



A mixture of three prebiotics does not affect vaccine specific antibody responses in healthy term infants in the first year of life

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ARTICLE INFO

Article history:

Received 7 March 2011

Received in revised form 11 July 2011

Accepted 25 July 2011

Available online 5 August 2011

Keywords:

Immune system

Prebiotic supplementation

Nondigestible carbohydrates

Vaccination antibody response

Healthy infants

Non-atopic

ABSTRACT

Background: Previous studies have shown, that prebiotics can modulate the immune response in infants at risk for allergy, leading to a lower incidence of atopic dermatitis. Few studies have evaluated the effect of prebiotic carbohydrates alone on the vaccine-specific antibody response as a marker for the development of the immune system in healthy infants not at risk for allergy.

Aim: This study evaluates the effect of adding a specific prebiotic mixture of short chain galacto-oligosaccharides (scGOS)/long chain fructo-oligosaccharides (lcFOS) ratio 9:1 and pectin-derived acidic oligosaccharides (pAOS) to formula feeding on the specific immunoglobulin responses to Haemophilus influenza type b (Hib) and tetanus immunization in healthy non-atopic infants during the first year of life.

Methods: This substudy has been embedded in a multinational multicenter RCT ($n = 1130$ children) to evaluate the effect of study prebiotics on the incidence of fever episodes during the first year of life. The study prebiotics were administered throughout the first year of life. This is a substudy on the vaccine-specific immunoglobulin responses to Hib and tetanus immunizations. Only data of the Dutch children, 80 in the prebiotics group and 84 in the control group, were used for this substudy. They all followed the national vaccination schedule leading to a homogeneous group. Blood was sampled at 6 and 12 months of age.

Results: Hib immunizations: median values did not differ between groups at the age of 6 and 12 months. At the age of 12 months, 34 out of 37 (91.9%) infants in the prebiotics group and 31 out of 34 infants (91.2%) in the control group had Hib antibody levels $>1.0 \mu\text{g/ml}$.

Tetanus immunizations: median values did not differ between groups at the age of 6 and 12 months and were above the cut-off value of 0.1 IU/ml in all infants in both the prebiotics and the control group.

Conclusion: No effect of prebiotics supplementation on vaccination specific antibody levels was found in children up to the age of 12 months; the vaccine specific antibody levels in infants fed the study prebiotics or a control diet were similar during the first year of life. We hypothesize that this specific prebiotic mixture, which resembles the composition of oligosaccharides in human milk, mainly promotes Th1 and Treg dependent immune responses and induces a down regulation of IgE-mediated allergic responses, while the desired vaccine-specific serum antibody responses remain intact.

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Abbreviations: Th, T-helper; scGOS, short chain galacto-oligosaccharides; lcFOS, long chain fructo-oligosaccharides; pAOS, pectin-derived acidic oligosaccharides; DTH, delayed-type hypersensitivity; Hib, haemophilus influenza type b; MIPS, multicenter infection prevention study; Ig, immunoglobulin; RCT, randomized controlled trial.

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1. Introduction

Neonates are born with an immature immune system that is not fully adapted for postnatal life. The immune system develops during the first few years of life and is highly susceptible to many external factors including diet [1,2]. Markers to study the development of immune system in infants are scarce. Recently, it was advocated

¹ For the Multicenter Infection Prevention Study 1 (MIPS 1) Study Group. See Appendix A.

by an ILSI-expert group to use vaccination response parameters for studying immunomodulation in nutritional intervention studies [3]. A successful example of this has been presented by Vos, showing that supplementation of a specific prebiotic mixture enhanced especially the cellular Th1-dependent adaptive immune response in mice, while vaccine specific antibody levels remained unaffected [4]. The same prebiotic mixture inhibited respiratory allergy in a validated animal model [5].

