

ORIGINAL ARTICLE

Cow's milk and ovalbumin-specific IgG and IgA in children with eczema: low β -lactoglobulin-specific IgG4 levels are associated with cow's milk allergy

Emma M. Savilahti¹, Mirva Viljanen¹, Mikael Kuitunen² & Erkki Savilahti¹

¹The Hospital for Children and Adolescents; ²The Skin and Allergy Hospital, University of Helsinki, Helsinki, Finland

To cite this article: Savilahti EM, Viljanen M, Kuitunen M, Savilahti E. Cow's milk and ovalbumin-specific IgG and IgA in children with eczema: low β -lactoglobulin-specific IgG4 levels are associated with cow's milk allergy. *Pediatric Allergy Immunology* 2012; **00**.

Keywords

cow's milk allergy; eczema; IgG4; tolerance; β -lactoglobulin.

Correspondence

Erkki Savilahti, Biomedicum 1, POB 700, 00029 HUS (Helsinki) Finland.
Tel.: +358947171939
Fax: +358947171947
E-mail: erkki.savilahti@helsinki.fi

The study was supported by the Helsinki University Central Hospital Research Funds. Valio Ltd provided probiotics and placebo preparations.

Accepted for publication 11 January 2012

DOI:10.1111/j.1399-3038.2012.01277.x

Abstract

Tolerance to allergens may partly depend on allergen-specific IgG and IgG subclasses and IgA antibodies. We investigated whether specific IgG and IgG subclasses and IgA antibodies to β -lactoglobulin, α -casein, and ovalbumin differed between infants who had verified cow's milk allergy (CMA) and infants with cow's milk (CM)-associated eczema, but negative CM oral challenge. The study population comprised 95 infants with clinical eczema that was by history associated with the consumption of CM. After an elimination period, a double-blind, placebo-controlled (DBPC) CM oral challenge confirmed CMA in 45 infants. Skin prick tests (SPT) were performed with CM and hen's egg. Serum levels of IgE antibodies to CM and hen's egg were measured with UniCAP (Phadia, Uppsala, Sweden), and levels of IgA, IgG, IgG1, and IgG4 antibodies to β -lactoglobulin, α -casein, and ovalbumin were measured with enzyme-linked immunosorbent assay. We observed that infants with CMA had lower IgG4 levels to β -lactoglobulin than infants with negative DBPC CM challenge ($p = 0.004$). Positive CM SPT was associated with lower IgG4 levels to α -casein ($p = 0.04$). The relation of CM IgE to β -lactoglobulin and α -casein IgG4 was higher in CMA than in infants with negative challenge ($p < 0.002$ and 0.0001). Positive egg SPT was associated with elevated levels of specific IgG to ovalbumin, β -lactoglobulin, and α -casein as well as IgA to α -casein ($p < 0.04$). Our study thus shows that low β -lactoglobulin-specific serum IgG4 levels may differentiate eczematous infants with CMA from infants who have eczema with only suspected association with CM.

Eczema in infants is often associated with sensitization to food allergens, cow's milk (CM) proteins in particular (1, 2). It often indicates a predisposition to allergy also later in life (3). Eczema, on the other hand, is a predominant symptom of cow's milk allergy (CMA) (1), which affects 2–3 % of infants (3).

A reliable CMA diagnosis requires an elimination diet followed by a controlled oral challenge, the gold standard being double-blind placebo-controlled (DBPC) challenge (4, 5). A history of CM-related symptoms combined with specific IgE response to CM is sometimes considered sufficient, but may lead to false diagnoses (1, 5–7). Diagnosing CMA when it is not IgE mediated is even more challenging, especially as the symptoms are often delayed (1, 2).

Whether measuring CM-specific IgG and IgG subclass or IgA antibodies helps in CMA diagnostics is controversial

(8–10). Both basic and clinical studies support the notion that especially specific IgG4 may be a useful marker for tolerance and allergy. While the Th2-cytokine IL-4 induces class switching in B cells to both IgE and IgG4, IL-10 up-regulates the secretion of IgG4 and inhibits IgE production (11). One plausible source of IL-10 is secretion by T regulatory 1 cells (12). Specific IgG4 functions, at least partly, by blocking the binding of specific IgE to allergen (11). Antibodies of the IgG4 subclass seem, indeed, to be associated with specific tolerance. Individuals tolerating CM were shown to have higher CM-specific IgG4 levels than those with allergic reactions to CM (13). In a study on children with CM and/or egg allergy, prolonged elimination diet was required in children with low levels of IgG4 to β -lactoglobulin and/or ovalbumin (14). We previously reported that in a prospective study from birth to the age of 8–9 yr, children who spontaneously recovered

from CMA early had higher β -lactoglobulin-specific IgA levels at the time of diagnosis and higher β -lactoglobulin-specific IgG4 levels at later age compared with those who had persistent CMA (15). Mucosal IgA has properties that promote tolerance, and the regulation of its expression involves cytokines secreted by T regulatory cells, such as TGF- β (16).

