Clinical Evidence for the Role of Probiotics in Mucosal Immune Development and Impact on Respiratory Health and Allergy:
Probiotics in the Treatment and Prevention of Infantile Allergy

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Infections such as hepatitis A, mumps, and measles have decreased during the last 3 to 4 decades, whereas allergies and Th1 type diseases such as diabetes and Crohn's disease have increased during the same time.[1] The marked increase in allergic diseases is strongly correlated to fewer environmental microbial stimuli, especially during early childhood.

An inverse relationship between exposure to foodborne and orofecal microbes and respiratory allergies has been discovered.[2] Children growing up in a farming environment who are exposed to more microbes via animal-related contacts and consumption of unpasteurized farm milk experience a lower incidence of asthma and allergic rhinitis as well as to atopic sensitization.[3] Several studies show that the microflora of allergic infants is depleted of *Lactobacilli* and *Bifidobacteria* and contain more *Clostridia* than that of nonallergic infants, and this can be seen even before small children develop atopic eczema.[4] The same investigators also have shown that Estonian infants have a gastrointestinal microflora in many ways similar to that prevailing in Sweden in the 1960s.[5] Families such as those in Estonia who adhere to an anthroposophic lifestyle use more biodynamic and organic food and fermented vegetables that contain *Lactobacilli*, and they use fewer antibiotics and have fewer vaccinations than other families. A study from Sweden compared the incidence of atopy as measured objectively with skin-prick testing among pupils from Steiner schools and ordinary elementary schools. The Steiner school children experienced significantly less atopy than those attending ordinary schools, and a significant outcome measure was the use of organic or biodynamic food.[6] This

finding led to an interest in supplementation with *Lactobacilli* and other probiotics in the hope of treating and preventing allergic diseases.

A large amount of foreign proteins and microbes flow into the gut, which the gut needs to handle properly. The immune system of the gastrointestinal system—our body's largest immune system—has developed to perform this task. In addition, the commensal microflora is a large active organ comprising more than 1.5 kg of microbial mass and 10 times more organisms than the number of cells in the entire body. The gut microflora has many important functions. It promotes normal gut functions, protects from infections, and has effects on systemic metabolism and the immune system. It is important for the development of tolerance. In a mice study, it was shown that germ-free mice do not develop oral tolerance and the addition of *Bifidobacteria* restored their ability to develop oral tolerance.[7]

Probiotics are defined by the World Health Organization as "live micro-organisms that, when administered in adequate amounts, confer a health benefit on the host." Probiotics have been shown to digest food proteins to become less antigenic and have caused a decrease in gut permeability.[8] They have been shown to clearly affect the immune system in vitro, in animal studies as well as in humans. One widely available probiotic is *Lactobacillus rhamnosus* GG (LGG), an organism that was isolated by Sherwood Gorbach and Barry Goldin in 1985. LGG is of human origin, adheres to the intestinal epithelial cells, is resistant to acid and bile, colonizes the human gut, and has good growth characteristics. The first small clinical study using an extensively hydrolysed formula (EHF) and EHF fortified with LGG showed quite dramatic effects from LGG on severity of atopic eczema. More recent larger studies show mildly positive results in alleviating atopic eczema and food allergy. In the largest study to date, we gave LGG, a mixture of four probiotics (LGG $10x10^9$ cfu/day, *L rhamnosus* LC705 $10x10^9$ cfu/day,

Bifidobacterium breve Bbi99 $4x10^8$ cfu/day, and *Propionibacterium freundenreichii* ssp *shermanii* JS $4x10^9$ cfu/day) or placebo for 4 weeks to infants with suspected eczema and cow's-milk allergy. We assessed the severity of eczema by the SCORAD scoring system. All were put on a milk-free diet and treated with emollients and topical steroids as needed. After the intervention, the eczema was relieved very efficiently, but with no clear differences between groups. However, in infants with IgE-associated eczema, mean reduction in SCORAD of infants in the LGG group was significantly greater when compared to the placebo group.[9] The infants were efficiently colonized by the bacteria they were supplemented with, according to assessment of fecal samples. The results from probiotic treatment studies have proven most effective in IgE-mediated eczema and food allergy.[10] However, not all studies show an effect from using *Lactobacilli* (Table 1).[11]

