an opportunity to select more effective strains or combinations of strains. Also, the influence on allergy of the combination of food components and probiotics deserves further investigation. These will enable optimization of probiotic use for the allergic subject.



Quick Facts

There is no consensus about the minimum number of microorganisms that must be ingested to obtain a beneficial effect; however, a probiotic should typically contain several billion microorganisms to increase the chance that adequate gut colonization will occur [31].

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The Probiotics news

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Think BIG, Think micro

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In this 5th edition of The Probiotics News, we turn our attention on the roles of probiotics in **Alcoholic Liver Disease, Diabetes** and **Allergy.**

Spending just ten minutes of your reading time on these three major articles should provide you with additional information into these medical conditions.

We are indeed privileged to invite **Dr Irina A. Kirpich (PhD) from the University of Louisville Medical Center, Department of Medicine, Hepatology and Gastroenterology Division, USA** to contribute her interesting article on Alcoholic Liver Disease and Probiotics, a growing concern amongst some advanced economies in the world.

Enjoy & God Bless!

Melvin Wong
Editor-in-Chief

Alcoholic Liver Disease and Probiotics

by Dr Irina A. Kirpich, PhD
Northern State Medical University, Russia; University of Louisville, USA

Alcoholic liver disease (ALD) remains a major cause of morbidity and mortality worldwide with more than 75,000 deaths annually and the incidence increasing in the last decade[1]. The clinical spectrum of ALD includes alcoholic fatty liver, alcoholic steatohepatitis, alcoholic cirrhosis, and increased risk of hepatocellular carcinoma. There are currently no effective treatments for ALD. Other than abstinence and the correction of associated malnutrition, there are no preventive measures. Although all alcoholics consume ethanol, only about 30% develop clinically significant ALD. Why some heavy drinkers develop ALD and others are spared is unclear. However, it is generally accepted that interactions between the gut, the liver and immune system are critical components of ALD.

Gut-Liver Axis: Alcoholic Endotoxemia and Endotoxin-Mediated Liver Injury

The development of ALD can be attributed to many factors that cause damage to the liver and alter its function. Endotoxin LPS (lipopolysaccharide) derived from Gram-negative bacteria has been postulated to play a crucial role in the pathogenesis of alcohol-induced liver injury. Alcohol increases intestinal permeability to various macromolecules including endotoxin in both human subjects and animal models of ALD. Endotoxin stimulates different cells in the liver releasing cytokines, chemokines, and reactive oxygen species (ROS) via Toll-like receptor-4 (TLR-4)-mediated mechanisms[2]. Increased production of TNF-α by Kupffer cells, the resident liver macrophages, play a crucial role in the hepatic immune response to gut-derived endotoxins and alcohol-induced liver injury[3].

Endotoxemia in ALD was first recognized by the detection of antibodies against *Escherichia coli* in the plasma of patients with ALD[4]. Plasma endotoxin levels were significantly elevated in patients affected with different stages of ALD - fatty liver, hepatitis and

cirrhosis when compared with healthy control subjects[5]. Monocytes from alcoholics with ALD were shown to be primed for producing cytokines and oxidants due to endotoxin exposure[6]. Endotoxemia has been confirmed also in experimental models of alcoholic liver injury. Rats with ethanol-induced liver injury have high endotoxin levels in their portal vein, and there is a strong correlation between endotoxin levels and the severity of liver injury[7].

Mechanisms of Alcoholic Endotoxemia

Several mechanisms have been proposed to contribute to alcoholic endotoxemia: excessive production of endotoxin through ethanol-induced bacterial overgrowth[8]; disruption of intestinal barrier function[9]; delayed endotoxin clearance from the blood[10]; oxidative stress and alcohol-induced generation of nitric oxide may also contribute to increased intestinal permeability to endotoxin[11]; increased intestinal production of pro-inflamatory cytokines, such as TNF-α and IL-6, could be involved in disruption of gut integrity[12]. Abnormal gut microbiota composition (intestinal dysbiosis) can cause endotoxemia by both increasing the production of endotoxin and by chronic deleterious consequences on gut barrier function (initiation or worsening of gut leakiness)[13]. Studies in animal models of ALD have revealed that treatment with antibiotics to sterilize the gut and eliminate the source of endotoxin can prevent alcohol-induced liver injury[14].

Probiotics and ALD

There is a growing body of clinical and experimental studies suggesting beneficial effect of prebiotics, probiotics, and synbiotics in ALD[15,16,17]. It was shown that *Lactobacillus rhamnosus* Gorbach-Golding (LGG) treatment reduced endotoxemia and severity of experimental alcoholic liver disease[15]; ameliorated alcohol-induced oxidative stress and inflammation in both the intestine and liver, gut leakiness, and liver injury in a rat model of ASH[18]. It was suggested, that oral administration of heat-killed *Lactobacillus brevis* SBC8803 ameliorated the ethanol-induced liver injury possibly through the induction of cytoprotective heat shock proteins (HSPs), and enhancement of intestinal barrier function[19].