The immune system of an infant is skewed towards a Th-2 profile [6]. Evidence is increasing that the postnatal modulation towards a balanced Th1/Th2 immunity is influenced at least in part by human milk oligosaccharides [2,7–9]. Human milk oligosaccharides are multifunctional biomolecules which have the potential to promote health benefits, including modulating the immune system by influencing gut microbiota, or by affecting the immune system directly [10,11]. Prebiotics are non-digestible food components, which can promote the growth of the health promoting probiotic bacteria in the human gut, such as the lactobacilli and bifidobacteria [8,12–14]. Recently, a specific prebiotic mixture of short chain galacto-oligosaccharides (scGOS) and long chain fructo-oligosaccharides (lcFOS) in a ratio 9:1 (IMMUNOFORTIS®) has been developed. This mixture has the same molecular size distribution as the oligosaccharides in human milk.

ScGOS/lcFOS 9:1 has been shown to induce a beneficial immunoglobulin profile [15]. That means lower IgE levels without affecting the other antibody isotypes, and without affecting the response to vaccination in infants at risk for allergy. There even seemed to be a trend in changing IgE/Ig4 ratios, indicating an anti-allergic profile and a tolerance inducing immune environment. In this study, pectin-derived acidic oligosaccharides (pAOS) have been added to the scGOS/lcFOS 9:1 mixture. Human milk contains a mixture of neutral and acidic OS. Pectin-derived AOS were developed to represent a functional analogue in infant formula in order to better resemble human milk; they might reduce the adherence of bacteria on the epithelial surface. Recent data suggests that supplementation of these pAOS could be beneficial for the development of the infant's immune system [16]. A combination of neutral OS and pAOS enhanced the vaccine-specific T cell dependent delayed-type hypersensitivity (DTH) responses in a dose-dependent manner in a murine vaccination model. Antibody levels to the vaccines used were not affected. In this model, combinations of scGOS/lcFOS 9:1 and pAOS were more effective in enhancing vaccination induced DTH-responses than either of the OS alone, which suggests a synergistic interaction between the neutral OS and pAOS. The combination of both neutral as well as acidic OS might be one of the key reasons for the immunological protection provided by human milk. In parallel studies it was indicated that the scGOS/lcFOS/pAOS mixture was slightly better in the prevention of respiratory allergy in a mouse model for allergic asthma. In this study immune biomarkers as well as lung function biomarkers were improved significantly by the scGOS/lcFOS/pAOS mixture [5].

Few studies have evaluated the effect of prebiotic carbohydrates on the vaccine-specific antibody responses, and so far no significant effect was shown for scGOS and lcFOS in infants at risk for allergy. It was suggested that scGOS/lcFOS mixtures might act specifically by promoting or down regulating Th1 and/or Th2 activities, respectively [4,15,17]. More recently, new data indicate specific effects on regular T cells besides down regulation and upregulation of Th2 and Th1, respectively [6,15,16,18]. The effect of a specific mixture of scGOS and lcFOS, ratio 9:1, and pAOS on vaccination antibody responses has not been studied in a clinical trial with healthy infants so far. Therefore the aim of this current study was to evaluate the effect of adding this prebiotics mixture to formula feeding on the specific immunoglobulin-G responses to *Haemophilus influenza* type b (Hib) and tetanus immunization

in healthy non-atopic infants in their first year of life to analyze potential immune effects in terms of safety and efficacy.

2. Materials and methods

2.1. Study design

A randomized, placebo-controlled, double-blind multicenter trial was conducted. The primary endpoint of the study was to investigate whether formula feeding supplemented with GOS/FOS 9:1 and pectin-derived AOS reduces the incidence of fever episodes in the first year of life [19]. Seven study centers in five Western European countries (Italy, Austria, Switzerland, Germany, and the Netherlands) participated. In this trial, vaccine-specific serum antibody responses were included as a secondary parameter of immune efficacy and immune safety. This substudy assesses the effect of scGOS/lcFOS 9:1 and pAOS on the vaccine-specific immunoglobulin responses to Hib and tetanus immunizations. Because of heterogeneity in vaccination schemes and composition of vaccines among the other Western European countries, only Dutch infants were included in this analysis. They all received the same immunizations according to the national vaccination program, making this cohort a homogeneous group.