Table 1. Randomized Controlled Trials on the Effect of Probiotics in Treament of Childhood Allergy

Authors, Country, Published	N of Patients	Age	Eczema in Baseline	Sensi-tized in Baseline	Intervention and Amount of Probiotics (cfu)	Outcome Measures	Clinical Effect
Rosenfeldt et al Denmark *J Allergy Clin Immunol* 2003;111	A1=20 A2=23	1 to 13 years	SCORAD >15	63%	*L rhamnosus* $10x10^{10}$ and *L reuteri* $1x10^{10}$ twice daily 6 weeks	SCORAD Subjective symptoms	Reduced subjective symptoms, Reduced SCORAD in sensitized children
Weston et al Australia *Arch Dis Child* 2005;90	A=28 P=28	6 to 18 mo	Moderate to severe SCORAD ≥25	71%	*L fermentum* twice daily 8 weeks	SCORAD	Reduced SCORAD in sensitized infants
Brouwer et al the Netherlands *Clin Exp Allergy* 2006;36	A1=16 A2=13 P=13	<5 mo	Eczema and suspected cow's milk allergy	38%	*LGG* or *L rhamnosus*	SCORAD Sensi-tization	No effect
Sistek et al New Zealand *Clin Exp Allergy* 2006;36	A=29 P=30	1 to 10 years	All sensitized, SCORAD ≥10	100% (food A=66% P=80%)	*LGG* and *B lactis* $2x10x10^{10}$ daily 12 weeks	SCORAD	Reduced SCORAD in food-sensitized infants
Fölster-Holst et al Germany *Br J Dermatol* 2006;155	A=26 P=27	1 to 55 mo	Moderate to severe	38% (A=8 P=12)	*LGG* $10x10^{9}$ daily 8 weeks	SCORAD	No effect
Grüber et al Germany *Allergy* 2007;62	A=54 P=48	3 to 12 mo	Mild to moderate SCORAD 15-40	55% (A= 62% P=47%)	*LGG* capsules $> 5x10^{9}$ twice daily 12 weeks	Symptom load score SCORAD	No effect

A = active treatment goup, A1 = active treatment group 1, A2 = active treatment group 2,
P = placebo group

Four studies have been published in which prevention of allergic diseases was attempted with supplementation of probiotic bacteria (Table 2).[12-18]

Table 2. Randomized Controlled Trials on the Effect of Probiotics in Prevention of Childhood Allergy

Authors, Country, Published	N of Patients	Treatment Initiated	Follow-up	Intervention Amount of Probiotics (cfu)	Clinical Effect
Taylor et al Australia *J Allergy Clin Immunol* 2007;119:184	High-risk A=89 P=89	Newborn babies aged <48h	12 mo	*L acidophilus* (LAVRI-A1) $3x10^9$ daily for 6 months	No effect Increased sensitization (Pos. SPT 40% vs 24%; *P*=0.03)
Abrahamsson et al Sweden *J Allergy Clin Immunol* 2007;119	High-risk A=95 P=93	Pregnant mothers + newborn babies	2 y	*L reuteri* (ATCC55730) $1x10^8$ from 36 gw to 12 months after birth	No effect on cumulative incidence of eczema (36% vs 34%) Less atopic eczema during 2 year: 4% vs 14 %, *P*=0.02)
Kopp et al Germany *Pediatrics* 2008;121	High-risk A=50 P=44	Pregnant mothers + newborn babies	2 y	LGG (ATCCT53103) $5x10^9$ x2/day; before delivery, 6 mo after birth to lactating mothers, otherwise to bottle-fed infants	No effect: eczema 28% vs 27%) LGG increased current wheeze
Wickens et al Australia *J Allergy Clin Immunol* 2008;122	High-risk A1=144 A2=152 P=150	Pregnant mothers + newborn babies	2 y	*L rhamnosus* HN001, *B animalis* ssp *lactis* HN019	*L rhamnosus*: sign less 0.51 eczema at 2 y, *Bifido* no effect No effect on atopic sens