We investigated whether infants with CMA could be distinguished by IgG and IgG subclasses and/or IgA antibodies in a population of infants with eczema that parents associated with CM consumption. We hypothesized that based on its immunological function as well as on data from previous clinical studies, IgG4 levels to CM proteins would be lower in infants with CMA and could thus contribute to CMA diagnostics. We also measured specific antibody responses to hen's egg to assess the overall propensity of the study subjects to mount antibody responses to early food allergens.

Methods

The original study (17) investigated whether probiotic treatment would alleviate eczema. The study protocol has been described previously in detail (17) and is depicted in Fig. 1. Samples for this study were, however, collected prior to the treatment. The DBPC CM oral challenges were performed 4 wks after the 4-wk-long treatment. One parent of each participating infant gave a written informed consent. The local ethics committee approved of the study protocol.

Originally, 230 infants with eczema that was suspected to be associated with CM consumption were recruited between November 1999 and March 2002 at the Skin and Allergy Hospital, University of Helsinki. All infants were under the age of 12 months at the first visit to the clinic. For the current study, we had serum samples available from 95 infants. Clinical characteristics of the current study population are depicted in Table 1.

At every visit to the clinic, a physician (MV) met the infant with one or both parents. She evaluated the clinical status of eczema with the Severity Scoring of Atopic Dermatitis (SCORAD) at the first, and second visit 4 wks later, and at the third visit 8 wks after the first visit. Parents were advised to treat the infant's eczematous lesions with emollients continuously and with topical 1% hydrocortisone when needed but not for more than 2 wks continuously. The use of hydrocortisone was recorded at every visit.

Cow's milk elimination was started for infants and their breast-feeding mothers at the first visit. CM-based formula was replaced by extensively hydrolyzed whey formula (EHF), (Peptidi-Tutteli; Valio Ltd, Helsinki, Finland).

At the first visit to the clinic, we performed skin prick tests (SPTs) with commercial allergen extracts of egg white (Alyostal prick test; Stallergenes SA, Antony, France), cat, dog, and birch (Soluprick; ALK-Abello', Hørsholm, Denmark), according to the standard technique. We also performed duplicate tests with fat-free CM, with commercially available adapted CM and hypoallergenic infant formulas, as well as with cereal grains and purified gliadin. We considered a SPT to be positive if the mean wheal diameter was at least 3 mm larger than the one elicited by the negative control. We defined atopy as a positive SPT response and/or an antigen-specific IgE concentration of 0.7 kU/l or greater to any antigen tested. AD was thus defined as eczema that coincided with atopy. Infants with positive DBPC CM challenge and with a positive CM SPT or specific IgE to CM ≥ 0.7 kU/l were considered to have IgE-mediated CMA.

Infants were randomly allocated to receive one of the three products in a double-blind manner for 4 wks: (i) *Lactobacillus rhamnosus* GG [ATCC 53103; 53109 colony-forming units (cfu)], (ii) a mixture of probiotics (LGG, 53109 cfu; *Lactobacillus rhamnosus* LC705, 53109 cfu; *Bifidobacterium breve* Bbi99, 23108 cfu; and *Propionibacterium freudenreichii* ssp. shermanii JS, 23109 cfu), or (iii) placebo.

The elimination diet lasted 8 wks, that is, the 4-wk treatment period followed by 4 wks without treatment. All infants showed clinical improvement of eczema. We performed a double-blind placebo-controlled CM challenge. The placebo product was EHF (Peptidi-Tutteli; Valio Ltd). CM formula (Tutteli; Valio Ltd) was mixed with EHF 1:2 to make it indistinguishable from the placebo.