2.2. Participants

The details of the full study setup have been described elsewhere [19]. In summary, parents were invited to participate with their child between July 2005 and December 2007. Inclusion took place before the postnatal age of 8 weeks. Infants were included if they were born at a gestational age of 37–42 weeks with a birth weight > P10 for gestational age according to standardized Dutch growth charts. Infants at risk for atopic diseases (having a first degree relative with a positive history of asthma, hay fever, or atopic dermatitis) were excluded.

2.3. Nutritional intervention

To comply with ethical standards not to influence breastfeeding mothers in any way to use an instant formula instead, mothers were asked their consent to participate in the study and to be randomized to one of the two formula groups only if they could not or did not fully breast feed their infants and had fed at least one bottle of formula before. Only infants of mothers who indicated before the 8th week after birth to give only formula feeding at least during the first 4 months of age were randomized into the prebiotics group or the control group. Infants allocated to the prebiotics group received a standard non-hydrolyzed cow's milk-based formula to which a mixture of scGOS and lcFOS, ratio 9:1 (IMMUNOFORTIS®), as well as pAOS was added. This mixture is referred to as the study prebiotics. The total amount of OS was 8 g/l with 6.8 g/l neutral and 1.2 g/l AOS. A similar standard non-hydrolyzed cow's milk-based formula without the prebiotics was given to infants allocated to the control group. For both groups starter formula was provided during the first 6 months of life, thereafter follow on formula was provided. Infants randomized to the prebiotics group continued to receive the oligosaccharides in the follow on formula. All infants, either allocated to the prebiotics group or the control group, were fed with the study formula throughout the first 12 months of life.

2.4. Immunizations

In the Netherlands, infants are immunized at local well-baby clinics with DKTP-Hib vaccines (Pediaceel; Sanofi Pasteur MSD Limited, Maidenhead, Berkshire, the United Kingdom, containing

Table 1

Vaccination schedule in the Netherlands according to the National Institute for Public Health and the Environment (RIVM) [20].

Age	Regular immunizations	Hepatitis B target group (infants from endemic countries or regions)
2 months	DKTP-Hib-1 ^a + Pneu-1 ^b	DKTP-Hib-Hep B-1 ^c + Pneu-1
3 months	DKTP-Hib-2 + Pneu-2	DKTP-Hib-Hep B-2 + Pneu-2
4 months	DKTP-Hib-3 + Pneu-3	DKTP-Hib-Hep B-3 + Pneu-3
11 months	DKTP-Hib-4 + Pneu-4	DKTP-Hib-Hep B-4 + Pneu-4

^a Diphtheria, Pertussis, Tetanus, Polio, and Haemophilus influenzae type b vaccine

^b Pneumococcus vaccine.

^c Hepatitis B vaccine.

diphtheria and tetanus toxoid, acellular pertussis antigens, inactivated poliovirus and Hib polysaccharide-conjugate vaccines), as well as the pneumococcal vaccine (Prevenar®, WYETH PHARMACEUTICALS BV, Pfizer Inc., Capelle aan den IJssel, the Netherlands), following the national immunization program (Table 1) [20].

The immunogenicity of the Hib polysaccharide conjugate vaccine and tetanus toxoid of the DKTP-Hib vaccine was assessed in this study to be able to analyze the evocation of a B-cell (Hib) and a T-cell (tetanus) specific immune response. Furthermore, the Hib conjugate vaccine is effective in two ways: the type b polysaccharide capsule results in protection against Hib invasive disease by stimulating the production of serum antibodies by B-cells, and the

Hib conjugate vaccination primes for long-term memory to the Hib polysaccharide by developing memory B cells. Tetanus toxoid, on the other hand, is an antigen known to induce a strong T-cell specific immune response.

2.5. Antibody analysis

Venous blood was collected at the ages of 6 and 12 months. The immunoglobulin-G concentrations specific to Hib and tetanus immunizations were assessed.

