

ORIGINAL ARTICLE

Ovalbumin-specific immunoglobulins A and G levels at age 2 years are associated with the occurrence of atopic disorders

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Summary

Background Humoral responses to food antigens may reflect the propensity of a child's immune system to develop tolerance to innocuous antigens. Early nutrition as well as probiotics may influence these immunological responses.

Objective To study the association of humoral responses to early food antigens with the administration of prebiotics and probiotics, with the occurrence of allergy, and with the length of exclusive breastfeeding.

Methods In a randomized double-blind allergy prevention trial in high-risk children, 1018 mothers took probiotics or placebo from the 36th week of gestation, and their newborn infants received probiotics and prebiotics or placebo during 6 months. At 2 and 5 years, we evaluated the cumulative incidence of allergic diseases (food allergy, eczema, asthma, rhinitis) and sensitization (skin prick test ≥ 3 mm or serum antigen-specific IgE > 0.7 kU/L). In 688 infants at age 2, we measured in sera-specific IgA, IgG, IgG1, and IgG4 antibody levels to cow's milk (CM), α -casein (CAS), β -lactoglobulin (BLG), and ovalbumin (OVA) with ELISA, and specific IgE levels to CM and hen's egg with UniCap.

Results Probiotic treatment ($n = 342$) compared with placebo ($n = 346$) showed no effect on serum food-specific IgA, IgG, IgG1, or IgG4 concentrations at age 2. Atopic children had higher OVA-IgA ($P < 0.001$), OVA-IgG ($P = 0.001$), OVA-IgG1 ($P < 0.001$), and egg-IgE but lower OVA-IgG4/egg-IgE ratio ($P < 0.001$) than non-atopic children. Longer duration of exclusive breastfeeding (≥ 4 vs. < 4 months) was associated with reduced CM- and CAS-specific serum IgA ($P < 0.001$) and IgG levels ($P < 0.001$; $P = 0.003$).

Conclusion and Clinical Relevance Allergy was associated with more intense IgA and IgG responses to OVA. Breastfeeding depressed humoral responses, whereas prebiotics and probiotics supplementation showed no immunomodulatory effect. The effect of probiotics on allergies is not mediated through food-specific antibody responses. Furthermore, OVA-specific IgA and IgG antibodies may help in assessing the risk for atopy. [Trial registration: Clinicaltrials.gov NCT00298337]

Keywords allergy, atopy, cow's milk, food antigen, IgA, IgG, IgG1, IgG4, ovalbumin, probiotic
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Introduction

Infants encounter food allergens, notably cow's milk (CM) and hen's egg, in early life when closure of the gut and the maturation of the immune system are not yet complete. Physiological humoral response to early antigens consists of class IgA and IgG antibodies [1], and transiently of specific IgE antibodies [2]. Sensitization to hen's egg is relatively common in children, its cumulative incidence from birth to 6 years was 19% in a Danish study [3],

whereas sensitization leads to clinical allergy in only 0.5–2% of children [3–5]. Sensitization to CM is not as common; in the same study it was 11% [3]. It is frequently associated with clinical CM allergy (CMA), which affects 2–3% of children [4, 6].

The interaction between Th2 and regulatory T cells, such as those producing IL-10, is central in the process that leads either to allergy or to tolerance [7, 8]. Higher IL-10 production by T cells was reported in children who tolerated CM compared to children with CMA [9]. Specific

antibody concentrations reflect the T cell balance. Th2 cytokines promote both specific IgE and IgG4 production [10], whereas IL-10, secreted by inducible T regulatory cells, augments specific IgG4 production and inhibits IgE production [11–14]. High levels of specific IgG4 antibodies are associated with the development and maintenance of clinical tolerance [13, 15, 16]. Early clinical tolerance in CM and/or egg allergy was shown to associate with high levels of IgG4 to β -lactoglobulin (BLG) and/or ovalbumin (OVA) as well as with a high specific IgG4/IgE ratio [17]. In a study on hen's egg allergy, OVA-specific IgG4 increased and specific IgE decreased during the natural development of tolerance [18]. Serum-specific IgA has also emerged as a potential factor in the development of tolerance during successful allergen-specific immunotherapy [13, 16, 19]. The production of specific IgA seems to be regulated by local TGF- β secretion [19].

