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Supplementation of Mice with Specific Nondigestible Oligosaccharides during Pregnancy or Lactation Leads to Diminished Sensitization and Allergy in the Female Offspring[1–3]

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Abstract

Background: The maternal environment and early life exposure affect immune development in offspring.

Objective: We investigated whether development of food allergy in offspring is affected by supplementing pregnant or lactating sensitized or nonsensitized mice with a mixture of nondigestible oligosaccharides.

Methods: Dams were sensitized intragastrically with ovalbumin before mating, with use of cholera toxin (CT) as an adjuvant. Nonsensitized dams received CT only. Dams were fed a control diet or a diet supplemented with short-chain galacto oligosaccharides (scGOSs), long-chain fructo oligosaccharides (lcFOSs), and pectin-derived acidic oligosaccharides (pAOSs) in a ratio of 9:1:2 at a dose of 2% during pregnancy or lactation, resulting in 7 experimental groups. After weaning, offspring were fed a control diet and ovalbumin-CT sensitized. Acute allergic skin responses (ASRs), shock symptoms, body temperature, and specific plasma immunoglobulins were measured upon intradermal ovalbumin challenge. Th2/Th1− and regulatory T cells were analyzed with use of quantitative polymerase chain reaction and flow cytometric analysis in spleen, mesenteric lymph nodes, and blood.

Results: Supplementing sensitized pregnant or lactating dams with scGOS/lcFOS/pAOS resulted in lower ASRs in the offspring [offspring of sensitized female mice fed experimental diet during pregnancy (S-Preg): 48 ± 2.1 μm; offspring of sensitized female mice fed experimental diet during lactation (S-Lact): 60 ± 6.2 μm] compared with the sensitized control group (119 ± 13.9 μm). In the S-Lact group, this coincided with an absence of shock symptoms compared with the offspring of sensitized female mice fed control food during pregnancy and lactation (S-Con) and S-Preg groups, and lower ovalbumin-IgG1 [S-Con: 3.8 ± 0.1 arbitrary units (AUs); S-Preg: 3.3 ± 0.1 AUs; S-Lact: 2.4 ± 0.1 AUs] and higher ovalbumin-IgG2a concentrations (S-Con: 1.1 ± 0.1 AUs; S-Preg: 0.8 ± 0.1 AUs; S-Lact: 2.0 ± 0.1 AUs). Supplementing nonsensitized pregnant or lactating dams with scGOS/lcFOS/pAOS resulted in lower plasma ovalbumin-IgE [offspring of nonsensitized female mice fed experimental diet during pregnancy (NS-Preg): 1.6 ± 0.4 AUs; offspring of nonsensitized female mice fed experimental diet during lactation (NS-Lact): 0.3 ± 0.1 AUs vs. offspring of nonsensitized female mice fed control food during pregnancy and lactation (NS-Con): 3.1 ± 0.6 AUs] and ovalbumin-IgG1 (NS-Lact: 2.3 ± 0.3 AUs vs. NS-Con: 3.4 ± 0.3 AUs) concentrations in offspring. Ovalbumin-IgG2a plasma concentrations were higher in offspring of scGOS/lcFOS/pAOS-supplemented dams (NS-Preg: 1.1 ± 0.1 AUs; NS-Lact: 1.1 ± 0.1 AUs) than in those of unsupplemented, nonsensitized controls (0.4 ± 0.0 AUs).

Conclusions: These data show impaired sensitization in offspring of scGOS/lcFOS/pAOS-supplemented mice. A number of the analyzed variables are differentially affected by whether supplementation occurs during pregnancy or lactation, and the outcome of dietary supplementation is affected by whether the mother has been sensitized to ovalbumin and CT. *J Nutr* 2015;145:996–1002.

Keywords: programming, oligosaccharides, maternal dietary intervention, food allergy, hen's egg

Introduction

Both paternal and maternal factors might be associated with an increased risk of allergic disease, however, maternal factors seem to be more influential (1). This suggests that the immunologic environment during pregnancy and lactation might contribute to the increased susceptibility for allergy. Animal studies have