2.5.1. Hib antibody detection

Immunoglobulin-G antibodies against polyribosylribitolphosphate (PRP) of Hib were determined using Immunozyg Hib-IgG, a two step enzyme immunoassay (EIA) (PROGEN BIOTECHNIK GmbH, Heidelberg). Immunoglobulin levels above 1.0 µg/ml were considered protective Hib antibody titers [21].

2.5.2. Tetanus antibody detection

Immunoglobulin-G antibodies to tetanus toxoid were quantified by a classic EIA kit (Genzyme Virotech GmbH, Rüsselsheim). Infants were considered to be protected against tetanus if immunoglobulin-G levels were above 0.1 IU/ml [21].

2.6. Ethics

This study has been carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki). The local ethics committee approved the study protocol. Written

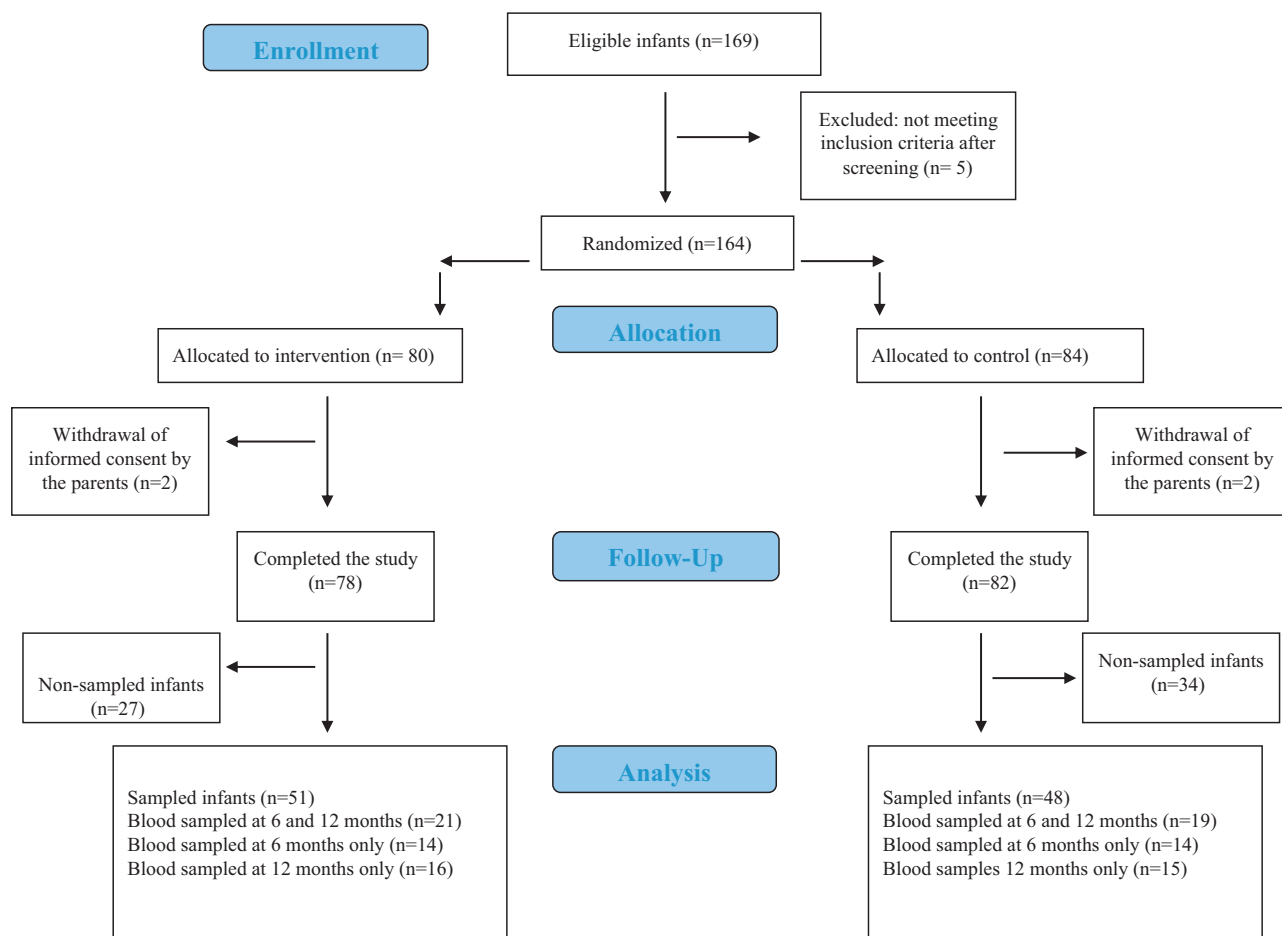


Fig. 1. Flow diagram inclusion.

consent was obtained from all parents before randomization. Parents were asked to give separate informed consent for venous blood sampling.

2.7. Statistics

No formal power calculation was made.

Statistical analyses were performed by SPSS for WINDOWS, version 16.0.

Continuous variables are presented in median values and the 25 and 75 percentiles.

A non-parametric test (Mann–Whitney *U*-test) was used to test for statistical significant differences between groups. A *p*-value of 0.05 was considered statistically significant. Using this test we compared the vaccine specific immunoglobulin-G antibody levels between the prebiotics group and the control group.

Fig. 1 presents the numbers of infants whose blood was sampled in both groups. The distribution of baseline characteristics [19] was compared between the infants in the prebiotics group versus the infants in the control group. Also, the distribution of baseline characteristics of the infants whose blood was sampled at 6 months and at 6 and 12 months were compared to those whose blood was not sampled. Furthermore, we compared infants whose blood was sampled at 12 months and at 6 and 12 months to infants whose blood was not sampled. And finally, infants whose blood was sampled at 6 months were compared to those whose blood was sampled at 12 months.

