

Serum immunoglobulin E, IgA, and IgG antibodies to different cow's milk proteins in children with cow's milk allergy: Association with prognosis and clinical manifestations

Hidvegi E, Cserhati E, Kereki E, Savilahti E, Arato A. Serum immunoglobulin E, IgA, and IgG antibodies to different cow's milk proteins in children with cow's milk allergy: Association with prognosis and clinical manifestations.

Pediatr Allergy Immunol 2002; 13:255–261. ©2002 Blackwell Munksgaard

Diverse pathogenic mechanisms elicit different clinical manifestations in cow's milk allergy (CMA). Our aim was to determine the concentration of serum immunoglobulin levels to different cow's milk proteins in patients with CMA and to determine how these values were related to clinical symptoms and prognosis. Fifty children (mean age 10.9 months, range: 1–34 months) with previously confirmed CMA were enrolled in this study. All had various clinical manifestations of CMA, including gastrointestinal, skin, and respiratory symptoms. At the diagnosis of CMA the serum total and the milk-specific immunoglobulin (Ig)E values were measured by enzyme immunoassay and fluoroimmunoassay, respectively, while the relative levels of serum IgA and IgG antibodies against different cow's milk proteins were determined by a sensitive enzyme-linked immunosorbent assay (ELISA). The results were compared to those of 30 non-atopic age-matched control children. On average, after 9.2 months (range 2–31 months) on a milk-free diet, a repeated challenge was performed in 38 children. At the re-challenge, 12 patients had clinical symptoms while the remaining 26 children were symptom-free. The IgG antibody level to bovine serum albumin (BSA) was significantly lower in the patients than in the controls (median: 0.36 vs. 2.94, $p < 0.01$). There was a close correlation among all individual IgA and IgG antibodies to different cow's milk proteins. The anti- α -casein IgG level (2.10) in children with a positive reaction at the re-challenge was significantly higher than in those with a negative reaction (0.89) ($p < 0.05$). The total IgE serum concentration was also significantly higher in those who had symptoms at the re-challenge compared to those who did not have any reaction at this time (22.9 vs. 6.8 kU/l, geometric mean, $p < 0.02$). There was no association between the clinical manifestations and the IgG and IgA antibody levels to the cow's milk proteins studied, except for the anti-BSA IgA level, which was higher in patients with gastrointestinal symptoms. The serum total IgE and anti- α -casein IgG levels could have prognostic values; their increase at the beginning of the disease may indicate the development of tolerance to cow's milk only at a later age and after a longer duration of CMA. However, as there is considerable overlap among the values observed in different groups of patients, there is a limitation of these tests for predicting the prognosis.

**Edit Hidvegi¹, Endre Cserhati¹,
Erzsebet Kereki², Erkki Savilahti³
and Andras Arato¹**

¹First Department of Pediatrics, Semmelweis University, Budapest, Hungary, ²Svabhegy Children's Hospital, Budapest, Hungary,

³Children's Hospital, University of Helsinki, Helsinki, Finland

Key words: cow's milk allergy; child; IgE, IgA, IgG antibodies

Edit Hidvegi, MD, First Department of Pediatrics, Semmelweis University, Bókay János u. 53, Budapest, Hungary, H-1083
Tel.: +36-1-3343186
Fax: +36-1-3138212
E-mail: hedit@gyer1.sote.hu

Accepted 13 November 2001

Cow's milk allergy (CMA) has become a common disease in early childhood. It is caused by the interaction between one or more milk proteins and different types of immune mechanisms, which can be classified into immunoglobulin (Ig)E-mediated and non-IgE-mediated forms (1,2). The symptoms commonly arise from the skin, and from the respiratory and gastrointestinal tracts, but the factors that determine the site of clinical disturbance remain unexplained, with some evidence suggesting that different immunopathogenic mechanisms may be involved in the variable clinical presentations (2). The symptoms may be elicited by several other diseases and are seldom pathognomic. Therefore, the primary test for establishing the diagnosis of food allergy is clinical: the symptoms cease on dietary elimination of cow's milk and re-appear upon challenge. The immune reactivity to cow's milk proteins (CMP) diminishes with age and clinical tolerance commonly occurs. About 85–90% of children with CMA will have remission by 3 years of age (3). It has been reported that in infants younger than 3 years of age the remission rate is lower in those with IgE-mediated CMA than in those with non-IgE-mediated reaction (4). Some laboratory tests may be useful in the prediction of prognosis. We recently showed that normalization of the previously elevated level of serum eosinophil cationic protein may indicate the cessation of CMA (5). Previously, a relationship between the development of tolerance to CMP and the ability to mount a local immune response was found, especially of IgA against CMP (6). The serum total IgE, milk-specific IgE, and IgA and IgG levels show great variability in children with CMA and therefore their diagnostic value is limited (7,8). However, it is possible that the level of these circulating antibodies at the onset of symptoms of CMA may help to predict the time at which the patients become clinically tolerant to cow's milk. Therefore, our aim was to find out whether these parameters really have a prognostic value and how they are related to the presenting symptoms.

