Fecal Secretory Immunoglobulin A Is Increased in Healthy Infants Who Receive a Formula with Short-Chain Galacto-Oligosaccharides and Long-Chain Fructo-Oligosaccharides\textsuperscript{1,2}

Petra A. M. J. Scholtens,\textsuperscript{3,5} Philippe Alliet,\textsuperscript{4} Marc Raes,\textsuperscript{4} Martine S. Alles,\textsuperscript{3} Hilde Kroes,\textsuperscript{3} Guenther Boehm,\textsuperscript{3,5} Leon M. J. Knippels,\textsuperscript{3} Jan Knol,\textsuperscript{3} and Yvan Vandenplas\textsuperscript{6}

\textsuperscript{3}Numico Research, Wageningen 6700 CA, The Netherlands; \textsuperscript{4}Virga Jesse Hospital, Hasselt 3500, Belgium; \textsuperscript{5}Sophia Children’s Hospital, Rotterdam 3015 GJ, The Netherlands; and \textsuperscript{6}University Hospital Brussel, Brussels 1090, Belgium

Abstract

In this double-blind, randomized, placebo-controlled study, we investigated the effect of an infant milk formula with 6 g/L short-chain galacto- and long-chain fructo-oligosaccharides [(scGOS/lcFOS) ratio 9:1] on the development of the fecal secretory immunoglobulin A (sIgA) response and on the composition of the intestinal microbiota in 215 healthy infants during the first 26 wk of life. The infants received breast milk or were randomized to receive an infant milk formula with or without scGOS/lcFOS. Stool samples were collected after 8 and 26 wk of intervention. The concentration of fecal sIgA was determined by ELISA, and the composition of the intestinal microbiota was determined by quantitative fluorescent in situ hybridization. The scGOS/lcFOS group and the control group were compared in the statistical analysis. A breast fed group was included as a reference. In total, 187 infants completed the study. After 26 wk of intervention, in infants that were exclusively formula fed, the concentration of sIgA was higher ($P = 0.006$) in the scGOS/lcFOS group (719 $\mu$g/g) than in the control group (263 $\mu$g/g). In addition, the percentages of bifidobacteria were higher in the scGOS/lcFOS group (60.4%) than in the control group (52.6%, $P = 0.04$). The percentages of Clostridium spp. were 0.0 and 3.27%, respectively ($P = 0.006$). In conclusion, an infant milk formula with 6 g/L scGOS/lcFOS results in higher concentrations of fecal sIgA, suggesting a positive effect on mucosal immunity. J. Nutr. 138: 1141–1147, 2008.

Introduction

Prebiotic oligosaccharides are specific types of dietary fiber that cannot be hydrolyzed by the digestive enzymes in the gastrointestinal tract. Hence, they enter the colon intact, where they can be fermented by bacteria of the intestinal microbiota (1). The intestinal microbiota consists of large numbers of different types of bacteria, such as bifidobacteria, lactobacilli, bacteroides, and Clostridium spp. Development of the intestinal microbiota is different in infants that received breast milk or formula milk (2). The intestinal microbiota of breast fed infants mainly contains bifidobacteria and lactobacilli, whereas the intestinal microbiota of formula fed infants contains more variable types of bacteria, such as bifidobacteria, lactobacilli, bacteroides, enterobacteria, and Clostridium spp. (2). An important determinant for the development of a bifidogenic microbiota are the human milk oligosaccharides (3). Human milk oligosaccharides are prebiotic oligosaccharides that are fermented by beneficial bacteria in the gastrointestinal tract. Due to the selective fermentation, the growth of beneficial bacteria is favored over the growth of potentially pathogenic bacteria.

Several studies have investigated the effect of a specific mixture of prebiotic short-chain galacto-oligosaccharides and long-chain fructo-oligosaccharides [(scGOS/lcFOS)\textsuperscript{7} also named IMMUNOFORTIS] on the composition of the intestinal microbiota in preterm, term, and weaning infants (4–8). These studies showed that scGOS/lcFOS induces an intestinal microbiota with high percentages of bifidobacteria (4–8), and a metabolic activity of the microbiota that is similar to that of breast fed infants (7). Infant milk formulas with scGOS/lcFOS have also been shown to have beneficial effects on the development of the immune system. In a double-blind, randomized, controlled clinical trial performed by Moro et al. (9,10), 206 infants at risk of atopy completed a 6-mo intervention period in which they received a hypoallergenic infant milk formula with 8 g/L scGOS/lcFOS or a control hypoallergenic formula without scGOS/lcFOS. The cumulative incidence of atopic dermatitis was significantly lower.

