

ORIGINAL ARTICLE

Duration of clinical reactivity in cow's milk allergy is associated with levels of specific immunoglobulin G4 and immunoglobulin A antibodies to β -lactoglobulin

E. M. Savilahti, K. M. Saarinen and E. Savilahti

Hospital for Children and Adolescents, University of Helsinki, Helsinki, Finland

Clinical & Experimental Allergy

Summary

Background The development of tolerance in IgE-mediated allergies has been associated with lower cow's milk (CM)-specific IgE levels, increasing levels of specific IgG4 and, more contestably, IgA.

Objective We investigated whether specific antibody responses to CM proteins differ over time between patients who recovered from cow's milk allergy (CMA) by the age of 3 years and those who developed tolerance only after the age of 8 years.

Methods The study population comprised of 83 patients with IgE-mediated CMA. They belonged to a cohort of 6209 healthy, full-term infants followed prospectively for the emergence of CMA. Serum samples were available at diagnosis (median age 7 months), 1 year later (median 19 months) and at follow-up (median 8.5 years). Age-matched control subjects with no history of CMA ($n = 76$) participated in the follow-up. Serum levels of IgE antibodies to CM were measured using UniCAP. Levels of IgA, IgG1 and IgG4 antibodies to β -lactoglobulin and α -casein were measured using ELISA.

Results Patients with persistent CMA at the age of 8 years ($n = 18$ at diagnosis, $n = 16$ at later time-points) had higher CM-specific IgE levels at all three time-points ($P < 0.001$) compared with patients who became tolerant by 3 years ($n = 55$ at diagnosis, $n = 54$ a year later, $n = 40$ at follow-up). They had lower serum IgA levels to β -lactoglobulin at diagnosis ($P = 0.01$), and lower IgG4 levels to β -lactoglobulin ($P = 0.04$) and α -casein ($P = 0.05$) at follow-up.

Conclusion High CM-specific IgE levels predict the persistence of CMA. Development of tolerance is associated with elevated levels of β -lactoglobulin-specific serum IgA at the time of diagnosis, and later increasing specific IgG4 levels to β -lactoglobulin and α -casein.

Keywords cow's milk allergy, IgA, IgG4, tolerance, α -casein, β -lactoglobulin

Submitted 15 July 2009; revised 22 September 2009; accepted 13 October 2009

Correspondence:

E. Savilahti, Hospital for Children and Adolescents, University of Helsinki, PO Box 281, Fin-00029 HUS, Finland.
E-mail: erkki.savilahti@hus.fi

Introduction

Cow's milk allergy (CMA) affects 2–3% of infants [1, 2]. Most children recover from CMA by the age of 3–4 years, but the allergy persists until school age in 10–20% of patients [1, 3]. While both atopic and non-atopic children produce low levels of IgE, IgG and IgA antibodies to food antigens, sensitization entails the production of higher specific IgE antibody levels [4, 5]. Patients with persisting CMA tend to have higher levels of IgE antibodies to cow's milk (CM) proteins than patients who recover early [6, 7].

Antibodies of the IgG4 subclass seem to associate with the development of tolerance. Low levels of IgG4 to β -

lactoglobulin and/or OVA indicated the need for prolonged elimination diet among children with CM and/or egg allergy [8]. Individuals tolerating CM had higher CM-specific IgG4 levels than those with allergic reactions to CM [9]. In patients with hen's egg allergy, an increase in OVA-specific IgG4 and a decrease in specific IgE levels were concurrent with the natural development of clinical tolerance to the allergen [10]. Data from immunotherapeutic settings are consistent with the observations from natural settings in food allergy, although they mostly address desensitization rather than long-term tolerance. Meglio et al. [11], however, followed children for over 4 years after oral desensitization to CM and reported

long-term tolerance to be associated with decreased specific IgE levels. In a study on patients with CMA but asymptomatic to heated CM, an increase in casein-specific IgG4 levels was observed after a 3-month period of consuming heated CM, whereas specific IgE levels did not change [12]. Similarly, during immunotherapy to CM that elicited desensitization, specific IgG, most notably IgG4, levels increased while specific IgE levels remained at baseline levels [13]. During peanut-allergy immunotherapy, desensitization was associated with increased specific IgG4 levels and decreased specific IgE levels [14].