The immune response to oral antigens is influenced by the timing of the first exposure [20–23]. Exposure to CM proteins during first 3 days [21] or first 3 months [20, 22] of life reportedly leads to relatively high specific IgG class responses. Evidence on the effect of early exposure on the development of allergy is, however, conflicting [6, 22]. Early feeding impacts immunological responses to food allergens both through the timing of exposure and through immunomodulatory factors present in breast milk.

Prebiotics and probiotics may influence the maturation of the immune system in infancy [24]. In children with CMA, probiotics increased IFN- γ production suggesting immune maturation towards Th1 responses [25]. We previously reported that children at high risk for atopy who received probiotics and prebiotics had increased CRP and serum IL-10 levels implying a low-grade inflammation status [26]. We furthermore observed increased IgA and IgE levels in children who remained non-atopic [26]. Although several studies indicate that probiotics may prevent the development of atopic eczema in children at high risk for atopy, the current research data are somewhat conflicting [27].

In a cohort of 688 high-risk children attending an allergy-prevention trial on probiotics and prebiotics, we studied antibody responses to CM and hen's egg proteins at the age of 2 years. We hypothesized that the immunomodulatory effects of these agents would be reflected in humoral responses to early food antigens. We furthermore investigated whether these responses were associated with early feeding patterns and with the development of allergies.

Methods

This double-blinded, placebo-controlled study took place in Helsinki, Finland between November 2000 and December 2007 and has been described in detail [28, 29]. We

recruited mothers in their early pregnancy to participate in a probiotic allergy-prevention trial in Helsinki suburban area in Finland. Mothers were eligible if at least one of the child's parents had a doctor-diagnosed allergic disease. Premature infants (<37 weeks of gestation) and infants born with major malformations were excluded. Randomization of the 1223 mothers to probiotic or placebo group took place at 35 weeks of pregnancy. The supplier (Valio Ltd., Helsinki, Finland) sent the blank packages directly to the mothers. The randomization code was not revealed to the study group until the end of the 5-year follow-up. During the 4 weeks before delivery, mothers in the probiotic group took twice daily one capsule containing *Lactobacillus rhamnosus* GG (ATCC 53103) 5×10^9 colony-forming units (CFU), *L. rhamnosus* LC705 (DSM 7061) 5×10^9 CFU, *Bifidobacterium breve* Bb99 (DSM 13692) 2×10^8 CFU, and *Propionibacterium freudenreichii* ssp. *shermanii* JS (DSM 7076) 2×10^9 CFU while controls took inert cellulose capsules. Mothers did not receive prebiotics. Newborn infants received daily one opened capsule containing the four probiotic strains mixed with an adequate amount of liquid containing prebiotic galacto-oligosaccharides (0.8 g/day), or placebo, in a teaspoon. Valio Ltd. supplied probiotic capsules, prebiotics, and placebo. Mothers signed a written informed consent. The ethics committee of the Hospital for Children and Adolescents of Helsinki University approved the study protocol. The trial was registered to Clinicaltrials.gov NCT00298337.

The study paediatrician examined the children at ages 3 and 6 months and 2 and 5 years. Parents responded to questionnaires concerning breastfeeding, complementary foods, environmental factors, infections and symptoms of allergic diseases at their child's age of 3 and 6 months, and at 1, 2, 3, 4, and 5 years.

The primary end-points were allergic and atopic diseases at the age of 5 years. Symptoms of allergic diseases were evaluated at the scheduled study visits. In addition, if the parents contacted by phone or e-mail, or if they reported their child having symptoms of allergic diseases in the questionnaires, the study nurse contacted them and organized an additional study visit to the child. In suspected food allergy, the child was put to a 2-week elimination diet. In case of relief of the symptoms, we diagnosed food allergy with an open food challenge [30]. A challenge was not required in case of doctor-diagnosed anaphylaxis. Emergence of tolerance was evaluated in the questionnaires (late-onset-symptoms) or in a re-challenge. We diagnosed eczema according to the UK Working Party's criteria [31], and asthma as two or more doctor-diagnosed wheezy episodes accompanied by persistent symptoms (persistent cough or exercise-induced symptoms) between wheezy episodes. In symptomatic children (>2 years of age) we used impulse oscillometry with exercise and bronchodilatation tests to diagnose bronchial

hyper-reactivity. If resistance was increased by 35% or more after outdoor running exercise or decreased by 35% or more after bronchodilatation test, the child was considered to have bronchial hyper-reactivity [32]. Allergic rhinitis was diagnosed according to the ARIA criteria [33].