\textsuperscript{1} Supported by Numico Research, Wageningen, The Netherlands.


\textsuperscript{5} To whom correspondence should be addressed. Email: petra.scholtens@numico-research.nl.

\textsuperscript{7} Abbreviations used: BM group, breast milk group; BSA, bovine serum albumin; Ig, immunoglobulin; scGOS/lcFOS, short-chain galacto-oligosaccharides and long-chain fructo-oligosaccharides; sIgA, secretory immunoglobulin A; Th, T-helper.
in the infants that received scGOS/lcFOS (9,10). These infants also had a lower incidence of infections (9,10). In another study, 326 healthy infants were randomized to receive an infant milk formula with 6 g/L scGOS/lcFOS or a control formula without scGOS/lcFOS (11). This open, prospective, randomized, placebo-controlled study also showed that infants that received scGOS/lcFOS had a lower incidence of infections (11). The exact mechanism behind the immune modulatory effects of scGOS/lcFOS is not yet fully understood, but the effects may be mediated by the composition of the intestinal microbiota. Newborn infants are mainly dependent on passive immune responses, such as the maternal immunoglobulin (Ig) G that was transported to the fetus during pregnancy, and the maternal secretory immunoglobulin A (sIgA), derived from the mother via human milk (12). Besides the passive transfer of sIgA via breast milk, the infant’s acquired immune system develops gradually. The intestinal microbiota may play an important role in the development of the acquired immune system of infants, especially in the development of the mucosal immune system and the production of endogenous sIgA (13). In a study by Bakker-Zierikzee et al. (14), a group of 38 healthy, formula fed infants was randomized to receive an infant milk formula with 6 g/L scGOS/lcFOS or a control formula without scGOS/lcFOS from birth until 32 wk of age. Infants that received scGOS/lcFOS had a significantly higher concentration of fecal sIgA after 16 wk of intervention than infants in the control group. Our study evaluated the effect of an infant milk formula with 6 g/L scGOS/lcFOS on the development of the fecal sIgA response and on the composition of the intestinal microbiota in healthy infants during the first 26 wk of life. An important difference between this study and that of Bakker-Zierikzee et al. is that the infants in our study were allowed to start with breast milk and to switch to 1 of the study formulas after cessation of breast feeding.

Materials and Methods

Study design. The study was a double-blind, randomized, placebo-controlled intervention trial, with a 6-mo intervention period. The results presented in this article are part of a larger trial (15). The study was approved by the medical ethics committee of the Virga Jesse hospital in Hasselt, Belgium.

Subjects. Pregnant women were recruited via pediatricians in the Virga Jesse hospital in Hasselt, Belgium and the University Hospital in Brussels, Belgium in the last 4 mo before their estimated delivery date. Infant eligibility was verified by the pediatrician during its stay in the hospital after birth. The study population consisted of healthy, term infants, with a normal birth weight (between the 5th and 95th percentiles). Infants with a double heredity for atopic diseases, congenital abnormality or disease, gastrointestinal disease, a strongly suspected cow’s milk intolerance, or with regular use of products with probiotic bacteria were excluded from the study. Participation in the study was voluntary, and written informed consent was obtained before the start of the study.

Nutritional intervention. The feeding intention of the mother was assessed before delivery. If the mother decided to start with formula, she was randomized to receive 1 of 2 types of infant milk formula: an infant milk formula with 6 g/L scGOS/lcFOS or a control formula without scGOS/lcFOS (provided by Numico Research). Both formulas were identical in appearance and taste. Mothers with the intention to start with breast milk were encouraged to continue breast feeding during the course of the study, and they were randomized to receive 1 of the 2 formulas at the time they decided to start with formula. Each mother/infant pair received a sequential number at study entry and kept that number throughout the study. The study products were coded on randomization number. A randomization number was allocated for those infants that started with formula, by assigning the next blank number of the randomization list. A randomization list was generated prior to the study, using a 2 block design, by the logistics manager of Numico Research, who was not involved in the study. Stratification was applied according to the duration of exclusive breast feeding, <3 mo or >3 mo.