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indicated that transplacental passage of Th2-related mediators may modify the developing fetal immune system (2). After birth, breastfeeding may affect immune development because breast milk from asthmatic mothers was shown to induce asthma susceptibility in murine offspring (3). Effects of breastfeeding and maternal asthma on allergic disease have also been observed in human studies. Longer duration of breastfeeding favorably influences lung growth, asthma, or recurrent wheezing in children; however, longer breastfeeding by asthmatic mothers is associated with decreased airflows and increased risk of asthma and recurrent wheezing. This indicates an association between the maternal allergic status and breastfeeding (4, 5). Besides maternal atopy, perinatal transmission of allergens has been implicated in several studies as a factor contributing to allergy development as well, however, these results are conflicting, with both increased (6) and decreased (7, 8) allergy outcomes. Moreover, complete allergen avoidance during pregnancy and lactation is difficult, and long-term studies did not show conclusive effects of avoidance strategies (9, 10). Studies focused on prevention of allergy development have gained more attention during recent years. Many nutrients are believed to have immune modulatory functions, and the maternal diet is still thought to be a very important determinant for allergy development in the offspring. Therefore, early life intervention with immune modulating ingredients might be a promising new way for allergy management. For example, maternal PUFA (11) and probiotic supplementation in mice was shown to decrease allergy development in the offspring (12). In addition, several studies in humans have shown beneficial effects of perinatal supplementation with PUFAs or probiotics on immune development as well (13, 14). Some studies did not show any effect after PUFA intervention if supplementation was performed during pregnancy only (15). A highly interesting group of immunomodulatory ingredients are the neutral and acidic oligosaccharides, which are abundantly present in human milk (16, 17). To mimic some of the health- and immune-promoting properties of human milk oligosaccharides, a specific mixture of nondigestible oligosaccharides containing short-chain galacto oligosaccharides (scGOSs)[6], long-chain fructo oligosaccharides (lcFOSs), and pectin-derived acidic oligosaccharides (pAOSs) was manufactured. In previous studies using these nondigestible oligosaccharides, it was demonstrated that an intervention diet stimulated the growth of *Bifidobacteria* and *Lactobacilli* similar to counts found in breastfed infants (16, 18–20). Clinical studies confirmed the positive effects, observed as a reduction in the incidence of atopic dermatitis and allergic manifestations in association with a beneficial immunoglobulin profile in high-risk children (21–23). However, not much is known about perinatal immune modulation via supplementation with oligosaccharides. In earlier studies, nondigestible oligosaccharides were observed to change the microbiota composition in both murine offspring and infants (24, 25). In another study, it was shown that the severity of atopic dermatitis–like skin lesions was decreased in offspring of NC/Nga mice supplemented with fructo-oligosaccharides during pregnancy and lactation (26). Results from a study carried out in our lab showed that a combination of fructo- and galacto-oligosaccharide supplementation during pregnancy changed various immune markers in mother and fetus (27). A significantly higher Th1-driven delayed-type hypersensitivity response was observed in fructo- and galacto-oligosaccharide–supplemented virgin mice but not in supplemented pregnant mice (27). Furthermore, IL10 mRNA expression levels were higher in spleens of supplemented dams and their fetuses, which may affect subsequent regulatory responses of the offspring later in life (27). In a follow-up study, we showed that supplementing pregnant mice with oligosaccharides reduces symptoms of allergic asthma in male offspring (37). Altogether, these data suggest that nondigestible oligosaccharides might be a promising dietary concept for early life intervention in the primary prevention against allergy. In the current study, the effect of maternal supplementation with nondigestible oligosaccharides during pregnancy or lactation on the development of food allergy in the offspring was investigated.

Methods

Chemicals. Ovalbumin (Grade V) was obtained from Sigma. Cholera toxin (CT) was purchased from Quadratech Diagnostics. PBS was obtained from BioWhittaker. Anti-IgE, −IgG2a, and −IgG1 were obtained from Pharmingen. All antibodies used for flow cytometric analysis were obtained from BD Biosciences, with the exception of Forkhead box P3 (Foxp3)-allophycocyanin, which was obtained from eBiosciences. Primers used for qPCR analysis were purchased from Qiagen and Isogen Life Science. 8-plex Luminex materials were purchased from BD Biosciences.

Diet. Semi-purified cow milk protein-free AIN-93G–based diets were composed and mixed with nondigestible oligosaccharides (28) by Research Diet Services.

The experimental diet was supplemented with a mixture of scGOSs (obtained by enzymatic elongation of lactose with galactose by β-galactosidase), lcFOSs (derived from chicory inulin), and pAOSs in a ratio of 9:1:2 at a dose of 2%. scGOS (Vivinal GOS, Domo) and lcFOS (Raftiline HP) consists of ~50% galacto oligosaccharide and fructo oligosaccharide, 19% maltodextrin (glucidex 2), 16% lactose, 14% glucose, and 1% galactose. The pAOS powder (Sudzucker AG) consists of ~75% galacturonic acid oligomers, 10% monomers, and 15% of moisture and ash. In the control diet, all oligosaccharides were exchanged for the same amount of maltodextrin, lactose, and glucose, resulting in a comparable carbohydrate composition in the diets. The diets were stored at −20°C before use.