Blood samples were drawn at the 2-year visit. Centrifuged serum samples were stored at -50°C . At 2 and 5 years, we performed skin prick tests according to a standard technique [34], and measured serum-specific IgE against a panel of common food and inhalant allergens with an immunoassay (ImmunoCAP[®] system, Pharmacia Diagnostics, Uppsala, Sweden).

We defined sensitization as any positive skin prick reaction ≥ 3 mm, and/or any serum antigen-specific IgE ≥ 0.7 kU/L and designated children who were sensitized as 'atopic'. When an allergic disease (eczema, food allergy, asthma, allergic rhinitis) was associated with sensitization, we used the term 'atopic disease'. Children who were not sensitized and who were not diagnosed with any allergic disease during the entire follow-up period from birth until the age of 5 were considered 'non-atopic, non-allergic'.

At the age of 2 years, we measured serum IgE (kU/L) specific to CM and hen's egg white with UniCap (Phadia, Sweden). We measured serum IgA, IgG, IgG1, and IgG4 isotype antibodies specific to CM, α -casein (CAS), BLG, and OVA using ELISA. IgA and IgG analyses are explained in detail in Savilahti et al. [35]. IgG1 and IgG4 measurement was adapted from the method by Ruiter et al. [15] and has been described previously [36]. Values were expressed as arbitrary units (AU).

Statistical analyses

Sample-size calculation was based to detect a 10% absolute reduction of allergic diseases by probiotics at age 5 years (significance level 5%, power 90%). We analysed all available serum samples at age 2 years. We assigned the value of detection limit/2 for antibody levels under detection limit. CM-, CAS-, BLG- and OVA-specific IgG antibodies were detectable in $>98\%$, and specific IgG1 in $>97\%$ of specimen. CM-IgA and CAS-IgA were detectable in $>98\%$, BLG-IgA in 85%, OVA-IgA in 84%, CAS-IgG4 in 74%, BLG-IgG4 in 89%, and OVA-IgG4 in 78%. Total IgE was detectable in 96% (detection limit 2 kU/L), hen's egg-specific IgE and CM-specific IgE in 100% (detection limit 0.02 kU/L). All distributions were skewed to the right, and we thus used logarithmic transformations for calculating geometric mean and 95% confidence intervals for the antibody concentrations. We compared groups with non-parametric tests (Mann-Whitney *U*, Kruskal-Wallis). We used ANCOVA to study possible interaction between duration of exclusive breastfeeding and atopic diseases at age 2 for serum antibody concentrations. Probiotic treatment was assessed as a covariate. We

assessed gender, probiotic treatment, allergy in both parents (compared with only one), and duration of exclusive breastfeeding as possible confounding factors for any atopic disease between 0–2, 0–5 years, and for food allergy at age 2 years using multivariate ANCOVA. We used SPSS, version 17.0 (SPSS Inc., Chicago, IL, USA) for all analyses.

Results

Participants

Of the 1223 randomized mothers, 1067 initiated the intervention and gave birth to 1018 intention-to-treat infants. Of them, 925 attended the follow-up visit at 2 years and 891 at 5 years. The cumulative incidence of any allergic disease from birth to age 2 and 5 years were 33% and 54%, respectively [28, 29]. Sera for this study were available from 342 children in the probiotic and 346 in the placebo group (Fig. 1). Baseline and clinical characteristics of the study groups are shown in Table 1.

Effect of probiotics and prebiotics on specific humoral responses to early food antigens

We observed no significant difference between the treatment groups on specific IgA, IgG, IgG1, or IgG4 antibodies to CM, CAS, BLG, or OVA (data not shown).