We started the challenge with drops on the skin. Next, we gave the formula orally in quantities of 2, 10, 50, and 100 ml at 30-min intervals. If no symptoms appeared during the challenge or 2-h follow-up, the infant received the same formula 400–600 ml daily at home for the next 4 days, unless an adverse reaction appeared. A washout period of 2–9 days followed. The challenge protocol was then repeated with the second formula. We compared the symptom diaries of the two challenge periods and decided on the symptomatic challenge period. We then opened the milk code. CMA diagnosis

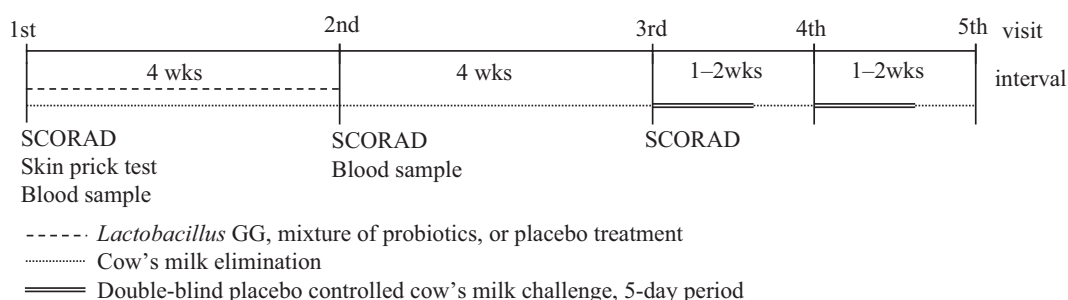


Figure 1 Protocol of the clinical trial.

Table 1 Clinical characteristics of the study population

	DBPC CM challenge		CM SPT	
	Positive	Negative	Positive	Negative
N	46	49	24	71
Age, months median (range)	5.6 (2.4–11)	6.6 (2.4–11)	6.1 (3.6–11)	5.6 (2.4–11)
Male N (%)	25 (54)	31 (63)		
Length of exclusive breastfeeding, months median (range)	1.6 (0–5.0)	0.75 (0–4.5)	2.3 (0.1–5.0)*	0.75 (0–4.5)
SCORAD at 1st visit mean (s.d.)	34 (14)	33 (15)	31 (14)	35 (15)
SCORAD at CM challenge, mean (s.d.)	14 (10)	12 (8)	12 (11)	13 (8)
CM IgE kU/L median (range)	0.1 (0–21)*	0.1 (0–2.1)	0.1 (0–21)*	0.1 (0–2.1)
Positive egg SPT N (%)	22 (48)	17 (35)	14 (58) †	25 (35)

CM, cow's milk; DBPC, double blind placebo controlled; SPT, skin prick test; SCORAD, Scoring of Atopic Dermatitis. *Denotes $p < 0.05$, Mann Whitney non-parametric test. †Denotes $p < 0.05$, χ^2 test.

was based on symptoms (urticaria, worsening of eczema, vomiting, diarrhea, physician-diagnosed wheezing, allergic rhinitis, and/or conjunctivitis) during the challenge with CM-containing formula.

Measurement of antigen-specific antibodies in serum samples

We had serum samples available from 95 infants, retrieved at the first visit to the clinic. Samples were stored at -80°C until analyzed. Cow's milk-specific IgE antibodies in sera were measured with enzymatic fluoroimmunoassay Pharmacia CAP system (Pharmacia Ltd, Uppsala, Sweden). Scarcity of samples prevented us from measuring β -lactoglobulin- and α -casein-specific IgE levels.

Serum antibodies of IgA, IgG, IgG1, and IgG4 isotypes to bovine- β -lactoglobulin and α -casein were measured with enzyme-linked immunosorbent assays (ELISA). Antigen-specific IgA and IgG were measured as previously described (18). Levels of antigen-specific IgG4 and IgG1 were measured with an ELISA protocol described in Savilahti et al., 2010 (15). The anti-human IgG4 antibody (clone MH164-4, HP6196) was purchased from Sanquin (Amsterdam, the Netherlands) and anti-human IgG1 antibody (clone HP6069) from Zymed (San Francisco, CA, USA). Values were expressed as arbitrary units (AU) deduced from the optical densities of the reference serum curve with a high level of antibodies after subtracting the blanks. The reference serum was a separate pool of sera collected for IgA and IgG isotypes; its concentration was assigned to 100 AU. Detection limit was 0.1 AU for the following antibody measurements (number of samples below detection limit, total $n = 95$): β -lactoglobulin-specific IgA (7), IgG (2), IgG1 (18), IgG4 (32), α -casein-specific IgG (1), IgG1 (16), IgG4 (42), and ovalbumin-specific IgG4 (20). Detection limit was 0.2 AU for α -casein-specific IgA (1) and ovalbumin-specific IgA (41) and IgG (22). For ovalbumin-specific IgG1, the limit was 0.3 AU (40).