Mice. Three-week-old pathogen-free female C3H/HeOuJ mice and 10-wk-old pathogen-free male C3H/HeOuJ mice were purchased from Charles River Laboratories and housed at constant temperature (20°C) and humidity (40–60%) at a 12:12 h light/dark cycle in the animal facility of Utrecht University. Animal care and use were performed in accordance with the guidelines of the Dutch Committee of Animal Experiments and the local university ethical committee (approval 2010. III.1.1.134).

Experimental set-up. An overview of the experimental set-up is provided in **Supplemental Figure 1**. Female mice were randomly assigned over 3 groups. One group of 9 mice was sensitized intragastrically once a week for 5 wk with hen's egg ovalbumin (20-μg/500-μL PBS) with use of CT (10-μg/500-μL PBS) as adjuvant. The second group, the non-sensitized dams, received PBS + CT (n = 9). The third group of dams received PBS only (see **Supplemental Table 1** for an overview of the groups). Ovalbumin-specific IgE and the acute allergic skin response (ASR) upon intradermal ovalbumin challenge was measured 1 wk after

[3] Supplemental Figures 1 and 2 and Supplemental Tables 1 and 2 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.

[6] Abbreviations used: ASR, acute allergic skin response; CT, cholera toxin; *Foxp3*, Forkhead box P3; lcFOS, long-chain fructo oligosaccharide; MLN, mesenteric lymph node; NS-Con, offspring of nonsensitized female mice fed control food during pregnancy and lactation; NS-Lact, offspring of nonsensitized female mice fed experimental diet during lactation; NS-Preg, offspring of nonsensitized female mice fed experimental diet during pregnancy; pAOS, pectin-derived acidic oligosaccharide; *Rps13*, Ribosomal protein S13; scGOS, short-chain galacto oligosaccharide; S-Con, offspring of sensitized female mice fed control food during pregnancy and lactation; S-Lact, offspring of sensitized female mice fed experimental diet during lactation; S-Preg, offspring of sensitized female mice fed experimental diet during pregnancy; *Tbet*, T-box transcription factor TBX21.

the last sensitization to determine whether the dams were allergic. Two weeks after the last sensitization, sensitized and nonsensitized mice were mated and fed either a control diet (AIN93G) or the AIN93G diet supplemented with scGOS/lcFOS/pAOS during pregnancy or lactation (Supplemental Table 1). After birth, the pups were randomly allocated to one of the nests within each experimental group (i.e., nonsupplemented, supplemented during pregnancy, or supplemented during lactation, n = 4–6; Supplemental Table 1). After weaning, all female offspring (in our lab, the mouse food allergy model has been validated for female mice; unpublished data) were transferred to the control diet and sensitized intragastrically once a week for 4 wk with ovalbumin + CT (Supplemental Table 1). One week after the last sensitization, the offspring were challenged intradermally with ovalbumin in the ear pinnea, with the exception of mice in the sham group, which received PBS intradermally. The ASR to ovalbumin was measured 1 h after intradermal ovalbumin challenge. Individual shock scores and body temperature were assessed 30 min after intradermal challenge. Six hours after the intradermal challenge, mice were challenged orally with ovalbumin (100-mg/500-μL intragastrically). Eighteen hours after the oral challenge, mice were killed by cervical dislocation and samples were collected for ex vivo analyses.

ASR. The ASR was determined in the offspring 1 h after intradermal challenge with 10-μg ovalbumin in the ear pinnae. Ear thickness, as readout for a Th2-mediated allergic reaction, was measured in duplicate before antigen challenge and 1 h afterward by using a digital micrometer (Mitutoyo Veenendaal). The ASR was calculated by subtracting the basal ear thickness from the value at 1 h after challenge, correcting for the ear swelling that occurred in the PBS-injected ear and expressed as Δ μm.

Anaphylactic shock score and body temperature. To evaluate the allergic response in the ovalbumin-sensitized offspring, anaphylactic reactions and body temperature were determined as a clinically related symptom after intradermal ovalbumin challenge. To establish the severity of the shock, a validated anaphylactic scoring table (see **Supplemental Table 2**) was used, as adapted from Li et al. (29). To measure changes in body temperature, all mice were implanted subcutaneously with an electronic ID transponder (Bio Medic Data Systems) on day 16 to measure individual body temperature.

Measurement of plasma-specific antibodies. Ovalbumin-specific IgE, IgG1, and IgG2a concentrations were measured in plasma by means of ELISA as described previously (28, 30). Results were analyzed with MicroplateManager PC software (Bio-Rad Laboratories). Concentrations in test sera were calculated in arbitrary units for each individual isotype, relative to a standard curve of pooled plasma.