The development of tolerance may also involve IgA antibodies, although the evidence to date is controversial. The relatively high prevalence of allergies among patients with IgA deficiency [15] advocates a role for IgA antibodies in the protection against allergies and/or in the development of tolerance. Studies on grass pollen immunotherapy reported that as allergic symptoms improved, specific IgA [16] or IgA2 [17] levels in serum increased. In a population of patients with IgE-mediated CMA, CM-specific IgE levels correlated positively with levels of specific Igs of other isotypes (IgA, IgG1, IgG4) [18]. Böttcher *et al.* [5] observed that infants who developed allergy had higher salivary levels of total and allergen-specific IgA antibodies, whereas sensitized infants without allergic symptoms had higher levels of secretory IgA.

Most studies on IgG4 and IgA antibody responses during the transition from allergy to clinical tolerance have investigated small numbers of patients undergoing immunotherapy. We approached the issue with a patient population ($n=83$) from a prospective study on the natural course of CMA. We investigated how antibody levels to β -lactoglobulin and α -casein developed over time in patients with IgE-mediated CMA, and whether they differed between patients who became tolerant to CM by the age of 3 years and those with persistent CMA at the age of 8 years.

Methods

Study population

We examined blood and salivary samples from 83 children with IgE-mediated CMA, who have been previously described [3]. Informed and written consent was received from all participants and their parents before any tests were performed. The Ethics Committee of the Hospital for Children and Adolescents, University of Helsinki, approved of the study.

Subjects were part of a population-based cohort of 6209 full-term newborn infants recruited between August 1994 and November 1995 to prospectively study the emergence of CMA [2]. An open oral challenge after a 2-week elimination period of CM confirmed CMA in 118 children at a mean age of 7 months [2]. CMA was

Table 1. Age and number of patients' serum samples at time points investigated within three study groups (patients who became tolerant to cow's milk by age 3 years, or at 3–8 years, and patients who had persistent cow's milk allergy at 8 years)

	Age median (range); N		
	Tolerant by 3 years	Tolerant at 3–8 years	Persistent at 8 years
At diagnosis (months)	6.4 (2.8–12); 55	6.4 (3.0–13); 10	7.0 (3.0–11); 18
1 year after diagnosis (months)	19 (15–24); 54	19 (16–24); 11	19 (12–23); 18
Follow-up (years)	8.7 (8.0–9.1); 40	8.8 (8.1–9.1); 10	8.5 (8.0–9.0); 18

considered IgE-mediated if skin prick test (SPT) with liquid CM formula (weal diameter ≥ 3 mm greater than the negative control) or specific IgE antibodies to whole CM (≥ 0.7 kU/L measured with UniCap, Phadia, Uppsala, Sweden) or both were positive at any time-point 0–12 months after diagnosis [3]. Negative control for the SPT was 50% glycerol solution (Soluprick, ALK, Denmark) and positive control was histamine dihydrochloride (10 mg/mL). We followed the 118 children at an outpatient clinic every 6–24 months until recovery, which was defined as regular use of CM before the visit or as having a negative open oral challenge. We furthermore invited the patients in a time frame from August 2003 to March 2004 for a follow-up study on their CMA status and other atopic manifestations; 94 (80%) patients participated [3]. Clinical data on the patients were available from previous studies on the population [2, 3, 19].

For the current study, we divided the patients with IgE-mediated CMA into three groups based on the pace of clinical recovery: patients (1) who became tolerant by age 3, (2) or by age 3–8 and (3) who had persistent CMA at 8 years (Table 1). Most patients had skin symptoms at the first, diagnostic CM challenge; the reaction was immediate (< 24 h) in approximately half of the patients (Table 2).

We studied the serum samples available collected at the time of diagnosis, a year after and at the final follow-up (Table 1). In addition, samples were available from 76 out of 80 control subjects from the same cohort who participated in the follow-up study (median age 8.6; range 8.1–9.3). Salivary samples were collected at the time of diagnosis before the CM challenge. They ($n=81$) were available from 54 patients tolerant by 3 years, 11 tolerant by 3–8 years and 16 persistent at 8 years.

Measurement of antibodies

Serum and non-stimulated salivary samples were stored at -80°C until analysed.