Statistical analysis

As data on antibody levels (IgE to CM and IgA, IgG, IgG4, IgG1 to β -lactoglobulin, α -casein, and ovalbumin) were skewed, we performed logarithmic transformations. Values under detection level were computed as lowest detection level divided by two. We calculated two measures of CM-specific IgE/IgG4 balance: the ratio of CM-specific IgE level to either β -lactoglobulin or α -casein-specific IgG4 levels. On the basis of our previous studies, both β -lactoglobulin or α -casein-specific antibody levels closely correlate with specific antibody levels to whole CM. We compared antibody levels, measures of CM-specific IgE/IgG4 balance as well as continuous clinical variables between groups with Mann–Whitney non-parametric test. We applied chi-squared test for comparing dichotomic clinical variables. We considered a p -value of 0.05 or less statistically significant. Statistical analysis was performed with SPSS 15.0.1 for MicroSoft Windows.

Results

Statistics of clinical variables are depicted in Table 1. Infants with positive CM SPT were exclusively breast-fed longer than those with negative CM SPT, whereas CMA was not associated with the length of exclusive breast-feeding (Table 1). CM-specific IgE response was associated with CMA and positive SPT to hen's egg (Table 1). However, 59 % of CMA cases had negative CM SPT, and on the other hand, 10% CM SPT positive cases were not diagnosed with CMA (Table 1). CMA diagnosis was not significantly associated with probiotics or placebo treatment ($p > 0.07$, χ^2 test); the frequency of positive DBPC CM challenges in LGG group was 14/31 (45%) and MIX 21/33 (64%) and in the placebo group was 11/31 (35%).

Infants with CMA had significantly lower β -lactoglobulin-specific IgG4 levels, and they showed a tendency to lower

α -casein-specific IgG4 levels, compared with infants with negative CM challenge (Table 2). Positive CM SPT was associated with low α -casein-specific IgG4 levels (Table 2). Also in the placebo group alone similar, statistically significant ($p < 0.02$, Mann–Whitney test) differences were observed: among infants with CMA ($n = 11$) β -lactoglobulin-specific IgG4 levels were 0.13 (geometric mean), 0.05–0.31 (95 % confidence interval) and among infants with negative CM challenge ($n = 20$) 0.65, 0.25–1.65. Neither β -lactoglobulin and α -casein-specific IgG, IgG1, IgA levels nor ovalbumin-specific IgG, IgG1, IgG4, IgA levels differed significantly between these groups (data not shown). None of the measured antibody levels differed between IgE-mediated and non-IgE-mediated CMA (data not shown).

We calculated two measures of CM-specific IgE/IgG4 balance: the ratio of CM-specific IgE level to either β -lactoglobulin or α -casein-specific IgG4 levels. Both measures of CM-specific IgE/IgG4 balance differed significantly between infants with CMA and those with negative CM challenge: Infants with CMA had higher ratio of specific IgE to IgG4 (Fig. 2). Similar, statistically significant ($p < 0.03$, Mann–Whitney test) difference was also seen in the placebo group alone for the ratio of CM IgE to β -lactoglobulin IgG4.

Infants who were exclusively breast-fed for <1 month had higher ($p = 0.001$, Mann–Whitney test) β -lactoglobulin-specific IgG4 levels (geometric mean, 0.5; 95 % confidence interval, 0.3–0.8; $n = 46$) than those who were exclusively breast-fed for 1 month or longer (0.1; 0.09–0.2; $n = 49$). Similar difference was observed in α -casein-specific IgG4 levels (0.3; 0.2–0.4 vs. 0.1; 0.07–0.1, $p = 0.001$).

Infants with positive SPT to hen's egg had higher levels of specific IgG to ovalbumin, β -lactoglobulin, and α -casein as well as IgA to α -casein (Table 3). In IgG4 and IgG1 subclasses separately, no significant difference was observed (data not shown).

Infants who were atopic had higher levels of ovalbumin-specific IgG compared with non-atopy (geometric mean, 9.0; 95 % confidence interval, 4.3–19; $n = 58$, vs. 1.1; 0.5–2.5; 37, Mann–Whitney test p -value of 0.0002). Hen's egg SPT was positive in 39 (67%) of infants with any sensitization, that is, atopy.

The severity of AD as measured with SCORAD was not associated with any of the specific antibody levels we measured (data not shown).

Discussion

Our study population consisted of infants who had eczema associated with the consumption of CM. We showed that infants who were diagnosed with CMA based on a DBPC CM oral challenge had lower β -lactoglobulin IgG4 levels compared to infants with a negative challenge. They, furthermore, had higher ratio of CM-specific IgE to β -lactoglobulin- and α -casein-specific IgG4 antibodies. Positive CM SPT was also associated with lower β -lactoglobulin and α -casein IgG4 levels. Specific IgG to β -lactoglobulin, α -casein, and ovalbumin levels, on the other hand, was higher in infants with positive SPT to hen's egg.