Data collection. Breast milk or formula intake was recorded in a daily diary by the parents, and adverse events were recorded by the pediatrician in a case report form. The parents were asked to collect a fecal sample from their infant before their visits to the pediatrician at the age of 8 and 26 wk. They were carefully instructed on the method for the collection of the samples before the study. Fresh fecal samples were collected in plastic feces containers, without a medium or buffer (Greiner Bio-One), frozen immediately after collection by the parents, and stored at −20°C until transport to the pediatrician. Fecal samples were transported to the pediatrician in a cooling bag with ice packs, without unfreezing the samples. At the end of the study period, the frozen fecal samples were transported to Numico Research.

Fecal homogenates. The frozen fecal samples were defrosted on ice, and 10% wt/vol fecal homogenates were made by weighing 1 g of feces, adding 9 mL of PBS (pH 7.4) and homogenizing this suspension for 10 min in the stomacher (IUL Instruments). One milliliter of the homogenized fecal suspension was directly fixed in 3 mL freshly prepared 4% (wt/vol) paraformaldehyde in PBS and incubated overnight at 40°C. Fixed samples were separated into aliquots and stored at −20°C to be used for the microbiota analysis. The remainder of the homogenates were aliquoted and stored at −80°C until used for the analysis sIgA.

Measurement of sIgA response by ELISA. sIgA was determined from the single feces samples that were collected at the age of 8 and 26 wk. The analysis of sIgA was performed as described before, with minor modifications (14). NUNC 96-well Immuno-maxisorp plates were coated overnight at 4°C with mouse α human secretory component (Sigma, clone GA-1), 1:10,000 in PBS. After thoroughly washing with wash buffer (0.005% Tween-20 in PBS), the plates were incubated for 1 h at room temperature with PBS containing 1% of bovine serum albumin (BSA) [PBS/BSA (Sigma, BSA Fraction V)] to block nonspecific protein binding sites. Supernatants of the fecal homogenates (defrosted on ice, vortexed, and centrifuged at 16,000 × g for 5 min at 4°C) were diluted in PBS/BSA between 2,500 and 125,000 times. Purified human IgA isolated from Colostrum (Sigma, 1-1010) was used as a standard calibration curve and serial diluted in PBS/BSA starting from 80 μg/L. After blocking, the plates were washed and the samples and standards were incubated for 2 h at room temperature. Plates were then washed and biotin conjugated mouse α human IgA1/IgA2 monoclonal antibody (Pharmingen, clone G20–359) was added to the plates at a concentration of 1 mg/L PBS/BSA. After 1 h of incubation at room temperature, the plates were washed again and finally incubated with Streptavidin conjugated horseradish peroxidase (CLB, M2051), 1:10,000, diluted in PBS/BSA for 30 min at room temperature. Plates were washed and incubated with 1-step Ultra TMB substrate solution (Pierce, 34028), 2 times diluted with 0.1 mol/L sodium acetate, pH 5.5 (Merck, 1.06268). The enzymatic color development was stopped by adding stop solution (1.8 mol/L H2SO4), and then the absorbance was measured at 450 nm in a plate reader. sIgA concentrations of the fecal samples were calculated from a calibration curve. Samples were assayed at 2 successive days in duplicate in 2 different dilutions.

Analysis of intestinal microbiota. The composition of the intestinal microbiota was analyzed with quantitative fluorescent in situ hybridization, as described previously (2,16,17). Fecal samples were applied to gelatin-coated glass slides [8-well object slides with square-shaped wells (1 cm2/well); CBN Labsuppliers], air dried, and hybridized with 10 ng/KL Cy3-labeled Bifidobacterium specific probe Bif164 (16), Cy3-labeled Clostridium histolyticum/Clostridium litusebrense specific probe Chis150/Clit135, or Cy3-labeled Escherichia coli–specific probe
Ec1531, or incubated with 0.25 ng in 1 μL 4',6-diamidino-2-phenylindole for total cell counts. Slides were automatically counted using an Olympus AX70 epifluorescence microscope and image analysis software. The percentage of bifidobacteria per sample was determined by counting all cells with a 4',6-diamidino-2-phenylindole filter set and counting all bifidobacteria using a Cy3 filter set (SP100 and 41007, respectively; Chroma Technology).