Whole-blood assay. Whole-blood stimulations, using ovalbumin or anti-CD3, were carried out with blood samples as previously described (31). Concentrations of IFN-γ, TNF-α, IL-13, IL-4, IL-5, IL-10, IL-17, and IL-12(p70) were determined as described previously (28) with use of a Luminex assay according to the manufacturer's instructions.

mRNA expression level analyses. Total RNA was isolated from spleen with use of the RNeasy mini kit (Qiagen). Potentially contaminating DNA was digested with use of an RNase-free DNase set (Qiagen) before RNA cleanup. cDNA was prepared with use of the iScript cDNA synthesis kit (Bio Rad). Quantitative real-time PCR analysis of Ribisomal protein S13 (*Rps13*), Forkhead box P3 (*Foxp3*), *Gata3*, and T-box transcription factor TBX21 (*Tbet*) was performed on a CFX96 real-time PCR detection system (Bio Rad) with use of iQ SYBR green supermix (Biorad). Validated qPCR primers for *Tbet*, *Gata3*, and *Foxp3* were obtained from SABiosciences (Qiagen). For *Rps13* the following primers from Isogen Life Science were used: forward 5′-GTCCGAAAG-CACCTTGAGAG-3′ and reverse 5′- AGCAGAGGCTGTGGATGACT-3′. mRNA expression levels were calculated with CFX Manager software (version 1.6) and corrected for the expression of the housekeeping gene *Rps13*.

Flow cytometric analysis. Single-cell suspensions of the spleen and mesenteric lymph nodes (MLNs) were prepared, and the cells were stained as described previously (28, 31). The following monoclonal antibodies were used: anti-mouse CD3-PerCPCy5.5, rat anti-mouse CD4–fluorescein isothiocyanate, Armenian hamster anti-mouse CD69-phycoerythrin, rat anti-mouse CD25-phycoerythrin, and rat anti-mouse FoxP3-allophycocyanin. Flow cytometric analysis was carried out on a Becton Dickinson fluorescence-activated cell sorting Canto II flow cytometer, and data were analyzed with use of BD FACSDiva Software.

Statistics. All data are expressed as means ± SEMs. Statistical analyses were performed with use of SPSS 20. Levene's test was used to evaluate equality of variance among treatment groups. One-factor ANOVA was used to detect differences between multiple treatment groups. If