All infants in our study underwent a DBPC oral CM challenge. Prior to the oral challenge, infants and their breastfeeding mothers followed a rigorous elimination diet. Thus, the CMA diagnoses are most reliable. Serum samples were collected at the first visit at our clinic before any intervention, that is prior to elimination diet, probiotic treatment, and DBPC oral CM challenge. As spontaneous recovery is characteristic of CMA (3), we cannot exclude the possibility that infants who had negative oral CM challenge would not have had a very transient form of CMA. We might speculate, however, that most cases of CMA were diagnosed at an early stage given the age at diagnosis, that is, 12 months or under. Furthermore, a relatively large proportion of infants who had a positive DBPC CM oral challenge had no detectable IgE-mediated response to CM. This may reflect diagnosis at an early stage of CMA when IgE response has not yet fully developed (3). The availability of serum samples limited the number of subjects to 95 of the original cohort of 230. Clinically the study population corresponded well to the original cohort (17). Both in the original clinical trial (17) and in the current subpopulation, the probiotics treatments showed no effect on the outcome of DBPC CM oral challenge.

The result that low specific IgG4 levels to CM proteins are associated with CM sensitization and CMA is in line with the concept that specific IgG4 antibodies are associated with tolerance to food allergens (13, 14). We furthermore showed that in infants with eczema and CMA, the ratio of specific IgE to IgG4 was higher than in eczematous infants without CMA. The result conforms with the studies on natural course of food allergy as well on specific immunotherapy. A shift in the balance between specific IgE and IgG4 antibodies occurs

Table 2 β -lactoglobulin (BLG) IgG4 levels in infants with or without cow's milk allergy (CMA) and α -casein (CAS) IgG4 levels in infants with positive or negative skin prick test (SPT) to cow's milk (CM)

	BLG IgG4 geometric mean (95% confidence interval) AU	p Mann Whitney	CAS IgG4 geometric mean (95% confidence interval) AU	p Mann Whitney
CMA ($n = 46$)	0.14(0.09–0.22)	0.004	0.12(0.08–0.16)	0.06
No CMA ($n = 49$)	0.42(0.25–0.70)		0.23(0.14–0.35)	
CM SPT positive ($n = 24$)	0.13(0.07–0.26)	0.06	0.11(0.06–0.18)	0.04
CM SPT negative ($n = 71$)	0.30(0.07–0.26)		0.19(0.05–0.27)	

The specific IgG4 levels are expressed as arbitrary units (AU). p -values are based on non-parametric Mann Whitney test.

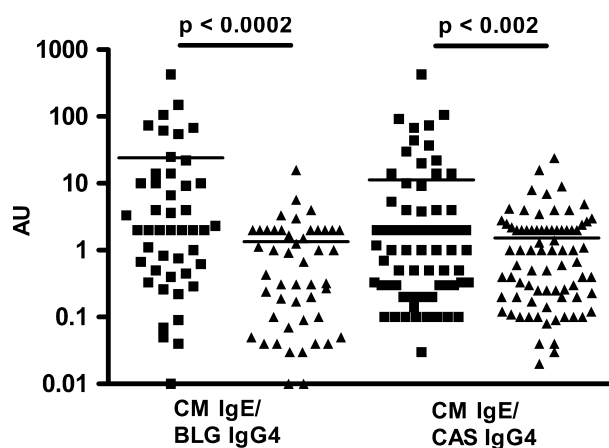


Figure 2 Ratio of cow's milk (CM) specific IgE to β -lactoglobulin (BLG) IgG4 and to α -casein (CAS) levels in infants with (■) or without (▲) cow's milk allergy. These measures of IgE/IgG4 levels are expressed as arbitrary units (AU) on a logarithmic scale. p-values are based on non-parametric Mann Whitney test. Bars denote the mean.

in the natural development of tolerance in CMA (15, 19) and in hen's egg allergy (20). Studies on specific oral immunotherapy also show that specific IgG4 antibody levels increase when desensitization to the allergen is achieved (21, 22).