Patients and methods

Fifty children with CMA were enrolled to our study. Children who were considered to have CMA had clinical symptoms which ceased upon dietary elimination of cow's milk and re-appeared on open challenge. The detailed clinical data of patients are shown in Table 1. Urticaria, atopic

dermatitis, repeated wheezing episodes, failure to thrive, vomiting, diarrhea, and bloody stools were the presenting symptoms after the introduction of CMP.

During the process of diagnosis of CMA, 4 ml of blood was drawn from every child in this study. From the serum samples, the total IgE level was measured using enzyme immunoassay (Phadezym IgE PRIST; Kabi Pharmacia Diagnostic AB, Uppsala, Sweden) and the milk-specific IgE was determined by fluorimmunoassay (Pharmacia CAP System FEIA; Kabi Pharmacia Diagnostic AB). The relative levels of IgA and IgG antibodies to different CMP, such as α -casein, bovine serum albumin (BSA), β -lactoglobulin and CMP, were measured using enzyme-linked immunosorbent assay (ELISA) methodology (9). These values were expressed as a ratio to a positive reference serum. Skin-prick tests were performed using an Epipharm food allergen kit (Epipharm, ALK/AS, Horsholm, Denmark).

After diagnosing the CMA, the patients received cow's milk-free diet: either extensively hydrolyzed formula or soya-based formula. One patient received an amino acid solution. After an average of 9.2 months (range 2–31 months) on a cow's milk-free diet, the cow's milk re-challenge test was made in 38 children using cow's milk-based formula containing a low concentration of lactose (Nutrilon low lactose; Nutricia). At the time of re-challenge, all patients were symptom free. After the CM re-challenge test, positive reactions were observed in 12/38 individuals (positive group, mean age 17.5 months). They had early (skin rash, vomiting) and delayed (atopic dermatitis, wheezing, slow weight gain) reactions. In the remaining 26 children (mean age 15.3 months) there was no reaction (negative group). (In 12 patients no re-challenge was made because of their bad compliance.)

Thirty non-atopic children represented the control group. The mean introduction time of CMP into their diet was similar to that of CMA patients: 3.3 months (range: 0.1–10 months) vs. 3.8 months (range: 0.1–12 months), controls and CMA patients, respectively (Table 1). The first introduction of CMP in the children with a positive (at 3.5 months of age) and negative (at 3.9 months of age) reaction at re-challenge was not significantly different.

Ethical permission for this study was given by the Ethical Committee of Semmelweis University, Budapest. Parents received detailed information about the examination before they signed consent forms.

Table 1. Clinical data of the cow's milk allergic patients and controls

Number of children with cow's milk allergy at diagnosis	50
Mean age*	10.9 (1–34)
Girls/boys†	20/30
Mean introduction time of CMP*	3.8 (0.1–12)
Presenting symptoms	
Skin†	37
Gastrointestinal†	23
Respiratory†	20
Combination of presenting symptoms	
One organ involved†	26
Two organs involved†	18
Three organs involved†	6
Number of children at the repeated challenge†	38
Mean age at diagnosis*	6.6 (1–34)
Mean age at re-challenge*	16.0 (6–41)
Positive reaction†	12
Negative reaction†	26
Controls†	30
Mean age*	8.7 (1–24)
Girls/boys	9/21
Introduction time of CMP*	3.3 (0.1–10)

*Months. Results are expressed as mean value (range).

†n.

CMP, cow's milk protein.