Atopic diseases and antibody responses to early food antigen

Of the children, 215 were non-atopic, non-allergic from birth to age 5, 113 suffered from any atopic disease by age 2 and 217 by age 5. Compared with non-atopic, non-allergic children, those diagnosed with any atopic disease during 0–2 or during 0–5 years, had higher OVA-specific IgA, IgG, IgG1, and egg white-specific IgE, but lower OVA-IgG4/IgE ratio (Fig. 2). CAS-IgG4 was higher in children with any atopic disease during 0–2 years (geometric mean 0.36; 95%CI 0.21–0.63; $P=0.001$) and during 0–5 years (0.32; 0.21–0.47; $P=0.001$) compared with non-atopic, non-allergic children in whom CAS-IgG4 was 0.11 (0.08–0.17). BLG-IgG4 was also higher in children diagnosed with any atopic disease during 0–5 years compared with non-atopic, non-allergic children (0.66; 0.45–0.96 vs. 0.37; 0.26–0.54; $P=0.023$). The other antibodies did not differ between the groups (data not shown).

Food allergy and antibody responses to early food antigens

During the first 2 years of life, food allergy was diagnosed in 47 children (5.1%). Of them, 42 were allergic to CM, six to hen's egg, and five suffered from wheat allergy. Six children had multiple food allergies. Eighteen (16 with

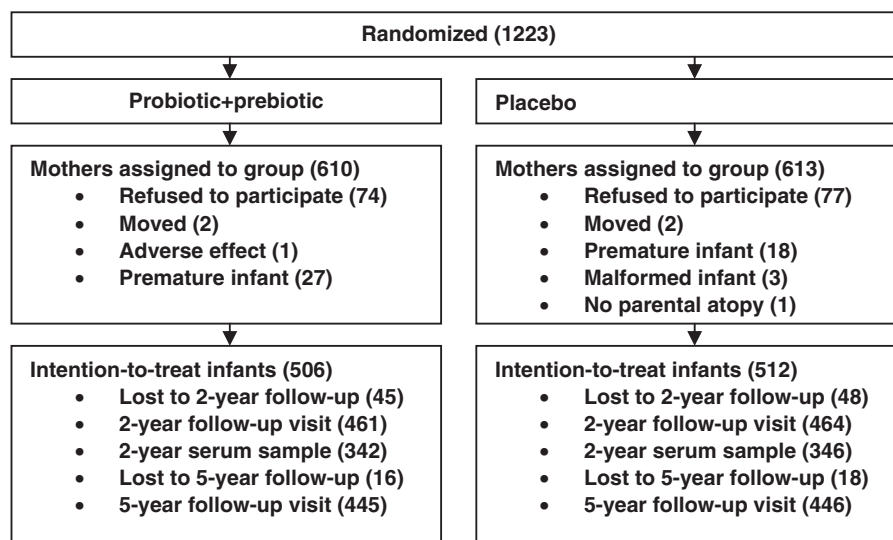


Fig. 1. Flow chart of the study population from randomization until the end of the 5-year follow-up.

Table 1. Clinical characteristics and main outcomes of the study children with serum samples at age 2 years

	Probiotic+ prebiotic (N = 342)	Placebo (N = 346)
Males, N (%)	173 (50)	169 (49)
Birth weight, g mean (SD)	3606 (481)	3587 (488)
Caesarean delivery, N (%)	58 (17)	63 (18)
Exposed to tobacco smoke 0–2 years, N (%)	104 (30)	104 (30)
Duration of breastfeeding, months mean (SD)	8.8 (5.3)	8.4 (5.0)
Duration of exclusive breastfeeding, months mean (SD)	2.1 (1.9)	2.1 (1.9)
Age of introducing solid foods, months mean (SD)	4.0 (0.8)	4.0 (0.7)
Non-atopic, non-allergic 0–5 years, N (%)	113 (33)	102 (29)
Any allergic disease 0–2 years, N (%)	110 (32)	122 (35)
Any atopic disease 0–2 years, N (%)	47 (14)	66 (19)
Food allergy 0–2 years, N (%)	22 (6)	25 (7)
Sensitized 0–2 years, N (%)	96 (28)	115 (33)
Any allergic disease 0–5 years, N (%)	181 (53)	186 (54)
Any atopic disease 0–5 years, N (%)	105 (31)	112 (33)
Sensitized 0–5 years, N (%)	139 (41)	37 (40)

Allergic diseases comprise eczema, food allergy, asthma, and rhinitis. Atopic disease refers to any allergic disease combined with sensitization (any specific-IgE ≥ 0.7 kU/L and/or skin prick test ≥ 3 mm bigger than the negative control). Non-atopic, non-allergic is a child without any allergic disease or sensitization.

