

# Clinical Evidence for the Role of Prebiotics in Mucosal Immune Development and Impact on Respiratory Health and Allergy

Guido Moro, MD, University of Milan, Italy

## Background and Hypothesis

The broad consensus is that breastfed infants grow and develop differently than artificially fed infants.<sup>1</sup> Breastfed infants have a reduced incidence of allergic or atopic diseases,<sup>2-4</sup> as well as of infections,<sup>5-7</sup> in comparison to bottle-fed infants, indicating a major impact of breastfeeding on the development of the immune system.

Increasing evidence shows that the composition of the intestinal microbiota plays a key role in the postnatal development of the immune system.<sup>8,9</sup> Before birth, the infant's gut is sterile. During vaginal delivery, the natural colonization of the infant starts with bacteria mainly from the vaginal and intestinal microbiota of the mother. The infant's diet plays an important role in further development of the intestinal microbiota.<sup>10</sup> During breastfeeding, the composition of the gut microbiota develops within a short period and becomes dominated by *Bifidobacteria*, whereas formula-fed infants develop a flora of a more adult type.<sup>11</sup>

Because of the importance of the intestinal microbiota for the development of the gut physiology and the immune system, many attempts have been made to mimic the intestinal microbiota of breastfed infants in bottle-fed infants. Recently, a mixture of neutral short-chain galactooligosaccharides and long-chain fructooligosaccharides (scGOS/lcFOS) were shown to reduce the incidence of atopic dermatitis (AD) and infectious episodes during the first 6 months of life.<sup>12-14</sup> This dual protection occurred through the intervention period. The hypothesis of the following study was to evaluate whether these protective effects would last beyond the intervention period.

## **Study Population and Methods**

In a prospective, randomized, double-blind, placebo-controlled study, 259 healthy term infants with a parental history of atopy were fed either prebiotic-supplemented (0.8 g/100 mL scGOS/lcFOS) or placebo-supplemented (0.8 g/100 mL maltodextrin) hypoallergenic formula during the first 6 months of life. A total of 102 infants in the prebiotic group and 104 infants in the placebo group completed the study. All infants were examined for clinical evidence of AD and incidence of infections. Following this intervention period, blinded follow-up continued until 2 years of life. Primary end points were cumulative incidence of allergic manifestations (AD, recurrent wheezing, and allergic urticaria). Secondary endpoints were rate (incidence) of infections (physician-diagnosed infectious episodes, fever episodes, and antibiotic prescriptions) and growth.

## **Results**

Two hundred and fifty-nine term infants were enrolled, and 206 infants completed the first 6-month part of the study. Parents of 152 completers gave consent to participate in the 2-year follow-up trial.

During the intervention period (first 6 months of life), 10 infants in the intervention group (9.8%) and 24 infants in the placebo group (23.1%) developed AD. The severity of AD was not affected by the diet. During the study period, infants in the scGOS/lcFOS group had fewer episodes of infections. They also tended to have fewer episodes of upper respiratory tract infection and fewer infections requiring antibiotic treatment.

Out of 152 participants in the 2-year follow-up, 134 infants (68 in placebo, 66 in intervention group) completed the study period. During this period, infants in the scGOS/lcFOS group had significantly lower incidence of allergic manifestations. Cumulative incidences of AD, recurrent wheezing, and allergic urticaria were respectively 27.9%, 20.6%, and 10.3% in the placebo; 13.6%, 7.6%, and 1.5% in the intervention group ( $P<0.05$ ).<sup>15</sup>

Infants in the scGOS/lcFOS group had fewer episodes of infections overall (respiratory, urinary, gastrointestinal infections, and otitis media), fewer episodes of fever, and fewer antibiotic prescriptions. The reduction in the number of infectious episodes reached a statistical significance for physician-diagnosed overall infections (5.9 vs 4.0 episodes/infant,  $P<0.01$ ), for fever episodes (4.0 vs 2.2 episodes/infant,  $P<0.000001$ ), for upper respiratory tract infections (3.2 vs 2.1 episodes/infant,  $P<0.01$ ), and for antibiotic prescriptions (2.7 vs 1.8,  $P<0.05$ ).<sup>15</sup>

Growth was normal and similar in both groups.

## **Conclusions**

Early dietary intervention with oligosaccharide prebiotics has a protective effect against both allergic manifestations and infections. This effect lasts beyond the intervention period until 2 years of life with the achievement of adequate growth.

Thus, the evidence shows that prebiotics have a significant and biologically relevant effect on the postnatal development of the immune system. The present data indicate that prebiotics can serve as an effective and safe tool to strengthen the immune system during infancy, which may offer a new method to prevent allergy and infections. However, long-term studies are needed to test the hypothesis that the influence of dietary factors on the immune system early in life might have beneficial consequences later in life.