Statistical analysis

Statistical analysis was performed by the SAS statistical software package (SAS/STAT Software Release 6.12; SAS Institute Inc., Cary, NC, USA). The total IgE and milk-specific IgE values showed a normal distribution only after logarithmic transformation, therefore they are expressed as geometric mean and 95% confidence intervals (CI). The level of different IgA and IgG antibodies did not show a normal distribution, even after logarithmic transformation, and therefore are expressed as median and 95% CI. Between all patients and controls the Mann–Whitney *U*-test was used, while the analysis between the values of children who had positive or negative reactions to cow's milk after the re-challenge (positive and negative groups) and the controls was performed by using Kruskal–Wallis

test. When a significant difference was found between the groups, the statistical analysis was continued by pairwise comparisons using the Mann–Whitney *U*-test. For the determination of correlations among immunoglobulins, the Spearman rank test was used. A *p*-value of <0.05 was considered statistically significant.

Results

The serum total IgE concentration was significantly elevated in children with CMA at diagnosis (geometric mean: 11.0 kU/l; 95% CI: 0.8–21.2 kU/l), compared to controls (mean 4.3 kU/l; 95% CI: 0.7–26.1 kU/l, $p<0.02$). In children who showed a positive reaction to cow's milk at the second challenge, the total IgE values at diagnosis were significantly higher (mean 22.9 kU/l; 95% CI: 8.7–60.7 kU/l) than in the negative group (mean: 6.8 kU/l; 95% CI: 0–13.9 kU/l; $p<0.02$). Figure 1 shows all individual measurements in the different groups.

The milk-specific IgE level in the children with CMA (mean 0.51 kU/l; 95% CI: 0.13–1.99 kU/l) correlated with the total IgE concentrations ($r=0.58$, $p<0.001$). In the positive group the milk-specific IgE levels were significantly increased (mean 0.87 kU/l; 95% CI: 0–1.5 kU/l) compared to those of control group (mean: 0.35 kU/l; 95% CI: 0.2–0.6 kU/l; $p<0.01$). There was a slight, but not significant, difference in the serum milk-specific IgE levels of controls and the negative group (mean: 0.49 kU/l; 95% CI: 0–2.7 kU/l). The individual values of milk-specific IgE are shown in Fig. 2. Five of 12 positive cases, and 16 of 26 negative, as well as 28 of 30 controls, did not have detectable levels of milk-specific IgE. The serum concentrations of neither the total IgE nor the milk-specific IgE showed any correlation with the clinical symptoms of CMA. The skin-prick test with milk protein was positive in 12 patients with CMA and

Table 2. The relative levels of immunoglobulin (Ig)A and IgG antibodies to different cow's milk proteins (CMP) in cow's milk allergic children and in controls

Antibodies to:	Patients with CMA (n=50)	Positive group (n=12)	Negative group (n=26)	Controls (n=30)
Alpha-casein IgA	0.61 (0–3.7)	0.70 (0–7.8)	0.37 (0–1.5)	0.54 (0–4.8)
Alpha-casein IgG	1.43 (0–5.0)	2.10 (0–16.7)*	0.89 (0–4.5)	0.72 (0–3.6)
BSA IgA	0.50 (0–5.0)	1.36 (0–3.8)	0.04 (0–1.9)	0.17 (0–5.3)
BSA IgG	0.36 (0–0.8)**	0.88 (0.2–1.9)	0.24 (0–0.7)**	2.94 (0–11.3)
Beta-LG IgA	0.70 (0–3.3)	0.70 (0.5–0.9)	0.70 (0.5–0.9)	0.70 (0–4.2)
Beta-LG IgG	0.55 (0.3–0.8)	0.70 (0.4–1.0)	0.40 (0–0.8)	0.60 (0–0.3)
All CMP IgA	0.65 (0–1.9)	0.67 (0–4.1)	0.35 (0–1.0)	0.71 (0–2.9)
All CMP IgG	5.16 (0–14.5)	5.67 (0–30.6)	3.03 (0–13.2)	3.20 (0–5.9)

Results are presented as median values (95% confidence intervals).

Statistical analysis was performed by using the Kruskal–Wallis test.

Beta-LG, β -lactoglobulin; BSA, bovine serum albumin.