Table 2. Clinical symptoms, cumulative volume of cow's milk (CM) that elicited symptoms and reaction time at the diagnostic oral cow's milk challenge in three study groups (patients who became tolerant to cow's milk by age 3 years, or at 3–8 years and patients who had persistent cow's milk allergy at 8 years)

	N (%)		
	Tolerant by 3 years	Tolerant at 3–8 years	Persistent at 8 years
Skin	52 (93)	10 (91)	18 (100)
Gastrointestinal	8 (15)	2 (18)	0 (0)
Respiratory	3 (5.5)	2 (18)	5 (28)
Anaphylaxis	0 (0)	1 (9.1)	0 (0)
Cumulative volume of CM < 20 mL	28 (51)	5 (45)	12 (67)
Cumulative volume of CM > 200 mL	7 (13)	1 (9)	4 (22)
Reaction time > 24 h	6 (11)	0 (0)	4 (22)

CM-specific IgE antibodies in sera were measured using enzymatic UniCap fluoroimmunoassay (Phadia) [19].

Serum antibodies of IgA, IgG1 and IgG4 isotypes to bovine- β -lactoglobulin and α -casein were measured using ELISA. The method was also applied for measuring CM-specific IgA levels in saliva. Protocols for antigen-specific IgA and IgG measurement have been described previously [20, 21]. Levels of antigen-specific IgG4 and IgG1 were measured with a protocol adapted from Ruiter et al. [9]. Microtitre plates (Nunc Maxi Immunoplate, Thermo Fisher Scientific, Roskilde, Denmark) were coated either with bovine- β -lactoglobulin (Sigma-Aldrich, St Louis, MO, USA) at a concentration of 1 μ g/mL, or with bovine- α -casein (Sigma-Aldrich) at a concentration of 2 μ g/mL in phosphate-buffered saline (PBS) and incubated at 4 °C overnight. After washing with PBS, a blocking solution of 2% sheep serum in PBS was applied to the plates, followed by an incubation of 1 h at 37 °C and washing with PBS–0.05% Tween (PBS–Tw). Sera were diluted to 1 : 20 in 1% sheep serum in PBS–Tw, and applied in duplicate to the antigen-coated plates and to control plates (coated only with blocking solution). The plates were incubated for 1 h at 37 °C. After washing, 100 μ L of horseradish peroxidase (HRP)-conjugated anti-human IgG4 (Sanquin, Amsterdam, the Netherlands) at dilutions of 1 : 30 000 for α -casein or 1 : 10 000 for β -lactoglobulin, or anti-human IgG1 (Zymed Laboratories, San Francisco, CA, USA) at a dilution of 1 : 1000 was applied to wells, followed by an incubation of 1 h at 37 °C. After washing, 100 μ L of tetramethyl-benzidine (TMB) liquid substrate (Sigma-Aldrich) was applied to the plates and incubated, excluded from light, for 10 min at room temperature. The reaction was stopped with 1 M H₃PO₄, and the optical density of the end product was immediately measured at 450 nm with a semiautomatic photometer (Labsystems Multiscan, Frankfurt, Germany). 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.

Statistical analysis

Logarithmic transformations of antibody levels (IgE to CM and IgA, IgG4, IgG1 to β -lactoglobulin and α -casein) were compared with one-way ANOVA between the three patient groups, and including control subjects at follow-up. The comparison between the two extreme patient groups (tolerant by 3 years and persistent at 8 years) was performed with *t*-test of independent variables. General linear model (GLM) for repeated measures was applied to test whether antibody levels changed significantly over time and whether the temporal profiles of antibody levels differed between the groups. Spearman's correlation was calculated for specific IgA levels in serum and salivary samples. A *P*-value of 0.05 or less was considered statistically significant. Statistical analysis was performed with SPSS 15.0.1 for MicroSoft Windows.