## References

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## Q & A

Q: How did you decide on this particular blend of prebiotics?

Dr Moro: We did not decide on the blend, the company that produced the mixture decided. After several animal studies, they evaluated different ratios between the short-chain galactooligosaccharide (scGOS) component and the long-chain fructooligosaccharide (lcFOS) component, and at the end, they found that the best ratio was nine to one scGOS to lcFOS. Several studies demonstrated that this level—0.8 g/100 mL of infant formula—was best for producing positive effects in infants.

Q: You fed both scGOS and lcFOS in your blend, and lcFOS contributed only 10%. How great an impact do you believe that 10% had on the outcome?

Dr Moro: That is a good question, because it is a complex topic. The oligosaccharide composition of human milk is completely different from that of the blend. lcFOS, as you know, are not present in human milk. The mixture of scGOS and lcFOS used in these studies resembles the molecular size distribution of human-milk oligosaccharides. The main objective was not to mimic the complex chemical structure of human milk oligosaccharides, but to mimic the bifidogenic effects of human-milk oligosaccharides in formula-fed infants. Some research groups

are trying to replicate the oligosaccharides that are present in human milk, but they are very far from the solution.

Q: I would like to follow up on a previous question. Dr Moro, you saw significant differences between the formula groups, with or without the oligosaccharides, in high-risk infants. Is there a cohort of infants fed human milk to show how they would have done over the course of time?

Dr Moro: No. In our study, we only compared the group receiving the standard formula to the group receiving the scGOS/lcFOS-supplemented formula in high-risk infants. They were high risk because at least one of the parents was positive for allergy. We did not use a breastfed group for comparison. However, a breastfed group was used in the Multicentre Immuno Programming Study (MIPS). I showed you the results related to allergies and atopic dermatitis. We had more than 300 infants receiving mother's milk, and we used them for comparison. You cannot use breastfed infants as a control group, because then it is not a blinded study—you know those infants are receiving the mother's milk. You can, however, use them as a reference to evaluate whether the results you obtain in the formula-fed groups are similar to the results in breastfed infants. So we used a breastfed group in the MIPS, but not in our allergy study.

Q: In your last study, you saw a reduction in the number of episodes of fever. Do you think it might be a consequence of the reaction of the episodes of infection, or do you think that the prebiotics might have an effect on reduction of fever in the children independently of whether they have infection. To me, fever is a consequence of the reaction of the immune system to the infection; that might not be good.

Dr Moro: We decided to use fever as an outcome measure because it is an objective element to evaluate. Definitions of infection always depend on the person who is evaluating the infants. So we were not involved in the diagnosis of infection; the diagnosis was made by the family

pediatrician. This is the reason why we decided to look at fever and number of infections. Fever generally appears in the most severe cases of infection. You can have infection without fever, but when you have fever, you have infection.

Q: Was the scGOS/lcFOS formula extensively hydrolyzed?

Dr Moro: It was somewhere between partially and extensively hydrolyzed, but we can consider it extensively hydrolyzed.

Q: Were you surprised that, in the placebo group, the incidence of atopic dermatitis was relatively high? Twenty-three percent, then 28% at 2 years.

Dr Moro: Yes, but in Italy, when there is a family history of allergy and the infant is not receiving mother's milk, the pediatrician generally starts the infant on hydrolyzed formula. The incidence of atopic dermatitis in these infants (~20%-22%) is similar to the incidence we found in our group receiving the standard formula.

Q: Do you think that the hydrolyzed formula helped lower the incidence?

Dr Moro: No, it did not help lower the incidence, but it probably helped reduce the severity of the disease. If you look at the results obtained with the Scord score at 3 and 6 months of age, the severity of the score was similar in the two groups—between 9 and 13.

Q: In the MIPS, was the formula hydrolyzed?

Dr Moro: No, a standard starting formula was used. All those infants were normal healthy babies with no family history of allergy.

Q: When you looked at the vaccine responses, you saw no significant differences. However, if you look at the immunoglobulin (Ig) G2 results in the control group vs that in the scGOS/lcFOS-formula group, it looks like there was a difference.

Dr Moro: There was a small difference, but without statistical significance.

Q: Were you concerned about that since IgG2 is the most predominant immunoglobulin?

Dr Moro: Of course, but we were interested in finding significant differences.

Q: Was there was a trend in the *P* value?

Dr Moro: I cannot tell you whether there was a trend, but for IgG1, IgG2, and IgG3 there were no significant differences. If you look at the results, IgG1 values were higher in the standard-formula group compared to those in the scGOS/lcFOS-formula group, so it made no difference.