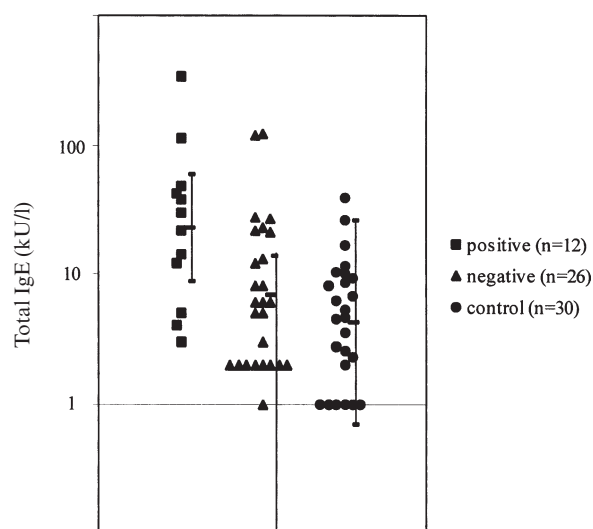


Fig. 1. Individual total immunoglobulin E (IgE) values in controls, and in children with cow's milk allergy who had a positive or a negative reaction to cow's milk at re-challenge. (The values are shown on a logarithmic scale.) Horizontal lines represent the geometric means of the different groups, vertical lines the 95% confidence intervals.

in all but one the milk-specific IgE level was elevated.

Table 2 summarizes the serum levels of specific IgA and IgG antibodies to the different CMP. In children with CMA, these values were similar to those of controls, except for the anti-BSA IgG concentration which was significantly lower in the patients. When the children with CMA were separated according to their positive or negative reaction to the second cow's milk challenge test, higher antibody values were observed in the

positive group, but this reached significance only for the anti- α -casein IgG. It should be emphasized that the values overlapped in the positive and negative groups. In the negative group the IgG antibody level to BSA was significantly lower compared to controls, while in the positive group the decrease of this antibody concentration was not significant. The other antibody levels were similar in children with early and delayed clinical reactions.

When the serum IgA and IgG antibodies to the different CMP were examined in patients with different clinical manifestations (skin, respiratory or gastrointestinal symptoms) the concentration of anti-BSA IgA antibodies was significantly elevated in children with gastrointestinal manifestation (relative ratio, median 1.76; 95% CI: 0–9.2) compared to those of controls (median 0.17; 95% CI: 0–5.3) ($p < 0.05$).

In children with CMA, close correlations were found between the specific IgA and IgG antibodies to different CMP in all comparisons (range of $r = 0.38$ – 0.86 , range of $p < 0.007$ to 0.0001). When the serum total IgE levels were compared with the levels of different IgG and IgA antibodies, correlations were found with anti- α -casein IgA ($r = 0.34$, $p < 0.02$) and IgG ($r = 0.36$, $p < 0.02$), anti-BSA IgA ($r = 0.34$, $p < 0.02$), and with IgA antibodies to all CMP ($r = 0.30$, $p < 0.02$) and IgG ($r = 0.39$, $p < 0.02$). Conversely, no correlation was found between the milk-specific IgE concentration and any types of IgA or IgG antibodies to the different CMP.

Discussion

The symptoms of CMA may be shared with several other diseases and therefore there is a requirement for a reliable laboratory test for this disease. However, considering that any one of the four types of hypersensitivity reactions can occur in its pathogenesis, it is obvious that no single test can diagnose patients with CMA (2). Degranulation of intestinal mast cells with the release of mediators acting on the gastrointestinal epithelium is an important mechanism for type I immunological reaction in CMA (10). Determination of the total serum IgE concentration may be useful for checking the global allergic status of children with CMA and its level is frequently elevated in CMA (11–13). This was confirmed also by our study, but we have to emphasize that many children had normal IgE values. Therefore, a low total IgE level does not rule out the diagnosis of CMA. It is unclear whether in CMA the milk-specific IgE antibodies contribute a considerable proportion of the total