Results

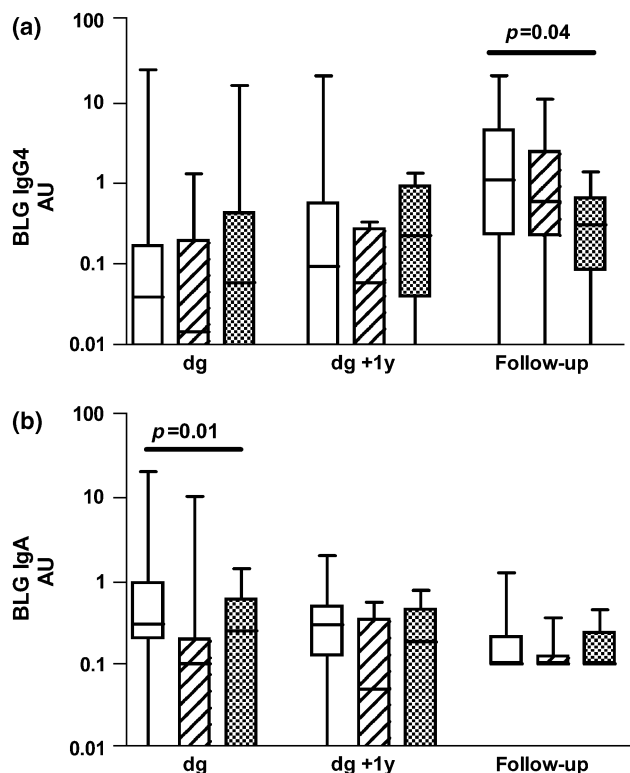
Children with persistent CMA at the age of 8 years had higher levels of CM-specific IgE at all three time-points compared with those who became tolerant by the age of 3 years (Table 3, Fig. 1). GLM of repeated measures indicated both time (*P* < 0.001) and group (as designated in Table 3; *P* = 0.005) to be significant factors to the differences. Levels of CM-specific IgE were higher at follow-up in all children who had a history of IgE-mediated CMA including those with early recovery, compared with control subjects (Table 3).

In contrast to CM-specific IgE levels, serum IgG4 levels to β -lactoglobulin were lower at follow-up among patients with persistent CMA at age 8 than among patients tolerating CM by 3 (Fig. 1a) or 3–8 years, and also in comparison with control subjects (Table 4). Comparison of serum IgG4 levels with α -casein resulted in similar, although less pronounced, differences between groups (Table 4). Time and group were both significant factors to differences in temporal profiles of β -lactoglobulin IgG4 levels (GLM of repeated measures: *P* < 0.01); the levels increased more over time among patients who became tolerant by the age of 3 years than among those with persistent CMA at the age of 8 years (Fig. 1a). Levels of IgG1 to β -lactoglobulin and α -casein did not differ between the groups (data not shown).

Serum levels of IgA to β -lactoglobulin were higher at the time of diagnosis among patients who recovered from CMA by 3 years compared with both groups where recovery was delayed until either 3–8 years or beyond 8

Table 3. Cow's milk specific IgE levels in three groups (patients who became tolerant to cow's milk by age 3 years, or at 3–8 years, and patients who had persistent cow's milk allergy at 8 years) at diagnosis, one year after and at follow-up, and control subjects at follow-up

	Cow's milk-specific IgE geometric mean (95% confidence interval) kU/L; <i>N</i>				
	Tolerant by 3 years	Tolerant at 3–8 years	Persistent at 8 years	Control subjects	<i>P</i> one-way ANOVA
At diagnosis	1.0 (0.7–1.4); 55	5.4 (2.2–13); 10	6.2 (2.8–13); 18	NA	< 0.0001
1 year after diagnosis	0.7 (0.5–1.0); 54	2.4 (1.1–5.0); 11	10 (5.2–20); 16	NA	< 0.0001
Follow-up	0.2 (0.1–0.2); 40	0.3 (0.1–0.8); 10	2.6 (1.0–6.9); 16	0.1 (0.08–0.1); 76	< 0.0001

**Fig. 1.** Levels of β -lactoglobulin (BLG)-specific IgG4 (a) and IgA (b) in two of three groups: patients who recovered from cow's milk allergy (CMA) by the age of 3 years (blank boxes), patients who recovered at 3–8 years (boxes with diagonal lines) and those who still had persistent CMA at 8 years (checked boxes). The y-axis represents (a) IgG4, and (b) IgA levels to BLG in arbitrary units (AU). Measurements are shown on a log10 scale; lines mark (from bottom up) the 5th percentile, lower quartile, median, upper quartile and the 95th percentile. The x-axis depicts the three time-points under investigation: at diagnosis (dg), approximately 1 year after (dg + 1 year) and at follow-up. *P*-values: one-way ANOVA.

years (Table 4, Fig. 1b). The two groups with delayed recovery did not significantly differ from one another (Table 4, *t*-test *P*-value > 0.5). Analysis with GLM of repeated measures showed time to be a significant factor (*P* = 0.04), with a downward trend over time (Fig. 1b), whereas the temporal profiles of specific IgA levels did not differ significantly between the groups.