CMA, two with wheat allergy) were already tolerant by age 2. At age 2, compared with non-atopic, non-allergic children, children with any food allergy or CMA had lower CM protein-specific IgA and IgG class antibody levels. They also had lower OVA-specific IgG4 levels and OVA-IgG4/egg-IgE ratio (Table 2).

Effect of early feeding on food-specific humoral antibody responses

Longer (≥ 4 months) exclusive breastfeeding was associated with lower levels of IgA and IgG specific to CM and to CAS, and lower IgG1 and IgG4 specific to CAS and BLG at age 2. Higher OVA-IgA levels were associated with longer exclusive breastfeeding; whereas OVA-IgG, IgG1 and IgG4 levels did not show as clear a trend (Kruskal–Wallis) (Table 3). The length of exclusive breastfeeding did not affect IgE specific to CM or to egg white at age 2. The 186 children who were exclusively breast-fed < 2 weeks had higher levels of IgA and IgG specific to CM and to CAS, and higher IgG1 and IgG4 specific to CAS and to BLG, but lower OVA-specific IgA and IgG at age 2 (Table 3). Adjustment for probiotics intervention and any atopic disease during 0–2 years did not significantly alter the results (Table 3).

Discussion

We studied humoral responses to CM proteins and hen's egg OVA at the age of 2 years in allergy-prone children who were followed from birth to the age of 5 years. They had received either a combination of probiotics and prebiotics or placebo for 6 months after birth. We found that atopic disorders were associated with high OVA-specific IgA, IgG, IgG1, and IgE levels. Children with food allergies, in contrast, had low IgA and IgG class responses to CM proteins. Lower antibody levels to CM proteins were associated with exclusive breastfeeding over 4 months. We did not observe any difference in these humoral responses between children who had received probiotics and prebiotics compared with the placebo group.

We studied a large population ($n = 688$) prospectively from birth until the age of 5 years. The sensitization