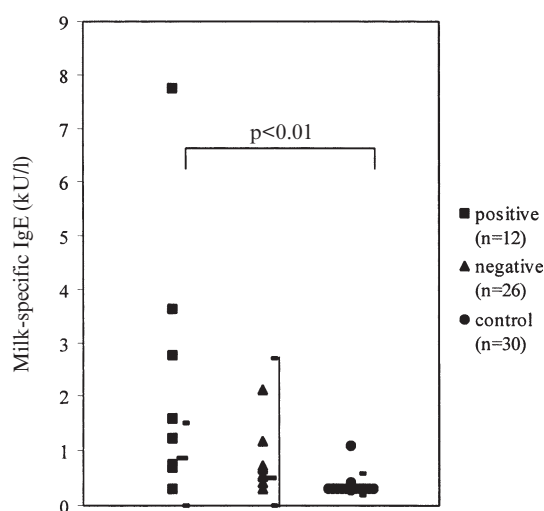


Fig. 2. Individual milk-specific immunoglobulin E (IgE) values in controls, and in children with cow's milk allergy who had a positive or a negative reaction to cow's milk at re-challenge. Horizontal lines represent the geometric means of the different groups, vertical lines the 95% confidence intervals.

IgE level; however, regardless of this, a close correlation was observed between the total and milk-specific IgE values. Our observation showed that the highly increased total IgE level at the diagnosis of CMA may indicate a prolonged course of this disease. Local production and systemic distribution of specific reagenic IgE antibodies to CMP is characteristic for CMA (11,14,15), but these antibodies can also be detected in 5% of healthy children (16). Radioallergosorbent testing (RAST) helps to diagnose CMA caused mainly by immediate reactions to CMP, but such antibodies are also found in CMA caused by delayed-type reactions. Our results also confirm this observation because no significant difference was found in the total and milk-specific IgE values in children with skin, respiratory, and gastrointestinal symptoms. CMA is often outgrown in children by the age of 3 years (17), and the recovery is earlier in children with cow's milk-induced enteropathy (18). According to previous studies, the development of tolerance to CMP in children with CMA is associated with a decrease in specific IgE antibodies (14,19). Our findings show that children developing tolerance to CMP earlier have only slightly, but not significantly, lower values of specific IgE antibodies, even at the time of diagnosis. In the recently published study of Sicherer & Sampson (20), the specific IgE antibody levels to CMP were lower in children with CMA, in whom the disease was not prolonged.

The diagnostic value of specific IgA and IgG antibodies to CMP is controversial because the production of these antibodies is a normal physiologic response following exposure to CMP (21–23). Japanese authors determined the milk-specific IgG levels in the sera of healthy children. The highest levels of milk-specific IgG were found in children 5–12 months of age; levels then decreased gradually to reach negligible values by adulthood (24). Keller et al. found that IgG antibody titers to casein, β -lactoglobulin, α -lactalbumin, and BSA, rose slowly in children who were exclusively breast-fed, and peaked rapidly in the cow's milk-based formula-fed group, especially during the first year of life. Consuming extensively hydrolyzed formula, the milk-specific IgG level is nearly the same as in the breast-fed group and significantly lower than that of the cow's milk-based formula-fed group (25). Jenmalm et al. found that exposure to CMP during the first 3 months of life causes an increased IgG antibody level to β -lactoglobulin, which gradually diminishes up to 8 years of age (26). Exposure to cow's milk during the first 3 days of life causes a strong stimulation of IgG

antibody production to CMP and this is still apparent at 2 years of age (27,28).

Previously, it was claimed that the antibody response was stronger among children with CMA than in controls (22,29,30). Later studies failed to show any difference in the level of IgA and IgG antibodies to cow's milk among children with CMA and healthy controls (6,31). Some authors have reported even low titers of IgG antibodies to cow's milk in patients with CMA (1,32). In our study only the anti-BSA IgG level was decreased compared to the control value, while the concentrations of other IgA and IgG antibodies were similar to those of controls. The close correlation in all comparisons among the relative level of IgA and IgG antibodies against different CMP also proves that the changes in the production of these immunoglobulins are proportional.

On the basis of our results, we conclude that the levels of these IgA and IgG antibodies do not have diagnostic significance in an individual child with CMA. The diagnostic value of IgG subclass antibodies to different CMP is uncertain, as shown by Host et al. (14) who did not find a significant difference in the levels of these antibodies between infants with CMA and controls.