Q: In the first part of your presentation, you talked about prebiotics and the intestinal flora. Did you omit any children who had received antibiotics?

Dr Moro: Yes. Infants who were receiving antibiotic for any reason were taken out of the study.

Q: I did not hear you say anything about asthma or the incidence of asthma in this population, and there has been such a difference of opinion about asthma in the prebiotic groups. What was your experience with wheezing or asthma?

Dr Moro: We followed the infants until the age of 2 years, and as you know, asthma usually occurs later. We were only able to evaluate the infants for wheezing, and the rate of wheezing was lower in the first 2 years of life in the group receiving scGOS/lcFOS formula. We are planning to follow the infants until the age of 5 years. Probably next year we will complete the follow-up and see whether there is any difference in the rate of asthma in the two groups.

Everyone is interested in this topic.

Q: At the end of your presentation, you strongly advocated the imprinting theory and a 6- month intervention. Do you see any place for oligosaccharides beyond 6 months, or does the food of Italian babies contain it naturally?

Dr Moro: I think all the conference participants talked about the intervention “window,” but no one knows how long it lasts. We do know that the earlier you intervene, the better the results. In

the MIPS, we continued the scGOS/lcFOS supplementation for the first year of life, but we decided that 6 months was a good time to evaluate the effect of the milk because during that time the infants in both groups were receiving only milk. We were able to evaluate exactly what nutrients and how much scGOS and lcFOS they were receiving. But you can continue this supplementation for a year or longer.

Q: It seems like the major outcome of your prebiotic treatment was to elevate the *Bifidobacteria* levels in the feces of the formula-fed infants to the same levels as those in breastfed infants. Would it be reasonable, then, to speculate that any formula that raises the level of *Bifidobacteria* to that of a breastfed infant would be efficacious against allergy?

Dr Moro: I think that you have to evaluate not only the number of positive bacteria you reach, but also the other aspect. That is, if you are giving *Bifidobacteria*, and the number of *Bifidobacteria* is similar to that found in the stools of breastfed infants, that is okay, but you also have to evaluate the number of pathogens—the different environment you are working with. Short-chain fatty acid levels, pH value, IgA levels—all these aspects—are extremely important. To have only the *Bifidobacteria* value, in my opinion, is not enough to evaluate the efficacy of the formula you are using.

Q: Do you think that the pathogen levels, or differences in pathogen levels, affect allergy, as well—not just infectious diseases?

Dr Moro: Yes. There is a clear correlation between some strains such as *Clostridium* and allergy. You also have to evaluate other aspects of the intestinal flora, not only the number of *Bifidobacteria* and *Lactobacilla*.

Q: Your presentation was about prevention, but based on your experience and your results, do you see any place for prebiotics in the treatment of atopic eczema or food allergy? Very few studies have been done on that.

Dr Moro: That is a good question, because prebiotics and probiotics have different roles. Prebiotics can have a preventive effect, and probiotics can have a therapeutic effect. Probiotics work efficiently and positively on diarrhea such as that caused by antibiotics. There is no evidence that prebiotics have such effects during diarrhea, but they may help prevent diarrhea.

Q: My question is about the hydrolyzed formula and the incidence of eczema observed in your control group. Studies such as the German Infant Nutritional Intervention (GINI) Study were not just about feeding a hydrolyzed formula, they were about a regimen—for instance, not feeding solid foods for a period of time and not exposing the infants to any milk-based formula before they entered the study. Have you considered that the feeding of the hydrolysate alone may have produced your results? Were other regimens incorporated into this study so that those things were avoided in your control group, and if so, could those be related to the higher incidence of eczema you observed in that group?

Dr Moro: The infants received only formula for the first 6 months of life. They did not receive any other foods or other substances during the treatment period. After that, they began weaning. There were no problems with the mothers because they were not breastfeeding; they were free to eat whatever they liked.

Q: You talked about the cumulative incidence of eczema in the MIPS 2-year follow-up. In studies such as GINI, the researchers continue to see differences in the cumulative incidence, because a lot of that is driven by what happens in the first 6 months. Is that what you saw in your

study, or did you continue to see a point prevalence difference at older ages, as well as early differences?

Dr Moro: We evaluated the difference and saw that the infants receiving the standard formula developed atopic dermatitis earlier than those receiving the scGOS/lcFOS formula. The majority of infants developed atopic dermatitis in the first 6 months of life.

Q: The difference you saw at 2 years was based on what was seen in the first 6 months?

Dr Moro: Yes, in our study on infants at high risk for allergy. In the MIPS, we treated healthy full-term infants without any family history of allergy infants for the first year of life, and now we are starting the follow-up. We will follow them until the age of 4 or 5 years to see the incidence of atopic dermatitis and other allergies, and after the first year of life, to also see the incidence of infection. We did not find any difference in the rate of infection during the study period, but we will continue to follow the infants after that.