Recent study demonstrated that daily alcohol consumption for 10 weeks altered colonic mucosa-associated bacterial microbiota composition in rats and was prevented by both oats and LGG supplementation[13]. Pretreatment with LGG also attenuated the suppressive actions of ethanol on mucus-secreting layer and transmucosal resistance, and reduced cellular apoptosis in the gastric mucosa[20]. Modulation of gut microbiota by selectively increasing *Bifidobacterium-spp* improves barrier function and function of TJA-proteins in diet-induced obesity and fatty liver[21]. Also, because alcohol-induced abnormalities in gut microbiome composition could contribute to intestinal tight junction disruption, these results provide one more beneficial effect of probiotics which should be considered in ALD.

Our recent study demonstrated that **alcoholics have altered bowel flora with reduced numbers of bifidobacteria, lactobacilli, and enterococci compared to non-drinkers.** After 5 days of probiotic therapy with *B. bifidum* and *L. plantarum* 8PA3, alcoholic patients had significantly increased numbers of both bifidobacteria (7.9 vs. 6.81 log CFU/g) and lactobacilli (4.2 vs. 3.2 log CFU/g) compared to the standard therapy arm. Despite similar values at study initiation, patients treated with probiotics had significantly lower AST and ALT activity at the end of treatment than those treated with standard therapy alone (AST: 54.67 vs. 76.43 U/L; ALT 36.69 vs. 51.26 U/L). In a subgroup of 26 subjects with well-characterized mild alcoholic hepatitis (defined as AST and ALT greater than 30 U/L with AST-to-ALT ratio greater than one), probiotic therapy was associated with a significant end-of-treatment reduction in ALT, AST, GGT, lactate dehydrogenase, and total bilirubin. In this subgroup, there was a significant end-of-treatment mean ALT reduction in the probiotic arm versus the standard therapy arm. Therefore, **short-term oral supplementation with *B. bifidum* and *L. plantarum* 8PA3 was associated with restoration of the bowel flora and greater improvement in alcohol-induced liver injury than standard therapy alone[22].**

Conclusion

In conclusion, the gut-liver axis, particularly gut-derived endotoxin, seems to play a crucial role in the pathogenesis of ALD. Although, the potential pathogenic role of the intestinal microflora and therapeutic effect of probiotics in ethanol-related liver disease require further investigation, the growing body of studies suggests that restoration of normal bowel flora is a rational target for the treatment of alcoholic liver disease.

Dr Irina Kirpich is a Professor of Biochemistry at the Northern State Medical University, Arkhangelsk, Russia. Currently, she is working for the University of Louisville, USA. Dr Kirpich has been working in the field of Alcoholism, Alcohol-Nutrient Interactions, Alcoholic Liver Disease, Gut-Liver Interactions for more than 15 years. Her early research focused mainly on alcohol withdrawal syndrome and vitamin B1 deficiency, where she studied the correlation of vitamin B1 deficiency with the severity of alcohol withdrawal syndrome, neurological disorders, duration of treatment and recovery. Her most recent work is related to the study of liver/gut interactions in connection with alcoholic and non-alcoholic fatty liver disease. She led clinical study investigating the role of altered bowel microflora in the pathogenesis of liver disease and the potential therapeutic role of probiotics supplementation in the treatment of alcoholic liver injury. Dr Kirpich has published numerous peer-reviewed research papers and presented her research at many scientific meetings in different countries.

Gut microflora and diabetes: Study suggests role for probiotics? [23-25]

Bacterial populations in the gut of diabetics differ from non-diabetics, says a new study from Denmark, unraveling a potential role of probiotics in the management of diabetes.

The study, published in the open-access peer-reviewed journal *PLos ONE*, examined the differences between gut microbiota in diabetics and non-diabetics. It builds on earlier studies that have linked gut microflora and obesity. A breakthrough paper published in Nature in December 2006 reported that microbial populations in the gut are different between obese and lean people, and that when the obese people lost weight, their microflora reverted back to that observed in a lean person, suggesting that obesity may have a microbial component.

36 men at ages 31 to 73 years with body mass indices (BMIs) ranging from 23 to 48 were recruited in the study. Half of the subjects had type 2 diabetes. Faecal samples were obtained from the participants and analysed by real-time quantitative PCR (qPCR) and in a subgroup of subjects (N = 20) by tag-encoded amplicon pyrosequencing of the V4 region of the 16S rRNA gene.