Data analysis. With a sample size of 65 infants per formula group, it was possible to detect a difference in the percentage of bifidobacteria between the groups of 22%, with a 2-sided test, assuming a standard deviation of 20% (our unpublished results), and α = 0.05 and a power of 80%.

Data were checked for normality by visual inspection of the normal probability curves, and with the Shapiro-Wilk test of normality. In the statistical analysis, infants that received the formula with added scGOS/lcFOS were compared with infants that received the control formula. A study group with infants that received at least 97.5% of the total number of feedings as breast milk was included as a reference (BM group). The 2 formula fed groups contained both infants that started with breast milk and changed to formula during the study period, and infants that received formula feeding for the whole 26-wk period (whole group analysis). To assess the differences between the 2 exclusively formula groups, a subgroup analysis was performed in which only children that received 97.5% of the total number of feedings as formula feeding were included. All analyses were performed on an intention-to-treat basis. Due to the skewed distribution of the data, nonparametric Mann-Whitney U tests were performed to assess differences among the groups. The breast fed group was not included in the pairwise comparisons, as these infants could not be randomized, and therefore deviated from the double-blind randomized design. The results are presented as medians, with the 10th and the 90th percentiles, unless noted otherwise.

Results

Subject characteristics. 215 infants were included in the study (Fig. 1). In total, 202 infants completed the first 8 wk of the study, of which 21, 25, and 90 exclusively received a formula with scGOS/lcFOS, a control formula, or breast milk, respectively (66 infants started with breast milk, and transferred to 1 of the 2 formulas before the age of 8 wk). 187 Infants completed the entire 26-wk study period, of which 22, 24, and 31 exclusively received a formula with scGOS/lcFOS, a control formula, or breast milk, respectively (110 infants started with breast milk and transferred to 1 of the 2 formulas between the age of 8 and 26 wk). The baseline and subject characteristics were comparable for the groups (Table 1).

Fecal slgA. After 26 wk of intervention, in the whole group of infants, the concentration of slgA in feces was significantly higher in the scGOS/lcFOS group (729 μg/g) than in the control group (377 μg/g, Fig. 2). The results of the subgroup of infants that were exclusively formula fed were comparable to those in the whole group, with concentrations of 719 μg/g in the scGOS/lcFOS group and 263 μg/g in control group (P < 0.001).

Intestinal microbiota. After 8 wk of intervention, the formula groups did not differ with respect to the percentages of bifidobacteria and Clostridium spp. (Table 2). In the subgroup of infants that were exclusively formula fed, the percentage of E. coli was lower in the scGOS/lcFOS group than in the control group. At the end of the study period, after 26 wk of intervention, in the whole group the percentage of bifidobacteria in the scGOS/lcFOS group was higher than in the control group. Furthermore, the percentage of Clostridium spp. was lower in the scGOS/lcFOS group than in the control group. The results of the subgroup of infants that were exclusively formula fed were comparable to those in the whole group.

The pH was lower in the scGOS/lcFOS group than in the control group after 8 and 26 wk of intervention in the whole group of infants (Table 2).

In total, 96 subjects reported adverse events during the study, of which 40 (of 85 infants) were in the scGOS/lcFOS group, 40 (of 89 infants) were in the control group, and 16 (of 38 infants) were in the breast fed reference group.

![FIGURE 1](https://example.com/figure1.png)  
Flow-chart of the study population after 8 and 26 wk of intervention.
Breast feedings, Formula feedings, Apgar score, cm, Head circumference, cm, Parental allergy (single heredity), Duration of breast feeding.

The results represent the median concentrations of sIgA in the group of exclusively formula fed infants that received a formula with scGOS/lcFOS or a control formula. The results represent the median concentrations of sIgA in the group of exclusively formula fed infants who received a formula with scGOS/lcFOS or a control formula. The results represent the median concentrations of sIgA in the group of exclusively formula fed infants who received a formula with scGOS/lcFOS or a control formula.

Discussion

In this study, infants who received an infant milk formula with added scGOS/lcFOS had significantly higher concentrations of sIgA than infants who received a control infant milk formula. This study is, to our knowledge, the first to demonstrate the effect of supplementation with scGOS/lcFOS on fecal sIgA concentrations after 26 wk of intervention.