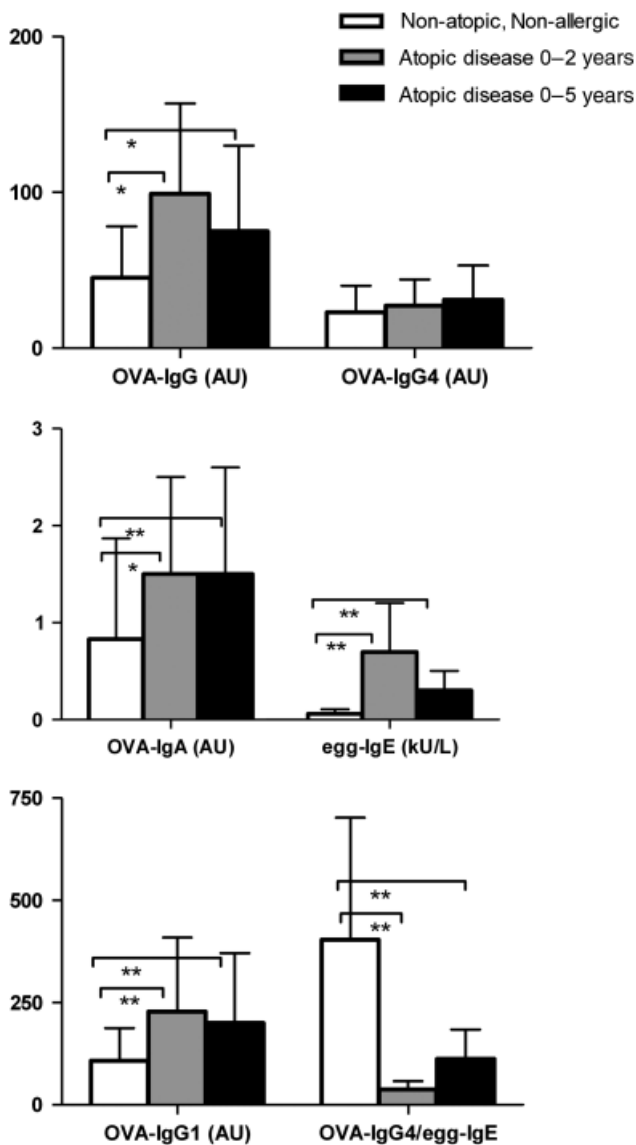


Fig. 2. Geometric mean values of antibody responses to ovalbumin (OVA) at age 2 years in children who were non-atopic, non-allergic from birth to age 5 ($n = 215$), in children with an atopic disease during 0–2 years ($n = 113$) and during 0–5 years ($n = 217$). (a) OVA-IgG and OVA-IgG4. (b) OVA-IgA and hen's egg white-specific-IgE. (c) OVA-IgG1 and ratio OVA-IgG4 to hen's egg white-specific-IgE. P -values were adjusted for the probiotic intervention (ANOVA) and appear as ** $P < 0.001$, * $P < 0.05$.

and allergic status of the children was meticulously followed at our outpatient clinic by the study paediatrician. The trial on a combination of probiotics and prebiotics was double-blinded and placebo-controlled. We gathered data on early feeding with frequent (at 3, 6, and 12 months) questionnaires throughout the first year of life. In this same population of children, we saw a reduction of atopic eczema at age 2 by pre- and probiotics. At age 5, however, the effect sustained in caesarean delivered children but not in the whole population [28, 29].

Our analysis on the association of food allergies and food-specific antibody levels was limited by the number ($n = 47$) of children who developed a food allergy. These children had met rigorous diagnostic criteria for food allergy. Children with food allergies had lower IgA and IgG class responses to CM proteins than non-atopic, non-allergic children. The allergens they were reactive to have been eliminated from the diet since the time of diagnosis until the emergence of tolerance. Reduced exposure may be one explanation why their humoral responses were subdued. Elimination diet may also explain why food antibody levels in children with food allergy were lower whereas in children with atopic disease overall levels were higher compared with non-atopic children. Most ($n = 42$) of the children with food allergy were reactive to CM, only six reacted to hen's egg. Specific IgG4 levels to OVA were, however, low in the entire group of children with food allergy, which may reflect a more general tendency towards subdued humoral responses to food allergens. The association of food allergy with lower IgA and IgG class responses to CM is in line with studies that have shown higher specific IgA and IgG class antibodies to be associated with the development and maintenance of tolerance [13, 15–18].

Exclusive breastfeeding for 4 months or more was associated with lower levels of specific IgA and IgG class antibodies to CM proteins. This may reflect later and/or lower antigen exposure compared with children who receive infant formula [20–22]. Infants do, however, get exposed to food antigens through breast milk [37, 38]. Antibody responses in breastfed infants are plausibly suppressed by the immunomodulatory factors in breast milk, such as secretory IgA and cytokines TGF- β and IL-10 [39, 40]. Furthermore, glycopeptides and oligosaccharides in breast milk induce the development of a balanced intestinal microbial flora [41, 42], which is a prerequisite for maturation of the gut immune system and of oral tolerance [43]. Prebiotics and probiotics have similar effects in maturation of the immune system [44]. Immunomodulatory effects of probiotics *in vivo* reportedly entail increased serum levels of IL-10, total IgA, and IgE [26]. We saw, however, no association between the intensity of specific humoral responses to early food antigens and prebiotics and probiotics supplementation. Similar findings have not been reported before. Probiotics increased IL-10, total IgE and IgA in non-allergic children without an effect on food specific antibodies of IgA, IgG, or IgE class implying a regulatory T cell response lying behind the allergy preventive effect [26]. Despite its preventive effect on allergy, probiotic treatment does not seem to affect sensitization [28, 45].

We saw that longer duration of exclusive breastfeeding was associated with lower antibody levels specific to CM proteins. Infants who received formula very early (<2 weeks of age) had the highest levels of CM-specific

Table 2. Specific antibody levels to cow's milk (CM), α -casein (CAS), β -lactoglobulin (BLG), and ovalbumin (OVA) at 2 years of age in children with food allergy during the first 2 years