The correlation between the time interval after immunization and vaccination response was tested using the non-parametric Spearman rho correlation coefficient.

3. Results

3.1. Participants

A total of 164 infants were randomized. 80 Infants were randomized to receive the formula supplemented with the study prebiotics and 84 infants to receive the standard non-hydrolyzed cow's milk-based formula (Fig. 1).

3.2. Immunizations

The distribution of immunizations of all participants is shown in Table 2. Approximately 60% and 5% of all infants received Prevenar and Hepatitis B immunization, respectively. A total of 4 (2.4%) infants were not immunized because of parental religious convictions. Of the non-vaccinated infants, no blood sample was obtained.

3.3. Blood samples

There were no statistical significant differences in the distribution of baseline characteristics in all comparisons between groups

Table 2

Distribution of immunizations in the Groningen cohort of the Multi Infection Prevention Study.

Type immunization, <i>n</i> (%)	Prebiotics group (<i>n</i> = 80)	Control group (<i>n</i> = 85)
DKTP-Hib ^a	25 (31.3)	33 (38.8)
DKTP-Hib + Pneu ^b	50 (62.5)	45 (52.9)
DKTP-Hib-Hep B ^c + Pneu	3 (3.8)	0 (0)
DKTP-Hib-Hep B	1 (1.3)	4 (4.7)
No vaccination	1 (1.3)	3 (3.5)

^a Pediacel: diphtheria, pertussis, tetanus, polio, and haemophilus influenzae type b vaccine.

^b Prevenar: pneumococcus vaccine.

^c Hepatitis B vaccine.

(Fig. 1), except a higher birth weight in infants of whom no samples were obtained at the age of 6 months (*n* = 61) versus infants who were sampled at 6 months and at 6 and 12 months (*n* = 68). We think this difference is based on coincidence because quite a lot of characteristics have been tested.

At the age of 6 months venous blood was collected from 35 of 80 (43.8%) infants in the prebiotics group and 33 of 84 (39.3%) infants in the control group. At the age of 12 months blood samples of 37 of 80 (46.3%) infants were obtained in the prebiotics group and of 34 of 84 infants (40.0%) in the control group.