Salivary CM specific IgA levels at diagnosis correlated positively with serum β -lactoglobulin-specific IgA levels

at diagnosis (Spearman's correlation coefficient 0.4; *n* = 53; *P* < 0.005) and a year later (Spearman's correlation coefficient 0.4; *n* = 52; *P* < 0.03) among patients who recovered from CMA by the age of 3 years. The same observation held for all those who recovered by the age of 8 years (Spearman's correlation coefficient at diagnosis 0.4; *n* = 63, and a year later 0.3; *n* = 61; for both *P* < 0.001), but not for patients with persistent CMA at the age of 8 years (Spearman's correlation coefficient at diagnosis 0.4; *n* = 16; *P* = 0.2 and a year later –0.04; *n* = 14; *P* = 0.9). Levels of salivary IgA antibodies to CM did not differ between the groups at any time-point.

Discussion

Our study on children with IgE-mediated CMA had a prospective setting of the natural course of CMA, a relatively large number of patients (*n* = 83) belonging to a population-based cohort of 6209 infants, and a long follow-up to the median age of 8.5 years. The main objective was to compare, at three time-points, patients who recovered by age 3 years with patients who had persistent CMA at age 8 years. In addition, we had samples of control subjects from the same original cohort at follow-up (median age 8.6 years), but not at earlier time-points. For technical reasons, the specific IgA and IgG4 levels were measured in arbitrary units, and values for different antibodies are thus not directly comparable.

Our study corroborates the relation of higher CM-specific IgE levels with prolonged clinical reactivity to CM [6, 7]. In agreement with observations by Skripak et al. [7], patients with persisting CMA had higher CM IgE levels than patients with earlier recovery, at all time-points from diagnosis until follow-up.

The association of increased allergen-specific serum IgG4 levels with the development of tolerance confirms similar observations in patients with egg allergy [10] as well as during oral desensitization in CMA [12, 13] and in peanut allergy [14]. Because β -lactoglobulin and α -casein-specific IgG4 levels were still increased years after the patients had recovered from CMA, and the CM-specific IgE levels remained elevated compared with controls, our data endorse the role of specific IgG4 antibodies in the maintenance of oral tolerance [9]. A further explanation

Table 4. β -lactoglobulin (BLG) and α -casein (CAS) specific IgG4 at follow-up, and β -lactoglobulin-specific IgA levels at diagnosis in three groups (patients who became tolerant to cow's milk by age 3 years, or at 3–8 years, and patients who had persistent cow's milk allergy at 8 years) and control subjects

	BLG IgG4, CAS IgG4, BLG IgA geometric mean (95% confidence interval) AU; N				
	Tolerant by 3 years	Tolerant at 3–8 years	Persistent at 8 years	Control subjects	<i>P</i> one-way ANOVA
BLG IgG4 at follow-up	0.9 (0.5–1.7); 40	0.6 (0.2–2.0); 10	0.2 (0.1–0.4); 16	0.8 (0.5–1.2); 76	0.04
CAS IgG4 at follow-up	1.2 (0.6–2.5); 40	5.4 (1.5–19); 10	0.5 (0.2–1.5); 16	0.7 (0.4–1.2); 76	0.05
BLG IgA at diagnosis	0.4 (0.3–0.5); 54	0.1 (0.04–0.2); 10	0.1 (0.1–0.3); 18	NA	0.01

for the higher specific IgG4 levels in patients with early recovery may be that after clinical reactivity has diminished, children most often include CM in their diet.

Specific IgA levels to β -lactoglobulin in serum were higher at diagnosis among patients who recovered from CMA by the age of 3 years than among patients whose CMA persisted beyond the age of 8 years, which is consistent with findings that specific IgA or IgA2 levels increase in allergic patients successfully undergoing specific immunotherapy [16, 17]. Moreover, β -lactoglobulin-specific IgA levels in serum correlated positively with salivary levels of CM-specific IgA at diagnosis in patients who became tolerant to CM by age 3 years. These results challenge previous studies that have suggested differential production of serum and secretory-specific IgA antibodies in allergy. In a mouse model of food allergy, tolerance to β -lactoglobulin was associated with higher levels of antigen-specific secretory IgA in feces, but lower serum levels of specific IgA [22]. In human subjects either with pollen allergy or no atopy, the antigen moieties recognized by serum IgA differed from those recognized by secretory IgA in tears [23]. Our results, thus, entice further research on the functional relation between secretory IgA and circulating IgA antibodies in allergy.