Fecal sIgA. The fecal concentration of sIgA after 26 wk of intervention was significantly higher in the infants that received an infant milk formula with added scGOS/lcFOS. Concentrations of sIgA in the group of exclusively formula fed infants reached 719 µg/g in the scGOS/lcFOS group, 263 µg/g in the control group, and 584 µg/g in the breast fed group after 26 wk of intervention. As the concentration of sIgA was determined in single feces samples, no information is available on the daily excretion of sIgA. Similar results were observed in a study by Bakker-Zierikzee et al. (14), in which healthy newborns were randomized to receive an infant milk formula with 6 g/L scGOS/lcFOS, or a control infant milk formula, for a period of 4 mo. A breast fed group was included as a reference. Concentrations of sIgA in feces in these infants reached 654 µg/g, 449 µg/g, and 767 µg/g in the scGOS/lcFOS, control, and breast fed groups, respectively, after 24 wk of intervention, but concentrations did not differ significantly at this time point. With samples sizes of 13, 13, and 12 infants in the scGOS/lcFOS, control, and breast fed groups, the groups may have been too small, and the variation may have been too high to detect statistically significant differences. An important difference between the study of Bakker-Zierikzee et al. (14) and this study is the comparison of the effect of scGOS/lcFOS in a group of infants that started with breast milk and were randomized to receive an infant milk formula after cessation of breast feeding, and in a group of infants that were exclusively formula fed from birth. Breast fed infants are provided with sIgA via human milk during the first few months of life. This was clearly observed in our study, with higher concentrations of sIgA in the breast fed reference group in the week 8 of intervention. The difference in the concentration of fecal sIgA between breast fed infants and formula fed infants was smaller after 26 wk of intervention, possibly indicating the decreased provision of sIgA via breast milk (18). In addition to the provision of sIgA, breast milk also contains other factors, such as sIgA-stimulating cytokines, that stimulate the infants’ own immune system (12,19). Kuitunen and Savilahiti (20) compared the concentration of fecal sIgA in term and preterm infants that received breast milk or formula. The concentration of sIgA was higher in breast fed infants throughout the study period. Probiotics also increase concentrations of fecal sIgA (21).

![FIGURE 2](image)

**FIGURE 2** Concentration of fecal sIgA after 8 and 26 wk of intervention in infants in the whole group and in the subgroups of exclusively formula fed infants who received a formula with scGOS/lcFOS or a control formula. The results represent the median concentration of fecal sIgA with the 10th and the 90th percentiles. *Different from control, P < 0.05.
Viljanen et al. (23) randomized infants with atopic eczema/dermatitis syndrome to receive a supplement with *Lactobacillus rhamnosus* *GG*, a supplement with a mixture of 4 types of probiotics, or a placebo supplement. Fecal concentrations of slgA tended to be higher in the infants that received 1 of the 2 probiotics supplements than in infants that received the placebo supplement. This difference was mainly due to a decrease in concentrations of fecal slgA in the placebo group during the study period. These studies indicate that the concentration of fecal slgA in breast fed infants is higher than that of formula fed infants, but that it is not always possible to detect differences in the effects of infant milk formulas on concentrations of fecal slgA.

The main function of fecal slgA is to agglutinate microorganisms and to prevent the adherence of pathogenic bacteria and viruses to the mucosal surface (12,13,24–26). Another important function of slgA is the maintenance of the intestinal microbiota. Animal studies have shown a substantial increase in anaerobic bacteria in the small intestine of mice in the absence of normal slgA, whereas normalization of the production of slgA resulted in a recovery of the regular composition of the intestinal microbiota (27). So on one hand, the composition of the intestinal microbiota regulates the production of slgA, but on the other hand, slgA regulates the composition of the intestinal microbiota to prevent an overstimulation of the immune system. At the same time, the continuous presence of commensal bacteria favors a constant stimulation of the immune system without invoking inflammatory responses. An interesting finding by Liepeke et al. (28) was that the secretory component derived from human milk may have bifidogenic properties, which can further enhance the growth of beneficial bacteria in the gastrointestinal lumen, and may thus also play a role in the microbial homeostasis. These studies indicate that there is a strong link between the composition of the intestinal microbiota and the mucosal immune system.