A = active treatment goup, A1 = active treatment group 1, A2 = active treatment group 2,
P = placebo group

The earlier and smaller studies show strong preventive effects from providing probiotics prenatally to the expectant mothers and their infants for 6 months. The incidence of atopic eczema was halved.[13] We studied the preventive effects of four probiotics (LGG $10x10^9$ cfu/day, *L rhamnosus* LC705 $10x10^9$ cfu/day, *B breve* Bbi99 $4x10^8$ cfu/day, and *P freundenreichii* ssp *shermanii* JS $4x10^9$ cfu/day) on more than 900 children at high risk for allergy. The study was randomized, prospective, and double blinded. The mothers consumed the combination of

probiotics or placebo 4 weeks before delivery, and their infants consumed the same probiotics and a prebiotic (galactooligosaccharide) from birth until 6 months. A prebiotic is nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of a limited number of bacteria in the colon, thus improving host health. The main outcome measure was the development of an allergic disease at age 2 years. A 30% decrease in the cumulative incidence of allergic disease at 2 years of age was observed (Fig 1).

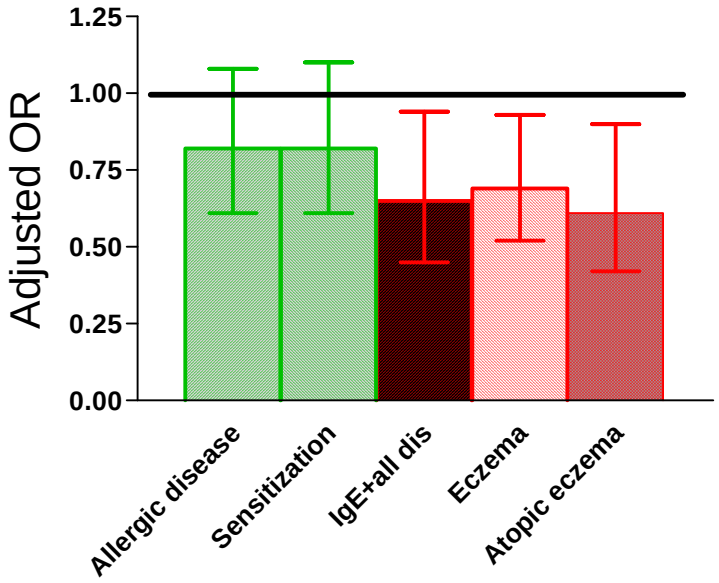

Fig 1. Development of allergic disease at age 2 years after supplementation with probiotics.

The supplemented bacteria were found in the feces of infants, confirming a successful intervention.[14] Another study from New Zealand failed to show any effect when *Lactobacillus acidophilus* was given only to the infants from birth until 6 months, but not to the mothers during pregnancy.[15] The type, dose, and mode of probiotic supplementation, as well as the setting and population studied might explain the differences. The strongest effects have been shown in high-risk infants demonstrating IgE-mediated atopic allergies.

Immune effects in vivo from supplementing with probiotics have clearly been shown. We have demonstrated an increased production of C-reactive protein (CRP) both in treatment of atopic eczema[19] and prevention of allergies.[20] The increased CRP was associated with a decreased risk of eczema and allergic disease at age 2 years (Fig 2).