	Geometric mean (95% CI), AU		<i>P</i> * (<i>P</i> adjusted [†])	Cow's milk allergy	
	Non-atopic, non-allergic <i>N</i> = 215	Any food allergy <i>N</i> = 47		<i>N</i> = 42	<i>P</i> * (<i>P</i> adjusted [†])
CM-IgA	1.0 (0.7–1.4)	0.2 (0.1–0.5)	<0.001 (<0.001)	0.2 (0.08–0.4)	<0.001 (0.001)
CM-IgG	9.2 (7.3–11.8)	2.8 (1.7–4.4)	<0.001 (<0.001)	2.4 (1.4–3.9)	<0.001 (<0.001)
CM-IgE	0.09 (0.08–0.10)	0.3 (0.13–0.22)	0.001 (<0.001)	0.34 (0.18–0.62)	<0.001 (<0.001)
CAS-IgA	3.2 (2.6–4.0)	1.7 (1.1–2.8)	0.031 (0.027)	1.6 (1.0–2.8)	0.033 (0.015)
CAS-IgG	5.2 (4.2–6.4)	2.6 (1.6–4.0)	0.006 (0.008)	2.3 (1.4–3.8)	0.003 (0.003)
CAS-IgG1	16 (13–18)	10 (7.0–15)	0.049 (0.068)	9.9 (6.6–15)	0.048 (0.048)
CAS-IgG4	0.1 (0.08–0.2)	0.07 (0.04–0.15)	0.285 (0.367)	0.06 (0.03–0.1)	0.104 (0.115)
CAS-OgG4/CM-IgE	1.23 (0.86–1.77)	0.25 (0.1–0.62)	0.004 (0.001)	0.17 (0.07–0.43)	<0.001 (<0.001)
BLG-IgA	0.2 (0.2–0.3)	0.1 (0.08–0.2)	0.052 (0.077)	0.1 (0.07–0.2)	0.027 (0.038)
BLG-IgG	0.5 (0.4–0.7)	0.3 (0.2–0.6)	0.084 (0.104)	0.3 (0.1–0.5)	0.033 (0.042)
BLG-IgG1	0.8 (0.7–1.0)	0.6 (0.4–1.0)	0.104 (0.306)	0.6 (0.3–1.0)	0.90 (0.266)
BLG-IgG4	0.4 (0.3–0.5)	0.1 (0.05–0.2)	0.004 (0.005)	0.08 (0.04–0.2)	0.001 (0.001)
BLG-IgG4/CM-IgE	4.1 (2.8–5.9)	0.34 (0.14–0.85)	<0.001 (<0.001)	0.24 (0.10–0.60)	<0.001 (<0.001)
OVA-IgA	0.78 (0.60–1.02)	0.95 (0.5–1.8)	0.660 (0.701)	0.73 (0.38–1.4)	0.783 (0.724)
OVA-IgG	45 (33–62)	26 (12–54)	0.122 (0.081)	21 (9–46)	0.045 (0.026)
OVA-IgG1	108 (90–129)	109 (68–175)	0.874 (0.971)	88 (54–147)	0.394 (0.394)
OVA-IgG4	23 (17–33)	3.7 (2.1–6.6)	<0.001 (<0.001)	3.5 (1.9–1.4)	<0.001 (<0.001)
Hen's egg-IgE	0.06 (0.05–0.06)	0.8 (0.39–1.64)	<0.001 (<0.001)	0.61 (0.29–1.29)	<0.001 (<0.001)
OVA-IgG4/egg-IgE	404 (298–548)	4.7 (2.0–11)	<0.001 (<0.001)	5.8 (2.3–14)	<0.001 (<0.001)

*Mann–Whitney test comparing children with any food allergy, or children with cow's milk allergy to non-atopic, non-allergic children. Measurements are in arbitrary units (AU).

[†]Adjusted *P*-value for probiotic intervention, gender, duration of exclusive breastfeeding, and biparental allergy.

Bold *P* values signify *P* < 0.05 and *P* < 0.001.

CI, confidence interval.

Table 3. The length of exclusive breastfeeding and specific antibody levels to cow's milk (CM), α -casein (CAS), β -lactoglobulin (BLG), hen's egg, and ovalbumin (OVA) at age 2