We found that a higher IgG antibody concentration to α -casein at diagnosis may predict a longer duration of CMA. This is in contrast to the results of James & Sampson who were not able to detect such a change in any type of IgG antibodies to different CMPs, which may help predict the duration of CMA (19). Isolauri et al. showed that the ability to mount a local IgA response to CMP may determine when the clinical hypersensitivity to cow's milk is lost (6). In our study, however, those patients who developed tolerance to cow's milk earlier had a low level of IgA antibodies to different CMPs. The observed elevated IgA antibody level to BSA in CMA patients with gastrointestinal manifestations may be explained by its increased production in the intestinal mucosa. The number of IgA-producing plasma cells is increased in children with cow's milk-induced enteropathy (33). The present observation that a close correlation exists between the production of total IgE and the levels of certain IgA and IgG antibodies to CMP may be indicative of a close relationship between the synthesis of IgE and other immunoglobulins (IgA, IgG), as previously suggested by Lilja et al. (34).

In conclusion, our study further confirms that laboratory methods have only an auxiliary role in the diagnosis of CMA, which must be based on

the clinical response to cow's milk elimination and provocation, as recommended by Goldman almost 40 years ago (35). The higher total IgE and the elevated level of IgG antibodies to α -casein may indicate a later development of tolerance to CMP, or even persistence of CMA.

Acknowledgments

This study was supported by the Hungarian Scientific Research Fund (OTKA-T22649 and T31791) and the National Scientific Council (ETT-555/1996 and 297/2000).

References

1. FIRER MA, HOSKING CS, HILL DJ. Humoral immune reaction to cow's milk in children with cow's milk allergy. *Int Arch Allergy Appl Immunol* 1987; 84: 173–7.
2. HOST A. Cow's milk protein allergy and intolerance in infancy. Some clinical, epidemiological and immunological aspects. *Pediatr Allergy Immunol* 1994; 5 (Suppl. 5): 5–36.
3. HOST A, HALKEN S. A prospective study of cow's milk allergy in Danish infants during the first 3 years of life. Clinical course in relation to clinical and immunological types of hypersensitivity reaction. *Allergy* 1990; 45: 587–96.
4. HILL DJ, FIRER MA, BALL G, HOSKING CS. Natural history of cow's milk allergy in children: Immunological outcome over 2 years. *Clin Exp Allergy* 1993; 23: 124–31.
5. HIDVÉGI E, CSERHÁTI E, KERÉKI E, ARATÓ A. Higher serum eosinophil cationic protein levels in children with cow's milk allergy. *J Pediatr Gastroenterol Nutr* 2001; 32: 475–9.
6. ISOLAURI E, SUOMALAINEN H, KAILA M, et al. Local immune response in patients with cow's milk allergy: Follow-up of patients retaining allergy or becoming tolerant. *J Pediatr* 1992; 120: 9–15.
7. SAVILAHTI E, KUITUNEN M. Allergenicity of cow's milk proteins. *J Pediatr* 1992; 121: S12–S20.
8. SAARINEN KM, SUOMALAINEN H, SAVILAHTI E. Diagnostic value of skin-prick and patch tests and serum ECP and cow's milk specific IgE in infants with cow's milk allergy. *Clin Exp Allergy* 2001; 31: 423–9.
9. VAARALA O, SAUKKONEN T, SAVILAHTI E, KLEMOLA T, AKERBLUM HK. Development of immune response to cow's milk proteins in infants receiving cow milk formula or hydrolysed formula. *J Allergy Clin Immunol* 1995; 96: 917–23.
10. CROWE SE, PERDUE MH. Gastrointestinal food hypersensitivity: Basic mechanisms of pathophysiology. *Gastroenterology* 1992; 103: 1075–95.
11. DANNAEUS A, JOHANSSON SGO. A follow-up study of infants with adverse reactions to cow's milk. I. Serum IgE, skin test reactions and RAST in relation to clinical course. *Acta Paediatr Scand* 1979; 69: 377–82.
12. SAMPSON HA, JOLIE PL. Increased plasma histamine concentrations after food challenges in children with atopic dermatitis. *N Engl J Med* 1984; 311: 372–6.
13. BJORKSTEN B, AHLSTEDT S, BJORKSTEN F, et al. Immunoglobulin E and immunoglobulin G₄ antibodies to cow's milk in children with cow's milk allergy. *Allergy* 1983; 38: 119–24.
14. HOST A, HUSBY S, GIESING B, LARSEN JN, LOWENSTEIN H. Prospective estimation of IgG, IgG subclass and IgE antibodies to dietary proteins in infants with cow's milk allergy. Levels of antibodies to whole milk protein, BLG and ovalbumin in relation to repeated milk challenge and clinical course of cow's milk allergy. *Allergy* 1992; 47: 218–29.
15. SAARINEN KM, SAVILAHTI E. Infant feeding patterns affect the subsequent immunological features in cow's milk allergy. *Clin Exp Allergy* 2000; 30: 400–6.
16. BJÖRKSTEN F, SAARINEN UM. IgE antibodies to cow's milk in infants fed breast milk and milk formulae. *Lancet* 1978; 2: 624–5.
17. BOCK SA. Prospective appraisal of complaints of adverse reactions to foods in children during the first 3 years of life. *Pediatrics* 1987; 79: 683–8.
18. KUITUNEN P, VISAKORPI JK, SAVILAHTI E, PELKONEN P. Malabsorption syndrome with cow's milk intolerance. Clinical findings and course in 54 cases. *Arch Dis Child* 1975; 50: 351–6.
19. JAMES JM, SAMPSON HA. Immunologic changes associated with the development of tolerance in children with cow's milk allergy. *J Pediatr* 1992; 121: 371–7.
20. SICHERER SH, SAMPSON HA. Cow's milk protein-specific IgE concentrations in two age groups of milk-allergic children and in children achieving clinical tolerance. *Clin Exp Allergy* 1999; 29: 507–12.
21. MAY CD, REMIGIO L, FELDMAN J, BOCK SA, CARR RI. A study of serum antibodies to isolated milk proteins and ovalbumin in infants and children. *Clin Allergy* 1977; 7: 583–95.
22. BURGIN-WOLFF A, SIGNER E, FRIESS HM, BERGER R, BIRBAUMER A, JUST M. The diagnostic significance of antibodies to various cow's milk proteins (fluorescent immunosorbent test). *Eur J Pediatr* 1980; 133: 17–24.
23. OLDAEUS G, BJORKSTEN B, JENMALM MC, KJELLMAN NI. Cow's milk IgE and IgG antibody responses to cow's milk formulas. *Allergy* 1999; 54: 352–7.
24. AHMED T, SUMAZAKI R, NAGAI Y, SHIBASAKI M, TAKITA H. Immune response to food antigens: Kinetics of food-specific antibodies in the normal population. *Acta Paediatr Jpn* 1997; 39: 322–8.
25. KELLER KM, BURGIN-WOLFF A, LIPPOLD R, LENTZE MJ. Quality assurance in diagnostics: Are there normal values for IgG-antibodies to cow's milk proteins? *Klin Paediatr* 1999; 211: 384–8.
26. 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.
27. 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.
28. SAARINEN KM, JUNTUNEN-BACKMAN K, JARVENPAA A-I, 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.
29. MAY CD, FOMON SJ, REMIGIO L. Immunologic consequences of feeding infants with cow's milk and soy products. *Acta Paediatr Scand* 1982; 71: 43–51.
30. FALLSTRÖM SP, AHLSTEDT S, CARLSSON B, LÖNNERDAL B, HANSON LA. Serum antibodies against native processed