Diagnosis of CMA currently requires a controlled, preferably DBPC, oral challenge (23). Finding laboratory methods for distinguishing CMA cases from other CM intolerance would reduce the burden on patients and the risk of anaphylaxis. Screening of IgE and IgG4 antibodies to CM epitopes may assist in diagnostics and prognostics (19, 24). Measuring immunoglobulin free light chains may also contribute to allergy diagnostics (25). Currently, however, CM SPT and specific CM IgE remain the main laboratory tests available in the clinical practice (7, 26). Relying on specific CM IgE measurement risks labeling mere sensitization as allergy (1, 6). On the other hand, also non-atopic infants may transiently produce allergen-specific IgE antibodies (27). CM IgE measurement leaves non-IgE-mediated CMA undetected. Moreover, IgE response may develop slowly during clinical allergy and be detected only at the later stages of CMA (3). In the current study, 59% of DBPC oral challenge verified CMA cases had negative CM SPT, whereas 10% of those with

positive CM SPT had negative oral challenge. Atopy patch test has been suggested to help in identifying CMA in patients with eczema, but it is of limited clinical value (6, 28).

As a working group of European Academy of Allergy and Clinical Immunology has stated, detectable specific IgG4 antibodies do not indicate food allergy, but rather exposure and possibly tolerance to the antigen (29). The position paper concludes that specific IgG4 should not be used in allergy diagnostics (29). In our study, exposure to CM did not explain differences in CM protein-specific IgG4 levels, whereas the data do support the role of specific IgG4 in the maintenance of tolerance. Specific IgG4 levels and IgE/IgG4 balance differed between infants with positive or negative CM challenge. More research on study populations meeting stringent CMA diagnostic criteria, preferably DBPC oral challenge verified diagnosis, is required to assess whether measuring specific IgG4 levels in addition to specific IgE in an individual patient has clinical value. In this study, the scarcity of serum samples drawn from infants prevented us from measuring IgE and IgG4 antibodies to the same CM antigens. We therefore settled for a ratio of CM IgE to β -lactoglobulin and α -casein IgG4.

Previous studies have reported that early exposure to CM is associated with pronounced IgG responses to CM proteins (30, 31). We have observed in another cohort that even when low β -lactoglobulin- and α -casein-specific IgG1, IgG4, and IgG levels were associated with IgE-mediated CMA, within the group of infants with IgE-mediated CMA, early exposure to CM was associated with higher β -lactoglobulin- and α -casein-specific IgG4 levels (32). Results of the current study were in line with these previous reports. As early exposure to CM is a possible etiological factor in CMA (33), we would not treat it as a confounding factor in statistical analyses. In the current study, the length of exclusive breast-feeding did not significantly differ between infants with positive or negative DBPC CM challenge.

In addition to CM sensitization, we looked at hen's egg sensitization, which often reflects atopic disposition more broadly (3, 34). The observation that positive SPT to hen's egg is associated with specific IgG to β -lactoglobulin, α -casein, and ovalbumin levels is in line with a study we published recently (35). It was based on another pediatric population and showed that atopy was associated with elevated levels of IgG and IgA antibodies to ovalbumin (35). Earlier reports on similar findings also point to the association of sensitization with high specific IgG antibody levels

Table 3 Ovalbumin (OVA), β -lactoglobulin (BLG) and α -casein (CAS) specific total IgG levels, and α -casein specific IgA levels in infants with positive or negative skin prick test (SPT) to hen's egg

	OVA IgG	BLG IgG	CAS IgG	CAS IgA
	Geometric mean (95% confidence interval) AU			
Hen's egg SPT positive (n = 39)	29 (15–58)	5.3 (3.5–8.2)	7.0 (4.6–10.4)	4.3 (2.8–6.6)
Hen's egg SPT negative (n = 56)	1.0 (0.5–2.0)	2.2 (1.3–3.2)	3.1 (1.9–5.1)	2.3 (1.6–3.5)
p	<0.0001	0.01	0.04	0.03

The specific antibody levels are expressed as arbitrary units (AU). p-values are based on non-parametric Mann Whitney test.

(36, 37). This association may be due to increased gut permeability, which results in high presence of food antigens in the gut immunological system and the generation of an IgG and IgA response. A further explanation may be that individuals prone to mount an IgE response to food antigens also have a pronounced general humoral response to these antigens (38).

Our study supports the role of specific IgG4 antibodies in the maintenance of tolerance to food allergens. The clinical implication of our data is that CM-specific IgG4 antibodies may assist in CMA diagnostics, especially in cases where

eczema is the only symptom and/or specific CM IgE antibodies are not detected.

Acknowledgments

We are grateful to Professor Tari Haahtela from the Skin and Allergy Hospital, University of Helsinki, for his valuable contribution. We warmly thank Elsa Valtonen for her excellent technical assistance in antibody measurements. We thank Helsinki University Central Hospital Research Funds for funding the study.