3.4. Vaccine-specific IgG antibody levels

3.4.1. Hib immunizations

Median values of the vaccine specific immunoglobulin-G antibody levels did not differ between the prebiotics group and control group at the age of 6 and 12 months (Table 3). Hib antibody levels >1.0 µg/ml were found in 29 of 35 (82.9%) infants aged 6 months in the prebiotics group and in 26 of 33 (78.8%) of infants in the control group. At the age of 12 months, 34 of 37 (91.9%) infants in the prebiotics group and 31 of 34 infants (91.2%) in the control group had Hib antibody levels >1.0 µg/ml. As expected, a higher percentage reached the cut-off level of 1.0 µg/ml at the age of 12 months than at 6 months.

3.4.2. Tetanus immunizations

Median values of the vaccine specific immunoglobulin-G antibody levels did not differ between groups at the age of 6 and 12 months. Median values were above the cut-off value of 0.1 IU/ml for both prebiotics group and control group (Table 3). Every infant reached a level of >0.1 IU/ml at 6 and 12 months of age.

The age of the infants, when the 6 months sample was taken, was 174 (168–224) days. The time interval between the third immunization and the 6-month sample was 53 days (35–94 days). This time interval was (negatively) correlated to the Hib antibody level (*p* = 0.001) and the tetanus toxoid antibody level (*p* = 0.005).

The age of the infants, when the 12 months sample was taken, was 369 (366–371) days. The time interval between

Table 3

Immunoglobulin-G antibody response to Hib and tetanus immunizations.

Immunization	Prebiotics group (<i>n</i> = 35)	Control group (<i>n</i> = 33)	Significance
Hib (µg/ml) ^a			
6 months of age	2.4 (1.2–6.2)	2.7 (1.3–6.8)	n.s.
12 months of age	10.4 (3.3–16.8)	11.2 (4.8–30.2)	n.s.
Immunization	Prebiotics group (<i>n</i> = 37)	Control group (<i>n</i> = 34)	Significance
Tetanus (IU/ml) ^a			
6 months of age	0.9 (0.5–1.2)	0.8 (0.5–1.1)	n.s.
12 months of age	1.7 (0.9–2.8)	2.0 (0.9–2.6)	n.s.

n.s., difference between groups not statistically significant (non-parametric test).

^a Median values (25–75th percentiles).

the fourth immunization and 12-month sample was 30 days (22–42 days). The Hib antibody level was (negatively) correlated to the interval between the fourth immunization and blood sampling at 12 months ($p=0.021$). The tetanus toxoid antibody level at 12 months was not significantly correlated to the interval between the fourth immunization and blood sampling at 12 months ($p=0.071$).

4. Discussion

In this study in a group of healthy non-atopic infants, the specific antibody responses to Hib and tetanus immunizations between a group of infants that received a formula supplemented with the study prebiotics and a group of infants who received a similar standard non-hydrolyzed cow's milk-based formula without the prebiotics during the first year of life were similar. Similar Hib and tetanus IgG concentrations were found in both study groups after 3 and 4 DKTP-Hib-doses at 6 and 12 months of age, respectively. No adverse side effects, i.e. effects out of the normal range, regarding vaccine specific antibody responses were demonstrated indicating the immune safety of this specific carbohydrate mixture. This study supports the safety of supplementing infants with this specific prebiotic mixture.

T-helper (Th) cells play a central role in balancing and steering the development of the infant's immune responses [23]. However, immunological studies show an increasing number of specific roles of the different T-cells, weakening the old black and white concept of Th1 and Th2 cells. The discovery of the involvement of the Treg cells in immune balancing suggests a complex etiology of atopy. Immune modulating effects of prebiotics have been shown in infants at risk for atopy. The reduction of the incidence of atopic diseases by prebiotics may be (partly) caused by promoting Treg activities [22,24,25].