Results showed that **type 2 diabetes was associated with compositional changes in the intestinal microbiota. In particular, the relative abundance of *Firmicutes* was significantly lower, while the proportion of *Bacteroidetes* and *Proteobacteria* was higher in the diabetics compared to their non-diabetic counterparts.** Accordingly, the ratios of *Bacteroides* to *Firmicutes* significantly and positively correlated with reduced glucose tolerance.

Several published studies have described the differences between gut microbiota in obese compared to lean persons. Many, on mice models and in humans provided evidence that increase in body weight were associated with a larger proportion of *Firmicutes* and relatively less *Bacteroidetes*.

Assuming that diabetes and impaired glucose tolerance are linked to obesity, positive correlation between ratios of *Bacteroidetes* to *Firmicutes* and BMI could be expected. However, the reverse tendency was observed in this study, indicating that overweight and diabetes are associated with different groups of the intestinal microbiota.

"Our data suggest that the levels of glucose tolerance or severity of diabetes should be considered while linking microbiota with obesity and other metabolic diseases in humans. It is especially important for developing the strategies to modify the gut microbiota in order to control metabolic diseases, since obesity and diabetes might be associated with different bacterial populations." The researchers concluded.

Statistics from the Health Promotion Board revealed that about 1 in every 10 Singaporeans has diabetes and the proportion is almost doubled among those 50 years and above. Among them, type 2 diabetes comprises about 90% of all diabetic patients.

Nadja Larsen from the University of Copenhagen and his team reported for the first time that the gut microbiota in diabetics differ from non-diabetics. Their findings open new doors to the management of patients diagnosed with type 2 diabetes probably by modifying the gut microflora. Although it is still immature at this stage, to consider the role for probiotics in diabetes prevention or management, the new research points to a potential use in the future.

Practical Insights:

Probiotics and Allergy [26-30]

Allergic diseases constitute the most common causes of chronic illness in developed countries and incidences are rising in developing countries. Although hereditary predisposition for allergy is thought to be involved, environmental factors appear to be required to trigger the disease. Reduced exposure to microbial allergens as a result of our hygienic lifestyle has been suggested as one of the possible causes of the development of allergy.

Experimental studies have provoked the suggestion that intestinal microbes are important regulators of immune responses and oral tolerance. In the human intestine, microflora have also been suggested to affect the development of the immune system and atopic sensitization in early infancy. As the world progresses with advancements in medicine and food processing, our contact with microbes has changed. Reduced family size, improved hygiene, vaccination, the use of antimicrobial medication, and the consumption of almost sterile food have reduced and changed our exposure to microbes. Humans have evolved in an environment with a heavy bacterial load and our immune system has been adapted to deal with that. The absence of such an appropriate microbial exposure may pose a problem for the development of a child's immune system.

The intestinal microbiota of allergic infants

The rationale for modulating the intestinal microbiota is supported by observations that allergic children have a different microbiota composition than healthy infants. **Children with allergy were found to have an aberrant microbiota even before the onset of allergy; they had higher levels of clostridia and lower levels of bifidobacteria. In addition to these quantitative differences in the *Bifidobacterium* microbiota, qualitative differences have also been observed. Infants with atopic dermatitis have been found to have a more-adult-like *Bifidobacterium* microbiota with high prevalence of *B. adolescentis*. Healthy infants, on the other hand, were found to be colonized mainly by *B. bifidum*, typical for breast-fed infants.**

Managing Allergy with Probiotics

On bacterial colonization of the colon after birth, the appropriate microbiological stimuli is essential to redress the balance of the skewed T-helper 2 immune response present in the newborn. By offering microbial stimulation of the infantile gut immune system, the use of probiotic therapy may help to prevent the manifestation of allergic reactions.

In a 4-year follow-up study, the efficacy of *Lactobacillus rhamnosus* GG on at-risk infants was studied. 159 pregnant women with a family history of atopic disease were given *L. rhamnosus GG* or placebo for 2 to 4 weeks before their expected delivery date in a randomized, double-blind trial. After delivery, the children received *L. rhamnosus* GG for 6 months. After 4 years, 46% of the children in the placebo group had developed atopic eczema, whereas in the probiotics group this was 26%; suggesting that the protective effect of probiotic on atopic eczema extends beyond infancy.

More recently, it has been demonstrated that children with IgE-mediated atopic dermatitis induced by cow's milk allergy have a reduced interferon-γ response that can be markedly increased by treatment with *L. rhamnosus* GG, thereby providing a strengthened Th1 cytokine response that could potentially reduce the Th2-mediated allergic potential in these individuals. Previous clinical study with *L. rhamnosus* GG and *B. lactis* BB-12 had also demonstrated benefits in infants with atopic eczema showing a significant improvement in skin condition after 2 months of probiotic-supplemented formula.