References

- Breuer K, Heratizadeh A, Wulf A, et al. Late eczematous reactions to food in children with atopic dermatitis. *Clin Exp Allergy* 2004; **34**: 817–24.
- Werfel T, Ballmer-Weber B, Eigenmann PA, et al. Eczematous reactions to food in atopic eczema: position paper of the EAACI and GA2LEN. *Allergy* 2007; **62**: 723–8.
- Saarinne KM, Pelkonen AS, Makela MJ, Savilahti E. Clinical course and prognosis of cow's milk allergy are dependent on milk-specific IgE status. *J Allergy Clin Immunol* 2005; **116**: 869–75.
- Bindslev-Jensen C, Ballmer-Weber BK, Bengtsson U, et al. Standardization of food challenges in patients with immediate reactions to foods—position paper from the European Academy of Allergology and Clinical Immunology. *Allergy* 2004; **59**: 690–7.
- Niggemann B, Beyer K. Diagnostic pitfalls in food allergy in children. *Allergy* 2005; **60**: 104–7.
- Saarinne KM, Suomalainen H, Savilahti E. Diagnostic value of skin-prick and patch tests and serum eosinophil cationic protein and cow's milk-specific IgE in infants with cow's milk allergy. *Clin Exp Allergy* 2001; **31**: 423–9.
- Celik-Bilgili S, Mehl A, Verstege A, et al. The predictive value of specific immunoglobulin E levels in serum for the outcome of oral food challenges. *Clin Exp Allergy* 2005; **35**: 268–73.
- Sletten GB, Halvorsen R, Egaas E, Halstensen TS. Changes in humoral responses to beta-lactoglobulin in tolerant patients suggest a particular role for IgG4 in delayed, non-IgE-mediated cow's milk allergy. *Pediatr Allergy Immunol* 2006; **17**: 435–43.
- Noh G, Ahn HS, Cho NY, Lee S, Oh JW. The clinical significance of food specific IgE/IgG4 in food specific atopic dermatitis. *Pediatr Allergy Immunol* 2007; **18**: 63–70.
- Hochwallner H, Schulmeister U, Swoboda I, et al. Patients suffering from non-IgE-mediated cow's milk protein intolerance cannot be diagnosed based on IgG subclass or IgA responses to milk allergens. *Allergy* 2011; **66**: 1201–7.
- Aalberse RC, Stapel SO, Schuurman J, Rispens T. Immunoglobulin G4: an odd antibody. *Clin Exp Allergy* 2009; **39**: 469–77.
- Satoguina JS, Weyand E, Larbi J, Hoerauf A. T regulatory-1 cells induce IgG4 production by B cells: role of IL-10. *J Immunol* 2005; **174**: 4718–26.
- Ruiter B, Knol EF, van Neerven RJ, et al. Maintenance of tolerance to cow's milk in atopic individuals is characterized by high levels of specific immunoglobulin G4. *Clin Exp Allergy* 2007; **37**: 1103–10.
- Tomicic S, Norrman G, Falth-Magnusson K, Jenmalm MC, Devenney I, Bottcher MF. High levels of IgG(4) antibodies to foods during infancy are associated with tolerance to corresponding foods later in life. *Pediatr Allergy Immunol* 2008; **20**: 35–41.
- Savilahti EM, Saarinne KM, Savilahti E. Duration of clinical reactivity in cow's milk allergy is associated with levels of specific immunoglobulin G4 and immunoglobulin A antibodies to beta-lactoglobulin. *Clin Exp Allergy* 2010; **40**: 251–6.
- Cerutti A, Chen K, Chorny A. Immunoglobulin responses at the mucosal interface. *Annu Rev Immunol* 2011; **29**: 273–93.
- Viljanen M, Savilahti E, Haahtela T, et al. Probiotics in the treatment of atopic eczema/dermatitis syndrome in infants: a double-blind placebo-controlled trial. *Allergy* 2005; **60**: 494–500.
- Savilahti E, Saukkonen TT, Virtala ET, Tuomilehto J, Akerblom HK. Increased levels of cow's milk and beta-lactoglobulin antibodies in young children with newly diagnosed IDDM. The Childhood Diabetes in Finland Study Group. *Diabetes Care* 1993; **16**: 984–9.
- Savilahti EM, Rantanen V, Lin JS, et al. Early recovery from cow's milk allergy is associated with decreasing IgE and increasing IgG4 binding to cow's milk epitopes. *J Allergy Clin Immunol* 2010; **125**: 1315–21.e9.
- Lemon-Mule H, Sampson HA, Sicherer SH, Shreffler WG, Noone S, Nowak-Wegrzyn A. Immunologic changes in children with egg allergy ingesting extensively heated egg. *J Allergy Clin Immunol* 2008; **122**: 977–83.e1.
- Skrupak JM, Nash SD, Rowley H, et al. A randomized, double-blind, placebo-controlled study of milk oral immunotherapy for cow's milk allergy. *J Allergy Clin Immunol* 2008; **122**: 1154–60.
- Pajno GB, Caminiti L, Ruggeri P, et al. Oral immunotherapy for cow's milk allergy with a weekly up-dosing regimen: a randomized single-blind controlled study. *Ann Allergy Asthma Immunol* 2010; **105**: 376–81.
- Niggemann B, Beyer K. Pitfalls in double-blind, placebo-controlled oral food challenges. *Allergy* 2007; **62**: 729–32.
- Wang J, Lin J, Bardina L, et al. Correlation of IgE/IgG4 milk epitopes and affinity of milk-specific IgE antibodies with different phenotypes of clinical milk allergy. *J Allergy Clin Immunol* 2010; **125**: 695–702, 702.e1–702.e6.
- Schouten B, van Esch BC, van Thuijl AO, et al. Contribution of IgE and immunoglobulin free light chain in the allergic reaction to cow's milk proteins. *J Allergy Clin Immunol* 2010; **125**: 1308–14.
- Verstege A, Mehl A, Rolinck-Werninghaus C, et al. The predictive value of the skin prick test wheal size for the outcome of oral food challenges. *Clin Exp Allergy* 2005; **35**: 1220–6.
- Hattevig G, Kjellman B, Bjorksten B. Appearance of IgE antibodies to ingested and inhaled allergens during the first 12 years of life in atopic and non-atopic children. *Pediatr Allergy Immunol* 1993; **4**: 182–6.
- Turjanmaa K, Darsow U, Niggemann B, Rance F, Vanto T, Werfel T. EAACI/GA2LEN position paper: present status of the atopy patch test. *Allergy* 2006; **61**: 1377–84.
- Stapel SO, Asero R, Ballmer-Weber BK, et al. Testing for IgG4 against foods is not recommended as a diagnostic tool: EAACI Task Force Report. *Allergy* 2008; **63**: 793–6.