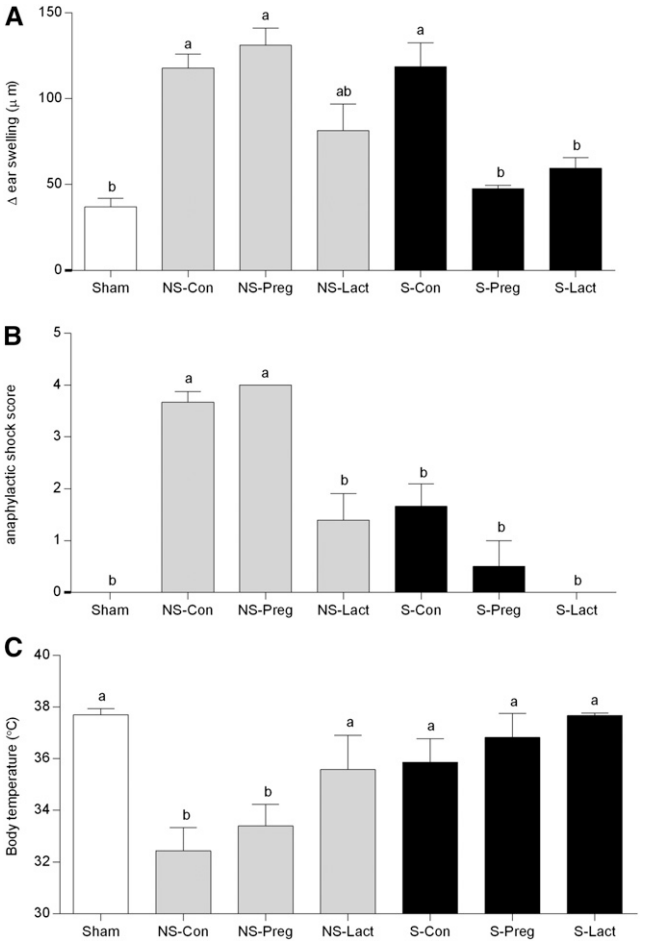

FIGURE 1 Acute effects of intradermal challenge with ovalbumin in female offspring of sensitized or nonsensitized mouse dams fed a control diet or scGOS/lcFOS/pAOS-supplemented diet during pregnancy or lactation. (A) ASR to ovalbumin. (B) Mean shock scores 30 min after intradermal challenge with ovalbumin. (C) Body temperature measured 30 min after intradermal challenge. Values are means ± SEMs, n = 4–6. Means not sharing a common letter differ significantly, $P < 0.05$ [(A) Bonferroni test; (B) Kruskal-Wallis test; (C) Kruskal-Wallis test]. ASR, acute allergic skin response; lcFOS, long-chain fructo oligosaccharide; NS-Con, offspring of nonsensitized female mice fed control food during pregnancy and lactation; NS-Lact, offspring of nonsensitized female mice fed experimental diet during lactation; NS-Preg, offspring of nonsensitized female mice fed experimental diet during pregnancy; pAOS, pectin-derived acidic oligosaccharide; scGOS, short-chain galacto oligosaccharide; S-Con, offspring of sensitized female mice fed control food during pregnancy and lactation; S-Lact, offspring of sensitized female mice fed experimental diet during lactation; S-Preg, offspring of sensitized female mice fed experimental diet during pregnancy.

significant differences were observed, Bonferroni post hoc analysis was used to identify significant differences between the means for the variables ASR and the immunoglobulins. If data were not normally distributed, Kruskal-Wallis was used to analyze the data (variables shock scores, body temperature, *Tbet* mRNA expression levels, flow cytometric analyses of MLN cell populations). Differences were considered significant at $P < 0.05$. Mean values between 2 groups (**Supplemental Figure 2**A–C) were compared to an independent *t* test.

Results

ASR. As expected, in female mice sensitized before breeding, the ASR was significantly higher than in the nonsensitized mice (Supplemental Figure 2A). In the offspring, the mean ASR was significantly higher in both the offspring of nonsensitized female mice fed control food during pregnancy and lactation (NS-Con) and the offspring of sensitized female mice fed control food during pregnancy and lactation (S-Con) compared to sham mice (**Figure 1**A). In the offspring of sensitized female mice fed the experimental diet during pregnancy (S-Preg) and the offspring of sensitized female mice fed the experimental diet during lactation (S-Lact), the ASR was significantly lower compared to the S-Con group (Figure 1A). In contrast to these results, supplementation during pregnancy did not affect the ASR in the offspring of nonsensitized female mice fed the experimental diet during pregnancy (NS-Preg). The ASR tended to be lower in the offspring of nonsensitized female mice fed the experimental diet during lactation (NS-Lact) compared to the NS-Con group ($P = 0.06$) (Figure 1A).

Shock score. In female mice sensitized to ovalbumin before breeding, anaphylactic shock symptoms (e.g., reduced activity, pilar erection, shortness of breath) 30 min after intradermal challenge were significantly higher compared to nonsensitized mice (Supplemental Figure 2B). In the offspring (all sensitized), moderate-to-severe shock symptoms were observed in the NS-Con group compared to the sham group mice, whereas the mice in the S-Con group underwent only minor anaphylactic shock symptoms (Figure 1B), indicating that sensitization of dams on a control diet affected the allergic response in the offspring. Compared to the NS-Con group, the outcome of the shock score in the NS-Preg group was not affected (Figure 1B). However, the NS-Lact group had significantly lower anaphylactic shock symptoms, and in the S-Lact group these symptoms were completely absent (Figure 1B).

Body temperature. A decrease in temperature after intradermal challenge is a quantifiable shock symptom as a result of acute allergic responses to ovalbumin. This drop in body temperature was observed in the NS-Con group 30 min after intradermal challenge compared to the sham group (endpoint measurement), whereas the S-Con group showed no significant drop in body temperature (Figure 1C). In the S-Preg group, no changes in body temperature were seen when compared to the S-Con group (Figure 1C). These findings are in line with the results depicted in Figure 1B because shock symptoms were also only significantly higher compared to the sham group in the NS-Con and NS-Preg groups.

Ovalbumin-specific antibodies. In mice sensitized to ovalbumin before breeding, ovalbumin-specific IgE concentrations were significantly higher compared to those in nonsensitized mice (Supplemental Figure 2C). In the offspring, ovalbumin-specific IgE concentrations were significantly lower in the NS-Lact group compared to the S-Con group. This difference was not observed for the NS-Preg group. Mean ovalbumin-specific IgE concentrations tended to be lower in the S-Lact group compared to the S-Con group (**Figure 2**, $P = 0.06$). Compared to the NS-Con and S-Con groups, ovalbumin-specific IgG1 concentrations were significantly lower in the NS-Lact and S-Lact groups, respectively, indicating that supplementation during lactation can affect the production of Th2-related immunoglobulins in the offspring regardless of the treatment of the dams (Figure 2). Interestingly, the Th1-related, ovalbumin-specific IgG2a concentrations were significantly higher in the S-Lact group when compared to the S-Con group. Compared to sham-treated mice, ovalbumin-specific IgE, −IgG1, and IgG2a concentrations were higher in the NS-Con and S-Con groups.