	Length of exclusive breastfeeding (months)			<i>P</i> *	<i>P</i> [†] adjusted
	Geometric mean with 95% CI (AU)				
	<0.5 <i>N</i> = 186	≥0.5 and <4 <i>N</i> = 293	≥4 <i>N</i> = 207		
CM-IgA	1.3 (0.86–1.9)	0.88 (0.66–1.2)	0.73 (0.55–0.98)	<0.001	0.020
CM-IgG	12 (9.0–16)	8.8 (7.1–11)	8.3 (6.7–10)	<0.001	0.019
CM-IgE	0.14 (0.12–0.17)	0.14 (0.12–0.16)	0.13 (0.11–0.15)	0.11	0.035
CAS-IgA	4.5 (3.4–5.9)	3.0 (2.5–3.6)	2.67 (2.2–3.3)	<0.001	0.002
CAS-IgG	6.9 (5.4–8.7)	5.1 (4.3–6.1)	5.0 (4.1–6.1)	0.003	0.014
CAS-IgG1	21 (18–25)	15.9 (14–18)	14 (12–16)	0.002	0.001
CAS-IgG4	0.35 (0.23–0.54)	0.22 (0.15–0.30)	0.11 (0.07–0.17)	<0.001	<0.001
CAS-IgG4/CM-IgE	2.5 (1.7–3.8)	1.54 (1.10–2.15)	0.90 (0.61–1.31)	0.002	0.004
BLG-IgA	0.17 (0.13–0.22)	0.18 (0.15–0.22)	0.24 (0.20–0.30)	0.57	0.06
BLG-IgG	0.55 (0.40–0.30)	0.58 (0.46–0.73)	0.60 (0.46–0.77)	0.78	0.98
BLG-IgG1	1.2 (0.99–1.6)	0.96 (0.79–1.17)	0.79 (0.63–0.99)	0.027	0.013
BLG-IgG4	0.75 (0.49–1.2)	0.55 (0.40–0.76)	0.26 (0.18–0.37)	<0.001	0.002
BLG_IgG4/CM-IgE	5.4 (3.6–8.3)	4.0 (2.9–5.5)	2.1 (1.4–3.0)	0.001	0.027
OVA-IgA	0.56 (0.42–0.77)	1.1 (0.88–1.4)	1.3 (1.0–1.7)	<0.001	0.014
OVA-IgG	40 (28–58)	59 (45–78)	56 (41–76)	0.08	0.19
OVA-IgG1	119 (98–143)	151 (128–178)	116 (95–143)	0.017	0.82
OVA-IgG4	20 (14–29)	30 (22–40)	20 (14–29)	0.11	0.60
Hen's egg-IgE	0.10 (0.08–0.12)	0.13 (0.11–0.16)	0.12 (0.09–0.14)	0.11	0.24
OVA-IgG4/egg-IgE	209 (146–300)	228 (164–316)	183 (126–266)	0.45	0.28

*Kruskal–Wallis test. Measurements are in arbitrary units (AU).

[†]Adjusted for any atopic disease (0–2 years) and probiotic intervention using ANCOVA.

Bold *P* values signify *P* < 0.05 and *P* < 0.001.

CI, confidence interval.

antibodies. Mothers of allergic children may have decided to exclusively breast-feed longer. However, it is unlikely that any symptom of an allergic disease will manifest in an exclusively breast-fed infant during neonatal period. Therefore, reverse causation is unlikely to fully explain the observation.

We observed higher IgA and IgG1 responses to OVA in children who developed an atopic disease by the age of 2 or 5 years compared with children who were non-atopic, non-allergic from birth to age 5. Several studies have reported that high levels of egg-specific IgE antibodies associate with the development of sensitization to other antigens and of allergic disorders [46–48]. Less is known about the association with other Ig subclasses. Our results show that in children who developed an atopic disorder, not only IgE but also IgA and IgG class responses to OVA were more intense than in non-atopic children. This may reflect that in addition to an excessive Th2 response, atopy is associated with a broader imbalance of effector Th cells and T regulatory cells. Intriguingly, antibody responses to CM proteins in the two study groups did not differ. The results implicate that specifically OVA has extensive immunogenicity in individuals who develop atopy. Timing and amount of first exposure may also play a role: CM is usually introduced to the diet earlier and in larger

quantities than hen's egg. We may speculate that the observed differences in humoral responses were largely influenced by environmental variables since all children in our study had a family history of allergy and furthermore double risk (both parents with allergy) was not a significant confounding factor.

Clinical relevance

Our study suggests that not only egg-specific IgE but also OVA-specific IgA and IgG antibodies may help in assessing the risk for atopy. The findings underscore the immunomodulatory effects of breastfeeding, whereas supplementary prebiotics and probiotics showed no association with humoral responses to early food antigens.

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