An interesting difference in the kinetics of specific IgA and IgG4 antibodies to β -lactoglobulin is revealed in our study comparing patients with a different pace of recovery from CMA. The specific IgA levels differed between the patients with persisting CMA and those with transient CMA as early as at the time of diagnosis. A difference in specific IgG4 levels, in contrast, was markedly delayed. This observation may reflect the distinctive mechanisms that regulate IgA and IgG4 expression, as well as their differing mechanisms of anti-inflammatory function in allergy.

Specific IgG4 promotes tolerance by blocking the binding of specific IgE to allergen [24–26], and the anti-inflammatory function of IgG4 relies on the Fab arm exchange [27]. IL-4 induces class switching in B cells to both IgE and IgG4, whereas IL-10 up-regulates the secretion of IgG4 and inhibits IgE production [28–30]. Antigen-specific Th1 and Th2 cells may shift to type 1 regulatory T cells secreting IL-10 when triggered by high antigen exposure, leading to tolerance [31]. Interestingly,

Francis et al. [16] observed that IL-10 production increased during grass pollen immunotherapy earlier than specific IgG4 levels. Our results indeed indicate that specific IgG4 levels rise slowly and remain at an increased level long after clinical tolerance is achieved.

Specific IgA, in contrast to IgG4, does not inhibit IgE binding [17, 24]. The regulation of specific IgA production appears to be independent of T helper cells [30], to be associated with local TGF- β expression and to induce IL-10 production from monocytes [17]. We observed that an increase in specific IgG4, which is stimulated by IL-10 production [31], was preceded by an increase in specific IgA levels in patients who achieved early tolerance to CM.

Our results suggest that in clinical practise, measurement of CM-specific IgE and also β -lactoglobulin-specific IgA may give insight in the prognosis of CMA. Specific IgG4 levels are less informative because the difference between patients with transient or persistent CMA arises only later. Our study supports, nevertheless, the role of both IgA and IgG4 subclasses in the development of tolerance in CMA. Our findings, thus, invite to investigate the mechanisms of different antibody classes further in the achievement of tolerance, and the possibilities for immunotherapeutic applications.

Acknowledgements

We are grateful to Elsa Valtonen and Terttu Louhio for their technical assistance in antibody measurements, to Bert Ruiter for his comments on the manuscript and to Caitlin Woo for proofreading. We thank Helsinki University Central Hospital Research Funds and the Foundation for Pediatric Research for funding the study.

The study was supported by the Helsinki University Central Hospital Research Funds and the Foundation for Pediatric Research.

References

- Host A, Halken S. A prospective study of cow milk allergy in Danish infants during the first 3 years of life. Clinical course in relation to clinical and immunological type of hypersensitivity reaction. *Allergy* 1990; 45:587–96.