**Composition of the intestinal microbiota.** Previous studies (4–8) have shown that the prebiotic mixture with scGOS/lcFOS in a ratio of 9:1 induces an intestinal microbiota with higher percentages of bifidobacteria, and an intestinal metabolic activity similar to that of breast fed infants in preterm, term, and weaning infants. Similar results were observed in this study, with higher percentages of bifidobacteria and lower percentages of *Clostridium* spp. in the scGOS/lcFOS group than in the control group after 26 wk of intervention in the subgroup and in the whole group of infants. All groups have a microbiota that is dominated by bifidobacteria, but surprisingly, the percentages of bifidobacteria appeared to be low in the breast fed group, when compared with both formula groups. In the subgroup of exclusively formula fed infants, percentages of 53.5 and 40.2 were observed in the scGOS/lcFOS and the control groups, respectively. In the breast fed reference group, a percentage of 33.2 bifidobacteria was observed in week 8 of intervention. Similar low percentages of bifidobacteria in breast fed infants were observed in a study of Bakker-Zierikzee et al. (29). In a recent study by Penders et al. (30), counts of bifidobacteria in breast fed infants equaled those in formula fed infants. Although the results of our study were unexpected, they may indicate regional differences in the composition of breast milk or in the composition of the intestinal microbiota. For instance, comparative studies in adults have shown differences in percentages of bifidobacteria in several countries (31). In this study, the percentage of *Clostridium* spp. was significantly lower in the scGOS/lcFOS group than in the control group after 26 wk of intervention. Percentages of *E. coli* were lower in the scGOS/lcFOS group after 8 wk of intervention, but the groups did not differ after 26 wk of intervention. The presence of *E. coli* and *Clostridium* spp. indicates the development of a more mature microbiota. The *Clostridium* spp. probes used in our study cover pathogenic species (*Clostridium perfringens* and *Clostridium difficile*), and the observed lower percentage thus indicates lower percentages of pathogens.

The composition of the intestinal microbiota is important in the skewing of the immune response in young infants (13). The

### Table 2

Intestinal microbiota and pH in the whole group and in the subgroup of exclusively formula fed infants, receiving an infant formula with scGOS/lcFOS or a control infant formula

<table>
<thead>
<tr>
<th>Week</th>
<th>scGOS/lcFOS (n)</th>
<th>Control (n)</th>
<th>BM reference (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bifidobacteria, %</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole group</td>
<td>8</td>
<td>49.5 [0.0, 83.6] (41)</td>
<td>40.4 [0.0, 75.0] (46)</td>
</tr>
<tr>
<td></td>
<td>26</td>
<td>59.8 [37.1, 88.0]* (66)</td>
<td>47.2 [12.1, 64.0] (74)</td>
</tr>
<tr>
<td>Subgroup</td>
<td>8</td>
<td>53.4 [0.0, 79.1] (16)</td>
<td>40.2 [0.0, 75.2] (24)</td>
</tr>
<tr>
<td></td>
<td>26</td>
<td>80.4 [37.8, 93.3]* (19)</td>
<td>52.6 [1.3, 68.7] (21)</td>
</tr>
<tr>
<td><strong>Clostridium spp., %</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole group</td>
<td>8</td>
<td>0.0 [0.0, 1.6] (42)</td>
<td>0.0 [0.0, 13.8] (47)</td>
</tr>
<tr>
<td></td>
<td>26</td>
<td>1.1 [0.0, 8.0]* (68)</td>
<td>3.9 [0.0, 10.4] (74)</td>
</tr>
<tr>
<td>Subgroup</td>
<td>8</td>
<td>0.0 [0.0, 2.9] (17)</td>
<td>0.0 [0.0, 15.5] (24)</td>
</tr>
<tr>
<td></td>
<td>26</td>
<td>0.0 [0.0, 3.6]* (20)</td>
<td>3.3 [0.0, 9.6] (21)</td>
</tr>
<tr>
<td><strong>E. coli, %</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole group</td>
<td>8</td>
<td>2.8 [0.0, 13.0] (42)</td>
<td>3.7 [0.0, 21.0] (47)</td>
</tr>
<tr>
<td></td>
<td>26</td>
<td>1.8 [0.0, 6.2] (68)</td>
<td>0.5 [0.0, 14.5] (74)</td>
</tr>
<tr>
<td>Subgroup</td>
<td>8</td>
<td>1.7 [0.0, 8.3]* (17)</td>
<td>5.8 [1.1, 25.3] (24)</td>
</tr>
<tr>
<td></td>
<td>26</td>
<td>0.8 [0.0, 4.8] (20)</td>
<td>0.5 [0.0, 11.2] (21)</td>
</tr>
<tr>
<td><strong>pH</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole group</td>
<td>8</td>
<td>5.9 [4.9, 7.5]* (47)</td>
<td>6.8 [5.7, 7.9] (60)</td>
</tr>
<tr>
<td></td>
<td>26</td>
<td>6.2 [5.4, 7.3]* (71)</td>
<td>6.5 [5.4, 7.7] (82)</td>
</tr>
<tr>
<td>Subgroup</td>
<td>8</td>
<td>6.3 [5.2, 7.8]* (19)</td>
<td>7.0 [5.6, 8.0] (26)</td>
</tr>
<tr>
<td></td>
<td>26</td>
<td>6.2 [5.4, 7.3] (21)</td>
<td>6.3 [5.6, 7.7] (24)</td>
</tr>
</tbody>
</table>