Recent studies show that supplementation with scGOS/lcFOS 9:1 stimulates the maturation of the Th1 response in infants at high risk for atopy by altering the microbiota amongst other actions. It was shown that supplementation of scGOS/lcFOS 9:1 resulted in a down regulation of total non-specific IgE levels and reduced the cow's milk protein-specific IgG1 which is early in life a major biomarker for allergy to food allergens [15]. The DTP-specific vaccination serum antibody response was not affected. Furthermore, the cumulative incidence of AD was significantly reduced by scGOS/lcFOS 9:1 during the first 6 months of life [26]. It seems that, in infants at risk for atopy, prebiotics might cause a switch from a Th2- to a Th1-profile, which is reflected in a down regulated IgE-level. Although total IgE levels were down regulated, the vaccine-specific antibody responses remained intact [15]. Another publication regarding infants at high risk for atopy indicated that early supplementation of scGOS/lcFOS 9:1 reduced the number of (respiratory) infectious episodes in early life [27,28]. It might be that infants at high risk for atopy may have a different immune profile than healthy non-atopic infants leading to a higher susceptibility for respiratory infections.

In sum the specific scGOS/lcFOS 9:1 mixture, being part of the current test diet, has been shown to stimulate the Th1 dependent delayed-type hypersensitivity response to vaccines in mice, while the specific vaccine serum antibody response was not affected [4]. Mainly cellular immune responses are involved in the etiology of delayed-type hypersensitivity responses. Also in animal studies the beneficial effect of scGOS/lcFOS was limited to the cellular response, while no effect was seen on the vaccine specific humoral response. However IgE levels were affected in animal models for allergy as well. In these studies animals at risk for allergy were used [5,18,29]. The effects on IgE were highly comparable to study

published by van Hoffer et al. indicating effects on IgE in infants at risk for allergy fed a prebiotic scGOS/lcFOS diet [15].

It was shown in a large multicenter study comprising 1130 infants that the study prebiotics reduced the occurrence of atopic dermatitis in the complete cohort in their first year of life; for the vaccination response study we included a subsample of 164 infants only [30]. This is the first study indicating a primary preventive effect of this specific prebiotics mixture in healthy non-atopic infants. This preventive effect was similar to the effect found in the previous study with high risk infants without the addition of pAOS [26]. Infants of our recent study are healthy non-atopic infants and their vaccine-specific serum antibody responses are comparable with the general non-atopic infant population. From this and other studies, investigating the immune modulating properties for non-digestible carbohydrates, we may conclude that the incidence in atopic dermatitis was down regulated leaving the response to vaccination intact. Thus dampening of immune reactivity such as that seen in atopic dermatitis was not associated with down regulation of desirable immune activity such as that involved in the response to vaccination.

PAOS were added to the scGOS/FOS 9:1 mixture to better resemble the OS in breast milk. Theoretically, the beneficial effects of prebiotics in breast milk may be mimicked by the addition of acidic fraction. A recent study in mice suggested that the combination of neutral OS and pAOS could be the key reason for the protective effect of OS in human milk [5]. It was shown that combinations of scGOS/lcFOS 9:1 and pAOS were more effective in enhancing vaccination induced DTH-responses than either of the OS alone in a murine vaccination model, which suggests a synergistic interaction between the neutral OS and pAOS [16]. This synergistic effect was validated in animal models for respiratory allergy as well [5].

In contrast to the studies with prebiotics, it has been shown that probiotics can improve vaccination antibody responses significantly. Kukkonen showed that supplementation of a combination of four probiotic strains and GOS may improve the Hib antibody responses in infants at increased risk for atopy [31]. These findings are consistent with a few other studies on the effect of probiotics on vaccine antibody responses. The oral rotavirus vaccination-induced IgA secretion was stimulated by *Lactobacillus casei* GG in 2- to 5-month-old infants and *Lactobacillus* F19 increased the capacity to raise immune responses to tetanus and Hib immunizations [32,33]. A study in healthy adult males by de Vrese et al. showed that clotted milk with *Lactobacillus rhamnosus* GG or *Lactobacillus acidophilus* CRL431 induced increased poliovirus-neutralizing antibody titers, as well as poliovirus-specific IgG and IgA in serum after being orally vaccinated against polio [34]. In a small study in 30 young infants an increased antipoliovirus response was shown by probiotic supplementation [35]. One study did not find any significant effect of a formula fermented by *Lactobacillus acidophilus*, *Lactobacillus casei*, *Streptococcus thermophilus*, oligofructose and inulin on vaccine-specific antibody titers to the tetanus and pneumococcal vaccine in 9 months to 10 years old children [36]. Another recent study showed no effect of a mixture of probiotics on the vaccination response in 8–10 months old infants; there were some suggestions for improvement of seroconversion [37]. These results indicate that probiotics, in contrast to prebiotics, can influence the humoral response in a detectable way. Disadvantages such as difficulties in storage and increased risk of infections in immune-compromised patients may limit the possibilities of probiotics for a broader use in the field of infant nutrition.