Ex vivo cytokine production. No differences were found in the production of IFN-γ, TNF-α, IL-13, IL-4, IL-5, IL-10, IL-17, or IL-12(p70) in supernatants of ovalbumin or anti-CD3–stimulated whole blood samples of the different experimental groups (data not shown).

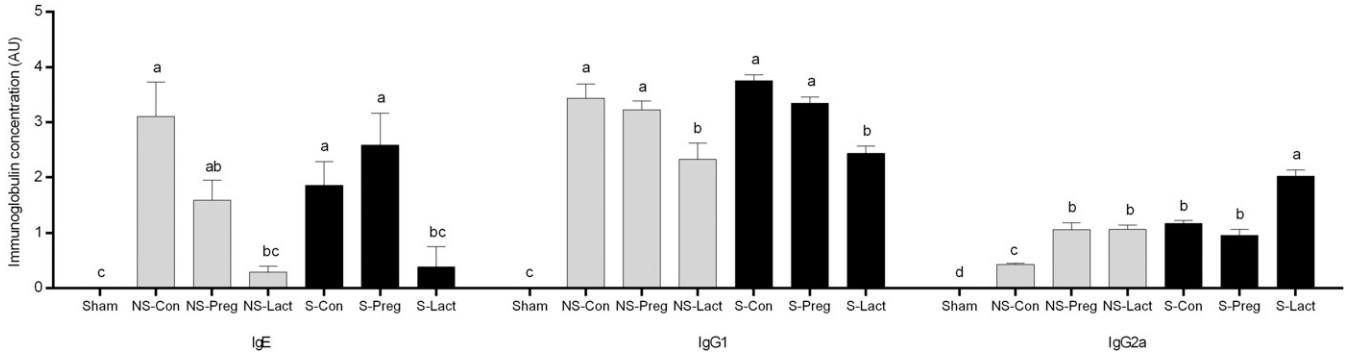

FIGURE 2 Concentrations of ovalbumin-specific IgE, IgG1, and IgG2a in female offspring of sensitized or nonsensitized mouse dams fed a control diet or scGOS/lcFOS/pAOS-supplemented diet during pregnancy or lactation. Values are means ± SEMs, $n = 4$–6. Means not sharing a common letter differ significantly, $P < 0.05$ (Bonferroni test). AU, arbitrary unit; lcFOS, long-chain fructo oligosaccharide; NS-Con, offspring of nonsensitized female mice fed control food during pregnancy and lactation; NS-Lact, offspring of nonsensitized female mice fed experimental diet during lactation; NS-Preg, offspring of nonsensitized female mice fed experimental diet during pregnancy; pAOS, pectin-derived acidic oligosaccharide; scGOS, short-chain galacto oligosaccharide; S-Con, offspring of sensitized female mice fed control food during pregnancy and lactation; S-Lact, offspring of sensitized female mice fed experimental diet during lactation; S-Preg, offspring of sensitized female mice fed experimental diet during pregnancy.

Tbet *expression in spleen.* Relative mRNA expression levels of the Th1 transcription factor *Tbet* are shown in **Figure 3**A. *Tbet* expression was significantly higher in the NS-Lact group compared to the NS-Con group. The *Tbet* mRNA expression in the NS-Con and NS-Preg groups did not differ significantly. In contrast, *Tbet* expression was significantly higher in the S-Preg group compared to the S-Con group. The *Tbet* mRNA expression in the S-Con group and the S-Lact group did not differ significantly (Figure 3A). Unfortunately, the expression levels of *Gata3* and *Foxp3* in our samples were too low to perform subsequent analysis (data not shown).

Flow cytometric analysis of activated T helper cells in MLNs. In spleen and MLNs, mean percentages of $CD3^+$ (T−) cells and $CD3^+CD4^+$ (T helper) cells did not differ significantly (data not shown). Percentages of $CD69^+$ cells within the $CD3^+CD4^+$ population (activated T helper cells) did not differ between the experimental groups when splenocytes were analyzed (data not shown). In the MLNs, the percentage of activated T helper cells was significantly lower in the offspring of sensitized dams, when compared to their respective nonsensitized counterparts (Figure 3B). Mean percentages of $CD3^+CD4^+CD25^+Foxp3^+$ cells (T regulatory cells) did not differ significantly in either tissue (data not shown).