- 2 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.
- 3 Saarinen 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.
- 4 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.
- 5 Bottcher MF, Haggstrom P, Bjorksten B, Jenmalm MC. Total and allergen-specific immunoglobulin A levels in saliva in relation to the development of allergy in infants up to 2 years of age. *Clin Exp Allergy* 2002; **32**:1293–8.
- 6 Vanto T, Helppila S, Juntunen-Backman K *et al*. Prediction of the development of tolerance to milk in children with cow's milk hypersensitivity. *J Pediatr* 2004; **144**:218–22.
- 7 Skripak JM, Matsui EC, Mudd K, Wood RA. The natural history of IgE-mediated cow's milk allergy. *J Allergy Clin Immunol* 2007; **120**:1172–7.
- 8 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* 2009; **20**:35–41.
- 9 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.
- 10 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**:977–983.e1.
- 11 Meglio P, Giampietro PG, Gianni S, Galli E. Oral desensitization in children with immunoglobulin E-mediated cow's milk allergy – follow-up at 4 yr and 8 months. *Pediatr Allergy Immunol* 2008; **19**:412–9.
- 12 Nowak-Wegrzyn A, Bloom KA, Sicherer SH *et al*. Tolerance to extensively heated milk in children with cow's milk allergy. *J Allergy Clin Immunol* 2008; **122**:342–7–347.e1–2.
- 13 Skripak 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.
- 14 Jones SM, Pons L, Roberts JL *et al*. Clinical efficacy and immune regulation with peanut oral immunotherapy. *J Allergy Clin Immunol* 2009; **124**:292–300, 300.e1–97.
- 15 Aghamohammadi A, Cheraghi T, Gharagozlou M *et al*. IgA deficiency: correlation between clinical and immunological phenotypes. *J Clin Immunol* 2009; **29**:130–6.
- 16 Francis JN, James LK, Paraskevopoulos G *et al*. Grass pollen immunotherapy: IL-10 induction and suppression of late responses precedes IgG4 inhibitory antibody activity. *J Allergy Clin Immunol* 2008; **121**:1120–1125.e2.
- 17 Pilette C, Nouri-Aria KT, Jacobson MR *et al*. Grass pollen immunotherapy induces an allergen-specific IgA2 antibody response associated with mucosal TGF- β expression. *J Immunol* 2007; **178**:4658–66.
- 18 Shek LP, Bardina L, Castro R, Sampson HA, Beyer K. Humoral and cellular responses to cow milk proteins in patients with milk-induced IgE-mediated and non-IgE-mediated disorders. *Allergy* 2005; **60**:912–9.
- 19 Saarinen KM, Savilahti E. Infant feeding patterns affect the subsequent immunological features in cow's milk allergy. *Clin Exp Allergy* 2000; **30**:400–6.
- 20 Saarinen KM, Vaarala O, Klemetti P, Savilahti E. Transforming growth factor- β 1 in mothers' colostrum and immune responses to cows' milk proteins in infants with cows' milk allergy. *J Allergy Clin Immunol* 1999; **104**:1093–8.
- 21 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.
- 22 Frossard CP, Hauser C, Eigenmann PA. Antigen-specific secretory IgA antibodies in the gut are decreased in a mouse model of food allergy. *J Allergy Clin Immunol* 2004; **114**:377–82.
- 23 Aghayan-Ugurluoglu R, Ball T, Virtala S, Schweiger C, Kraft D, Valenta R. Dissociation of allergen-specific IgE and IgA responses in sera and tears of pollen-allergic patients: a study performed with purified recombinant pollen allergens. *J Allergy Clin Immunol* 2000; **105**:803–13.
- 24 van Neerven RJ, Wikborg T, Lund G *et al*. Blocking antibodies induced by specific allergy vaccination prevent the activation of CD4+T cells by inhibiting serum-IgE-facilitated allergen presentation. *J Immunol* 1999; **163**:2944–52.
- 25 Wachholz PA, Soni NK, Till SJ, Durham SR. Inhibition of allergen-IgE binding to B cells by IgG antibodies after grass pollen immunotherapy. *J Allergy Clin Immunol* 2003; **112**: 915–22.
- 26 Nouri-Aria KT, Wachholz PA, Francis JN *et al*. Grass pollen immunotherapy induces mucosal and peripheral IL-10 responses and blocking IgG activity. *J Immunol* 2004; **172**:3252–9.
- 27 van der Neut Kolfschoten M, Schuurman J, Losen M *et al*. Anti-inflammatory activity of human IgG4 antibodies by dynamic Fab arm exchange. *Science* 2007; **317**:1554–7.
- 28 Jeannin P, Lecoanet S, Delneste Y, Gauchat JF, Bonnefoy JY. IgE versus IgG4 production can be differentially regulated by IL-10. *J Immunol* 1998; **160**:3555–61.
- 29 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.
- 30 Meiler F, Klunker S, Zimmermann M, Akdis CA, Akdis M. Distinct regulation of IgE, IgG4 and IgA by T regulatory cells and Toll-like receptors. *Allergy* 2008; **63**:1455–63.
- 31 Meiler F, Zumkehr J, Klunker S, Ruckert B, Akdis CA, Akdis M. In vivo switch to IL-10-secreting T regulatory cells in high dose allergen exposure. *J Exp Med* 2008; **205**:2887–98.