The strengths of this study lie in the setting and design. This study is a prospective, randomized, double-blind, placebo-controlled trial. To the current knowledge, no randomized controlled studies on the effect of adding the specific prebiotic mixture to formula feeding on vaccine antibody responses in healthy non-atopic infants have been performed before. The current cohort

consists of a homogeneous research group. The infants all received the same immunizations according to the national vaccination program. The infants were sampled in a narrow age range and time interval after vaccination. As expected we found a longer interval correlated with lower specific immunoglobulin levels.

A limitation of this study is the relative small sample size and the use of the optimal vaccination protocol. For detecting an increased response to vaccination the study was possibly underpowered since the study was not designed originally for the evaluation of vaccination responsiveness. The vaccine-specific serum antibody responses were included as a secondary parameter of efficacy and immune safety. Suboptimal vaccination protocols might be a better tool to detect immune stimulation as indicated by vaccination responsiveness.

5. Conclusions

In summary, in the current study no relevant detectable effect on vaccination specific antibody levels was found: the vaccine specific antibody levels in infants fed a specific prebiotics mixture or a control diet were similar during the first year of life.

We hypothesize that the specific prebiotic mixture, which resembles the OS-composition in human milk, mainly promotes Th1 and Treg dependent immune responses and induces a down regulation of IgE-mediated allergic responses, while the desired vaccine-specific serum antibody responses remain intact.

Acknowledgements

Conflict of interest: Garssen and Knipping received honoraria from Danone. *Funding:* This study is financially supported by Danone.

Appendix A.

The Multicenter Infection Prevention Study-1 (MIPS-1) Study Group consisted of Groningen, the Netherlands: Pieter Sauer, Margriet van Stuijvenberg, Annemieke Eisses, Jitty Streurman; Berlin, Germany: Ulrich Wahn, Christoph Grüber, Juliane Wauer, Kirn Parasher, Madeleine Wust, Ingrid Lawnitzak, Marion Trentmann, Gabi Schulz, Eckard Hamelmann; Mangiagalli Hospital, Milan, Italy: Paola Roggero, Fabio Mosca, Pasca Piemontese, Maria Lorella Gianni, Anna Orsi, Valeria Puricelli; Macedonio Melloni Hospital, Milan, Italy: Sertac Arslanoglu, Guido Moro, Silvia Rizzardi, Elisa Rigotti, Laura Tandoi, Michela Coppola; Zürich, Switzerland: Christian Braegger, Michael Friedt, Rebecca Koller, Petra Martel, Sarah Nussbaumer, Daniela Rogler, Tamar Stricker; Schwarzbach, Austria: Josef Riedler, Margret Macheiner, Christine Tyma; Brescia, Italy: Gaetano Chirico, Gasparoni Antonella, Garzoli Elena, Offer Chiara, Spinoni Vania, Iacono Graziella, Stellini Maria; and Danone Research: Jan van der Moeren, Chryssanthi Kiriakidou, Johan Garssen, Leon Knippels, Jan Knol, Kaouther Ben Amor, Günther Boehm, Jürgen Jelinek, Bernd Stahl, Esther König, Anja Frings, Anja Wittke, Yvonne Zens, Stephanie Cremers, Kathrin Friedrichs, Janine Gerloff, Gemma Bruno, Ayako Fischer.

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