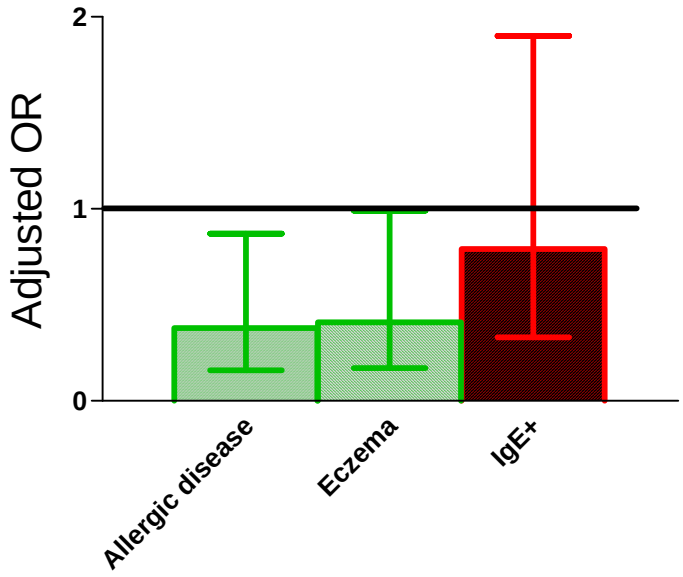

Fig 2. CRP value vs development of allergic disease at age 2 years.

This supports the view that a chronic low-grade inflammation protects from eczema. This increase in CRP was accompanied by an increase in IL-10 and total IgE and IgA, changes in regulatory mechanisms that resemble those seen in helminth infections. Chronic microbial exposure is an important immune modulator that protects from allergies.

Dendritic cells in the gut mucosa bearing receptors for bacterial components extend into the lumen where the stimulation from bacterial components leads to stimulation and changes in cytokine balance. Stronger and repeated or continuous stimulation can lead to permanent changes in immune balance. This has been shown in both treatment and prevention of atopic diseases. Probiotics are safe, and they may have beneficial effects on immune development of

infants, as seen in vaccine antibody-responses after supplementing with probiotics.[21] They have been shown to result in somewhat fewer infections and less need for antibiotics.[22, 23] They alleviate atopic eczema and reduce the appearance of allergic disease by age 2 years. They do not, however, affect the rate of IgE sensitization. The beneficial effect is strain-dependent. It seems that supplementation for prevention should start with the mother during pregnancy. The concept that supplementing with probiotics leads to immunologic and clinical effects has been proven. The microbial flora is important for the health of humans, and new research within this field, stimulated by probiotic studies, is most welcome.

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Q & A

Q: When you used your combination of probiotics as a treatment, you did not get positive results, but when you used the combination as prevention, you had a positive effect. However, you combined the probiotics with oligosaccharides. Do you think that the positive effects were due to

the utilization of this mixture of probiotics as a prevention, or to the combination of probiotics with a prebiotic?

Dr Kuitunen: That is a good question. There may have been a symbiotic effect. On the other hand, the dose we gave was small—0.8 g altogether. You gave much more in your studies. We wanted to be safe and not cause any harmful effects such as diarrhea in the children. We cannot conclude from our studies, however, that the positive effect resulted from symbiosis or just from the probiotics.

Q: No one has yet discussed the question of dose. In our rodent studies, we use around 10^8 or 10^9 cfu/day, and these are the same doses used in humans. We do not understand why we should use the same dose. Very few studies have compared doses in humans in these conditions. I presume you have been using about 10^9 cfu/day.

Dr Kuitunen: Yes.

Q: This is sort of the standard dose. My view is that we have to address this question, because a part of the problem we have comparing these organisms may be dose as much as anything else. Do you have any comments about this?

Dr Kuitunen: I have not seen any studies in humans comparing different doses, and this should be done. The problem is that we need large study populations. We thought about doing this with two intervention groups, but we would have needed more than 3,000 infants. This is too big for anyone to do. This should be studied in smaller animals. In the reported probiotic prevention studies, the dose has been 10^8 or 10^9. Researchers use this dose in studies of treatment as well. Dr Moro has told us that the prebiotic dose might affect the results; this suggests that we should probably give higher doses to get a maximum effect because we do not see side effects from higher doses.

Q: Since half of these experiment conclusions are translated to humans, we might as well do them in animals. Most of us have never done these experiments, even in animals. Some experiments do show clear dose-response curves in animals on effects on motility. Interestingly, they have a sigmoid-type response, which is amazing. We think there are possibly even bell-shaped curves; it is possible that if you give too great a dose or too little that you will not see an effect. Therefore, I think we should encourage whoever is involved in animal experiments to look at dose responses.

Dr Kuitunen: I agree. I am waiting for this to be done. I also encourage animal researchers to do this.