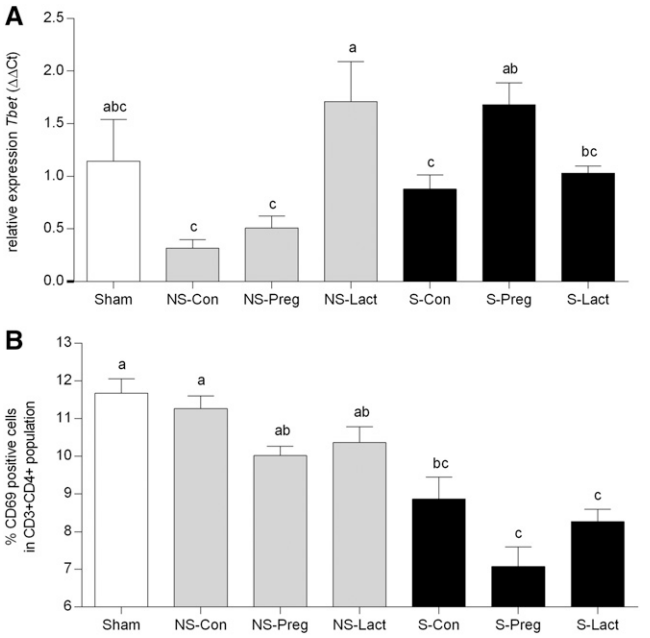

FIGURE 3 *T-bet* mRNA expression levels in spleen and percentage of activated helper T cells in MLNs of female offspring of sensitized or nonsensitized mouse dams fed a control diet or scGOS/lcFOS/pAOS-supplemented diet during pregnancy or lactation. (A) Mean relative mRNA expression levels of *Tbet*. (B) Mean percentages of $CD69^+$ cells within the $CD3^+CD4^+$ population (activated T helper cells). Values are means ± SEMs, n = 4–6. Means not sharing a common letter differ significantly, $P < 0.05$ (Kruskal-Wallis test). lcFOS, long-chain fructo oligosaccharide; MLN, mesenteric lymph node; NS-Con, offspring of nonsensitized female mice fed control food during pregnancy and lactation; NS-Lact, offspring of nonsensitized female mice fed experimental diet during lactation; NS-Preg, offspring of nonsensitized female mice fed experimental diet during pregnancy; pAOS, pectin-derived acidic oligosaccharide; scGOS, short-chain galacto oligosaccharide; S-Con, offspring of sensitized female mice fed control food during pregnancy and lactation; S-Lact, offspring of sensitized female mice fed experimental diet during lactation; S-Preg, offspring of sensitized female mice fed experimental diet during pregnancy; *Tbet*, T-box transcription factor TBX21.

Discussion

Early life exposure to factors such as maternal diet can affect immune development in offspring (11). Maternal dietary supplementation during pregnancy and/or lactation may therefore have a beneficial programming effect, protecting the offspring from developing allergic disease. In a recent study, we showed that symptoms of allergic asthma in male offspring were reduced when dams were supplemented with oligosaccharides during pregnancy (37). In the current study, we investigated whether it is possible to affect the development of hen's egg allergy in offspring by supplementing pregnant or lactating mice with a specific mixture of nondigestible oligosaccharides.

In the S-Con group, neither allergen-specific IgE and IgG1 nor the ASR were significantly affected compared with the NS-Con group. In addition, in the S-Con group, shock symptoms and body temperature upon ovalbumin challenge were not significantly affected compared with the sham group. Thus, the current data suggest that maternal sensitization of mice before mating is no risk factor for increased sensitization and subsequent allergic symptoms in the offspring. In a comparable study, it was shown that maternal sensitization before mating reduced IgE concentrations in the offspring (32), implying that instead of increasing the risk of early life sensitization, tolerance was induced in the offspring. Corresponding with these results, individual offspring mice of sensitized dams in the current study showed lower IgE, shock symptoms, and changes in body temperature with a reduction in the percentage of activated T helper cells in the MLNs. Also, in the NS-Lact group, ovalbumin-specific IgG2a concentrations were higher compared to the offspring of nonsensitized mice. Furthermore, *Tbet* mRNA expression levels were higher in these mice, pointing toward a regulatory Th1 response in the offspring of sensitized dams.