*Values are medians [10th, 90th percentiles]. *Different from control, *P* < 0.05.
immune system of pregnant women is skewed toward T-helper (Th)-2 responses, to avoid rejection of the fetus by the mother (32,33). Due to this preserved skewness, newborn infants are born with a skewed immune system toward Th2 responses (34). As Th2 responses have been shown to be involved in the onset of allergic disease, regulation of this skewed Th2 response is favorable. In the hygiene hypothesis, it was suggested that microbial stimulation was necessary to skew the immune system more toward Th1 responses (35,36). Regulatory T cells have also been suggested to play an important role in this process, by regulating both Th1 and Th2 responses (36). The development of a healthy microbiota may favor the production of regulatory T-cells, which in turn may regulate Th1 or Th2 responses (35–37). sgA can be produced in response to pathogenic bacteria via a Th2 response, or in response to commensal bacteria via a regulatory T-cell response. As sgA plays an important role in the defense against all types of pathogens in the gastrointestinal lumen (25,26), an increased production induced by a stimulation of commensal bacteria favors the defense against pathogens in the gastrointestinal tract. We cannot conclude from our study whether the increased concentration of fecal sgA was due to an increased level of commensal bacteria, such as bifidobacteria, or due to an increased level of pathogenic bacteria. However, as the percentages of Clostridium spp. and E. coli tended to be lower in the infants that received the scGOS/lcFOS formula, we can speculate that the increased percentages of bifidobacteria may have been involved in the stimulation of the production of sgA. Animal experiments have shown that supplementation with scGOS/lcFOS can also induce systemic immune effects (38,39). Although lymphocytes that were activated in the gut associated lymphoid tissue by commensal or pathogenic bacteria have been shown to be destined to return to mucosal surfaces, without cross-talk with the systemic circulation (40), it can be hypothesized that specific Th1, Th2, or regulatory T cells cytokines are transported to the systemic circulation via mesenteric lymph nodes, thereby also inducing systemic effects. In addition, scGOS/lcFOS may have direct effects on the immune system. For instance, a study performed by Roller et al. (41), showed that prebiotic inulin can directly stimulate the production of regulatory cytokines, such as interleukin 10, and protective cytokines, such as interferon-γ. Infant milk formulas with scGOS/lcFOS have also been shown to have beneficial effects on the development of the immune system (9–11). Hence, the provision of scGOS/lcFOS in an infant’s diet has more functional effects than the stimulation of sgA. However, this article focuses only on the production of sgA, and further studies are needed to evaluate other functional effects.

In conclusion, an infant milk formula with 6 g/L scGOS/lcFOS in a ratio of 9:1 results in higher concentrations of fecal sgA, suggesting a positive effect on mucosal immunity.

Acknowledgments
We thank Marie-Paule Verjans for her assistance in the practical execution of the study, Inge Peeters for data entry, and Rob Slump and Esmeralda van der Linde for the quantitative fluo-

Literature Cited