30. Jenmalm MC, Bjorksten B. Exposure to cow's milk during the first 3 months of life is associated with increased levels of IgG subclass antibodies to beta-lactoglobulin to 8 years. *J Allergy Clin Immunol* 1998; **102**: 671–8.
31. Juvonen P, Mansson M, Kjellman NI, Bjorksten B, Jakobsson I. Development of immunoglobulin G and immunoglobulin E antibodies to cow's milk proteins and ovalbumin after a temporary neonatal exposure to hydrolyzed and whole cow's milk proteins. *Pediatr Allergy Immunol* 1999; **10**: 191–8.
32. Savilahti EM, Saarinen KM, Savilahti E. Specific antibodies to cow's milk proteins in infants: effect of early feeding and diagnosis of cow's milk allergy. *Eur J Nutr* 2010; **49**: 501–4.
33. Saarinen KM, Juntunen-Backman K, Jarvenpaa AL, et al. Supplementary feeding in maternity hospitals and the risk of cow's milk allergy: A prospective study of 6209 infants. *J Allergy Clin Immunol* 1999; **104**: 457–61.
34. Nickel R, Kulig M, Forster J, et al. Sensitization to hen's egg at the age of twelve months is predictive for allergic sensitization to common indoor and outdoor allergens at the age of three years. *J Allergy Clin Immunol* 1997; **99**: 613–7.
35. Kukkonen AK, Savilahti EM, Haahtela T, Savilahti E, Kuitunen M. Ovalbumin-specific immunoglobulins A and G levels at age 2 years are associated with the occurrence of atopic disorders. *Clin Exp Allergy* 2011; **41**: 1414–21.
36. Jenmalm MC, Bjorksten B. Development of immunoglobulin G subclass antibodies to ovalbumin, birch and cat during the first eight years of life in atopic and non-atopic children. *Pediatr Allergy Immunol* 1999; **10**: 112–21.
37. Rowntree S, Cogswell JJ, Platts-Mills TA, Mitchell EB. Development of IgE and IgG antibodies to food and inhalant allergens in children at risk of allergic disease. *Arch Dis Child* 1985; **60**: 727–35.
38. Vance GH, Thornton CA, Bryant TN, Warner JA, Warner JO. Ovalbumin-specific immunoglobulin G and subclass responses through the first 5 years of life in relation to duration of egg sensitization and the development of asthma. *Clin Exp Allergy* 2004; **34**: 1542–9.