- and digested cow's milk proteins in children with cow's milk protein intolerance. *Clin Allergy* 1986; 16: 417–23.
31. BURKS AW, WILLIAMS LW, CASTEEL HB, FIEDOREK SC, CONNAUGHTON CS. Antibody response to milk proteins in patients with milk-protein intolerance documented by challenge. *J Allergy Appl Immunol* 1990; 85: 921–7.
 32. HILL DJ, FIRER MA, BALL G, HOSKING CS. Recovery from milk allergy in early childhood: antibody studies. *J Pediatr* 1989; 114: 761–6.
 33. PERKKIO M, SAVILAHTI E, KUITUNEN P. Semi-quantitative analysis of immunoglobulins and complement fractions 3 and 4 in the jejunal mucosa in coeliac disease and in food allergy in childhood: Immunohistochemical study by light and electron microscopy. *Pathology* 1981; 89: 343–50.
 34. LILJA G, MAGNUSSON CGM, OMAN H, JOHANSSON SGO. Serum levels of IgG subclasses in relation to IgE and atopic disease in early infancy. *Clin Exp Allergy* 1990; 20: 407–13.
 35. GOLDMAN AS, ANDERSON DW Jr, SELLERS WA, et al. Milk allergy. I. Oral challenge with milk and isolated milk proteins in allergic children. *Pediatrics* 1963; 32: 425–43.