Q: When we compare the results of clinical studies done in various diseases, we observe some of the effects you mentioned, or we observe something we did not expect. For example, some allergy studies show a bell-shaped relationship. However, in the case of diarrhea prevention, especially rotavirus-associated diarrhea, we do not see a dose-dependent relationship at all within the range of 10^7 to10^{11}. I suppose you would expect different effects in different diseases, but, as you say, someone should do a study verifying this.

Dr Kuitunen: There is a study from the UK that correlated cat allergen levels and sensitization; very small and very high doses of cat allergen decreased sensitization [Custovic A et al: *J Allergy Clin Immunol* 2001;108:537-539].

Q: I have a question about the inflammatory immune profile induced by the probiotics. You talked about increases in IL-6, C-reactive protein (CRP), and IgE. I noticed that those markers increased in both the allergic and the non-allergic infants. Do you think that may explain some of the side effects of the LGG with respect to increased wheezing and bronchitis, and potentially asthma, at the 7-year follow-up?

Dr Kuitunen: That might be one explanation, but I find it fascinating that these markers also increased in the nonallergic infants. We do not know how the immune system works when you have a genetically different "drive" to allergy, but we should explore this further.

Q: Particularly the increase in IgE in nonallergic infants.

Dr Kuitunen: Yes. It is fascinating to compare them to, as I said, the helminth-infested individuals who are not sick. If you treat them, they develop allergy quickly.

Q: Some people in the United States are monitoring CRP as a prelude to necrotizing enterocolitis in premature infants, and they want to keep the CRP levels as low as possible. Can you comment on that relative to the infant?

Dr Kuitunen: This is mild inflammation, of course. These CRP levels are not close to those we see in clinical symptoms with infections. CRP is a sensitive method that measures very small amounts (<0.4 mg/L). These levels are not related to clinical infections.

Q: Most of these prevention trials focus on *Lactobacilli* vs *Bifidobacteria*. Are there data driving the decision by investigators to focus on *Lactobacilli*? A limited number of studies, including yours, have used *Bifidobacteria* either in combination with *Lactobacilli* or something else, or by itself, and they have not shown any efficacy. I wonder whether *Lactobacilli* and *Bifidobacteria* are really different. When you "drill down" even further, only the *L rhamnosus* species shows any benefits. So are there real data, or enough data, to decide about *Bifidobacteria* vs *Lactobacilli*?

Dr Kuitunen: *Lactobacilli* have been proven more effective, but we do not know the right bacteria to study. In one Polish or Czech study, investigators gave a nonpathogenic *E coli* and had good effects [Lodinova-Zadnikova R et al: *Int Arch Allergy Immunol* 2003;131:209-211]. However, it was a retrospective study, designed to work against nosocomial infections; many

bacteria-related factors might have influenced the effect. A new study from New Zealand found no effects from *Bifidobacteria*, but it does not conclude that *Bifidobacteria* do not work [Wickens K et al: *J Allergy Clin Immunol* 2008;122:788-94].

Q: Have you heard anything from investigators who choose to study *Lactobacilli* about the reason for their choice?

Dr Kuitunen: I think their use of *Lactobacilli* is a matter of supply.

Q: So it is not related to potential mechanisms of interaction with the immune system?

Dr Kuitunen: I would not say that, but not much.

Q: You mentioned that, regarding probiotics, allergy prevention should start late in pregnancy. On the other hand, some data indicate that prebiotics are not needed during pregnancy. Why are probiotics important during pregnancy but prebiotics are not?

Dr Kuitunen: The data on prevention using prebiotics are quite limited. We have some preliminary results from our 5-year study that show that infants, especially those delivered by caesarian section, benefit from probiotic therapy. It is important to give these probiotics to mothers before their child is born.

Q: In the studies in which giving pregnant women probiotics before birth was efficacious, were most of the infants breastfed? Is there also a potential benefit from the different components of breast milk, as shown by Susan Prescott's reearch? Could that account for some of the effects, or do you think the effects are just from the initial colonization from birth?

Dr Kuitunen: I think that the breast milk potentially has some effect. As you point out, this has been nicely studied by Susan Prescott's group. It is an important matter.