Q: In the multicenter study, did the mothers pick their own choice of infant formula after the first 6 months, or did you specify a hydrolyzed or nonhydrolyzed formula?

Dr Moro: The mothers continued with the same formula until the infant was 1 year of age. The only difference was weaning, because the infants were weaned according to different styles of weaning.

Q: Studies such as GINI and others of this nature are fascinating because the control populations have such astoundingly different percentages of atopic dermatitis across the various studies. I believe that in the GINI Study of high-risk infants, 15% of those fed conventional cow's milk formula had atopic dermatitis at 1 year of age. In a similar population in northern Italy, however, 24% of those fed a hydrolyzed formula without scGOS/lcFOS had atopic dermatitis at 6 months of age. Can you speculate whether living north or south of the Alps has an effect, or is something

else working there? Is there a selection bias in how patients come into the studies in the two places? Is there a difference in how the entry criteria are written?

Dr Moro: When considering atopic dermatitis or other forms of allergy, we have to consider not only what the infant eats, but also where the infant lives. Milan, for instance, is a very polluted city; this is surely one of the main factors in allergy and atopic dermatitis. I am sure that pollution in industrialized cities is creating problems for these types of infants.

Q: If I understand correctly, your studies showed a reduction in the incidence of allergy after treatment with prebiotics. When you studied the specific IgE, you did not find any difference, but you saw a difference in the IgG1-specific level for cow's milk proteins. Might that suggest that the mechanisms of allergy in these infants are mainly mediated by IgG1 and not by IgE?

Dr Moro: We found a difference in IgG1 and total IgE. We found a significant difference between the group receiving the standard formula and the group receiving the scGOS/lcFOS formula; total IgE level was significantly higher in the standard-formula group. We did not see a difference in specific IgE levels, only in the level of IgG1.

Q: This process is interesting because it suggests that the mechanisms arise mainly in IgG1.

Dr Moro: This is intriguing, but we do not know the mechanism behind it.

Q: When we talk about probiotics, we talk in terms of species, but now it is getting to the point where we talk in terms of the importance of a given strain. When we do research on prebiotics, however, we talk more about the total level of *Bifidobacteria*. You saw a substantial increase in *Bifidobacteria* after feeding this particular blend. Did you look at the different species, and was there a difference in species in your standard formula compared to the treatment group?

Dr Moro: Yes, we looked at the different species, but I did not show those results. We were able to demonstrate that, for example, the number of *Bifidobacterium adolescentis* was decreased in

the scGOS/lcFOS group compared to the group receiving the standard formula. If you look at the composition of the subspecies of intestinal flora, you would find differences. This is important for atopic disease and other forms of allergies.

Q: Can you speculate on the use of oligosaccharides for premature infants relative to the programming approach that you suggested? Would you recommend them?

Dr Moro: Of course, the best choice is mother's milk and breastfeeding. If the infant is not receiving mother's milk, however, and particularly if he or she is at high risk for allergy, I suggest giving this mixture of prebiotics, not to save the infant, but to decrease the possibility that he or she will develop atopic dermatitis or other forms of infection in the first years of life.

Q: What about premature infants?

Dr Moro: A couple of studies have been done with premature infants. One study in Milan included a limited number of infants [Boehm G et al: *Arch Dis Child* 2002;86:178-181]. Ten premature infants received a standard formula, and another 10 premature infants received the same formula supplemented with the mixture of scGOS and lcFOS. The infants with the supplemented formula received 1 g scGOS/lcFOS/100 mL of milk. The researchers were able to demonstrate a significantly higher number of *Bifidobacteria* in the feces of the supplemented infants without any side effects. Also, Neena Modi will publish a study soon in which she evaluates a larger number of preterm infants, also with positive results.

Q: I noticed that among the baseline characteristics in this study is a difference between vaginal and caesarian births. It seems that you have enough of a population of caesarian births compared to vaginal births. Are you looking at what those differences might hold in any of these studies?

Dr Moro: We did not evaluate the difference between vaginal birth and caesarian delivery.

However, the rate of caesarian delivery was the same in the two groups. But you are right, there

is a significant difference at birth. This is why it is important to start oligosaccharide supplementation immediately, because we are able to modify the intestinal flora of an infant born by a caesarian section in a positive way.

Q: Do you have information in your studies that you could look at, even if it is retrospective, to see whether you have differences in *Bifidobacteria* but not in prebiotics between vaginal and caesarean births?

Dr Moro: We have these data available, because in the study we performed in high-risk populations we evaluated fecal flora at birth, at 3 months, and at 6 months of life. We have to take out these data and elaborate on them.