Perinatal exposure to maternal dietary factors may have epigenetic or immune regulatory effects on the promotion or prevention of allergic disease in offspring. In the current study, supplementing sensitized dams with a mixture of nondigestible oligosaccharides in an enriched diet during pregnancy affected acute allergic symptoms in the offspring. These effects coincided with higher expression of *Tbet* in spleen indicative of a Th1 response, which might change the Th2/Th1 balance toward a dampened Th2 response. Even more pronounced effects were observed in the S-Lact group with a significantly lower allergic skin response and complete abolishment of anaphylactic shock symptoms upon challenge. These reductions in clinically related allergic symptoms in offspring supplemented during lactation are consistent with the observed reduction in the Th2-inducing immunoglobulin IgE and IgG1 described to have an anaphylactic activity in mice (33, 34). Interestingly, the concentrations of allergen-specific IgG2a, a Th1-inducing immunoglobulin in mice, were higher in the offspring of sensitized dams supplemented during lactation. In contrast, no effect on *Tbet* mRNA expression levels was observed in these offspring mice.

These data indicate that nutritional supplementation during pregnancy is effective in reducing allergic symptoms in sensitized and challenged offspring with a possible regulatory role for Th1 cells. However, supplementing ovalbumin-sensitized dams during lactation reduced allergic symptoms and decreased IgG1/IgE while increasing the regulatory IgG2a, demonstrating diminished sensitization or tolerance to ovalbumin in the offspring.

Maternal supplementation in mice has been shown to decrease allergy development in offspring. Supplementation with fructo-oligosaccharides reduced atopic dermatitis or contact sensitivity in the offspring of NC/Nga mice (26, 35).

However, dams in this study were supplemented throughout pregnancy and lactation, thus making it impossible to distinguish which window would most contribute to the findings.

To our knowledge, the differential effects of maternal supplementation with scGOS/lcFOS/pAOS during pregnancy or lactation on the development of allergic disease have not been previously described. A mouse model is used to investigate the dietary intervention with scGOS/lcFOS/pAOS during pregnancy and lactation. Obviously, there is a difference between mice and man. However, the model provides an experimental set-up to distinguish between the dietary interventions during pregnancy and lactation. This is hard to accomplish in humans because it is not ethical to ask mothers to replace breastfeeding with bottle-feeding. However, we recommend confirming our data in a human study.

To investigate whether the observed effects on maternal supplementation with nondigestible oligosaccharides were different in sensitized vs. nonsensitized dams, we included an additional 3 groups in the study. Nonsensitized dams were fed a control diet or a diet supplemented with nondigestible oligosaccharides during pregnancy or lactation.

In nonsensitized dams, the protective effects in the offspring were limited to the lactation period. We observed fewer ASRs and anaphylactic shock symptoms in the NS-Lact group, although the difference with the NS-Con group was not statistically significant (P = 0.06 and 0.37, respectively); furthermore, significantly lower concentrations of allergen-specific IgE and IgG1 were seen in the NS-Lact group. This suggests that nondigestible oligosaccharides administered during lactation led to less sensitization and the development of allergic disease in both sensitized and nonsensitized dams.

Tbet expression was higher in these mice suggesting an increased Th1/Th2 balance as a possible regulatory mechanism. No effect was observed on either the percentage of regulatory T cells (data not shown) or activated T helper cells. In a mouse model for allergic asthma, we recently observed a decreased allergic response in the offspring of dams supplemented during pregnancy (37), indicating that both intervention during pregnancy and lactation might be effective, but there might be a different window of opportunity in food allergy vs. allergic asthma.

Maternal supplementation with nondigestible oligosaccharides may affect the maternal microbiome (24, 25), which could alter the availability of SCFAs. These are thought to be able to elicit epigenetic changes (36). Long-term immune programming effects, such as those observed in the current study, may be caused by histone modification and/or DNA methylation alterations to the epigenome of the fetus. New experiments are planned to further elucidate what the underlying mechanism of these immune programming effects may be.

Current data show that supplementation during pregnancy led to fewer allergic symptoms in the adult offspring of dams sensitized before mating. However, supplementing lactating mice led to fewer allergen-specific immunoglobulins in the offspring and less development of allergic symptoms independent of whether the dam had been sensitized before mating. Thus, the extent of the effect is differentially affected by whether the mother has been sensitized or not with an important additional nutritional window for putative preventive dietary strategies, especially during lactation. Effects coincided with higher concentrations of allergen-specific IgG2a and/or *Tbet* expression, implying a regulatory role for Th1 cells. Altogether, these data show that long-term immune effects can be established by supplementing pregnant or lactating mice with nondigestible oligosaccharides.

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AH and BCAMvE designed and conducted the research and wrote the paper; and LMJK and JG had primary responsibility for final content. All authors read and approved the final manuscript.

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