

Prospective estimation of IgG, IgG subclass and IgE antibodies to dietary proteins in infants with cow milk allergy

Levels of antibodies to whole milk protein, BLG and ovalbumin in relation to repeated milk challenge and clinical course of cow milk allergy

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Prospectively, serum levels of IgE, specific IgE antibodies (AB) to whole cow milk protein (CMP), bovine se-albumin, bovine immunoglobulin, bovine lactoferrin, bovine lactalbumin and beta-lactoglobulin (BLG), IgG and IgG subclass antibodies to ovalbumin (OA) and BLG, and IgG₄ RAST to CMP (bovine whey) were measured in 39 infants with cow milk protein allergy (CMPA) at birth (cord blood), at time of diagnosis and before and after milk challenge at the age of 12 months. Immunological measurements were also undertaken in 33 control infants without CMPA at birth, at 6 months and at 18 months. At no time, were differences found between the levels of IgG and IgG subclass AB to OA and BLG in control versus infants with CMPA. In the 39 infants with CMPA no correlation was found between the levels of IgE, IgG and IgG subclass AB in cord blood and subsequent levels of these values, irrespective of the type of CMPA (IgE-mediated (CMA) or non-IgE-mediated (CMI)), and irrespective of whether remission had occurred. In cord blood 25/33 (76%) of the infants with CMPA had specific IgE-AB to one or more of the bovine milk proteins indicating a prenatal intrauterine sensitization to cow milk protein. At 6 months the frequency of specific IgE-AB to bovine milk proteins was significantly ($p < 0.05$) higher in infants with CMA versus CMI, and at 12 months total serum-IgE and the increase of these specific IgE-AB and RAST to CMP were significantly higher ($p < 0.05$) in infants with persistent CMA. From 6 to 12 months withholding milk resulted in a significant fall in specific IgE-AB to CMP, and IgG, IgG₁ and IgG₄ anti-BLG followed by an increase after milk challenge. Decreasing levels of IgG anti-OA from birth to 6 months reflect passive maternal transfer of IgG through the placenta, and increasing levels of IgG anti-BLG, already from birth to 6 months, may represent an early exposure to CMP in all infants. Significantly higher levels ($p < 0.05$) of IgG anti-OA AB, IgG₁ and IgG₄ anti-BLG AB were found in infants with persistent CMA, indicating a close relation between the synthesis of IgE and IgG and between IgE and IgG subclasses (IgG₁ and IgG₄) in symptomatic cow milk-allergic individuals. Determination of IgG AB and IgG subclass AB (IgG₁ and IgG₄) to BLG and bovine whey in cord blood appears unable to discern infants at high risk of development of CMPA. However, infants with persistent CMPA have an increased antibody response of specific IgE and IgG subclasses (IgG₁ and IgG₄) to CMP exposure.

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Cow milk protein allergy (CMPA), i.e. reproducible adverse reactions to cow milk protein (CMP), is a common disorder in early childhood. In recent stud-

ies the incidence of CMPA during the first year of life has been reported to be between 2 and 5% (6, 33, 38). Adverse reactions to CMP may be due to the

interaction between one or more milk proteins and one or more immune mechanisms (3, 27). Immediate reactions to CMP are often IgE-mediated (Type I) (13, 21, 26, 35) as demonstrated by positive RAST and skin prick test (SPT) to CMP (13, 21, 26, 35, 50) or positive basophil histamine release test (44). Children with late reactions to CMP are usually without signs of IgE-mediated reactions (11, 12, 21, 26, 48), and the clinical significance of the available diagnostic tests for other types of immune mechanism (Types II – III – IV) seems to be limited. These tests have included investigation of activation of the complement system (31, 42), tests for cellular reactivity, such as stimulation of lymphocytes by BLG (49, 53), leukocyte migration inhibition factor (1, 51), neutrophil chemotactic activity (8), suppressor cell activity (18, 53), and determination of antibodies to CMP of immunoglobulin classes G,A,M (3, 15, 16, 17, 19, 27).

The role of IgG and IgG subclass antibodies in CMPA is controversial. Some authors have reported low titers of IgG antibodies to CMP in patients with CMPA and IgE antibodies to CMP (16, 27), and others have found high levels of IgG antibodies in combination with high specific IgE levels (11, 17, 50). In patients with delayed reactions to CMP high titers of IgG antibodies to BLG (19) have been demonstrated. IgG is transferred actively by the mother to the foetus via the placenta (20, 39, 40), and high levels of IgG antibodies to BLG in cord blood have been reported to protect against development of CMPA (9). Likewise, an increased IgG/IgE ratio indicated an earlier recovery from CMPA (14). Another study, however, showed an association between increased IgG level in cord blood and later development of atopic disease (37).

Also, much controversy exists on the possible pathogenetic role of IgG subclasses (30) and especially IgG₄ in patients with CMPA. It has been argued whether IgG₄ functions as a blocking antibody or as an anaphylactic antibody. One study shows some evidence for a pathogenetic role of IgG₄ antibodies in the provocation of both immediate and delayed reactions (45), whereas 2 reports do not confirm any evidence for specific IgG₄ antibodies playing a role in CMPA (4, 7).

The aims of the present prospective study were to evaluate the IgG and IgG subclass antibody response to dietary cow milk proteins and ovalbumin during 1 year follow-up of 39 infants with CMPA diagnosed by strict elimination/milk challenge procedures (2, 48). Immunologic measurements were made at birth (cord blood), at time of diagnosis, and before and after milk challenge at the age of 12 months. The immunologic changes were related to the results of milk challenge, IgE response to CMP and to acquisition of clinical tolerance to CMP.

Material and methods

The 39 infants (19 boys) with CMPA and the 33 controls in this study form part of a cohort of 1749 newborns from the municipality of Odense born during 1985 in the University Hospital of Odense and followed prospectively for the development of IgE-mediated and non-IgE-mediated CMPA during their first year (33). Subsequently, in 39 infants with CMPA, continued clinical sensitivity to CMP was assessed by rechallenging at the ages of 12 months, 18 months, 24 months and 36 months (35). The study protocol has been described in detail elsewhere (33, 35).

Diagnostic criteria

The diagnosis of CMPA was established by generally accepted criteria (2, 33, 48): a) definite disappearance of symptoms after each of 2 dietary eliminations of cow milk and cow milk products, b) recurrence of identical symptoms after one challenge, and c) exclusion of lactose intolerance and coincidental infection.

Milk challenges

A milk-free diet was kept for at least 4 weeks before challenge. All challenges were undertaken in a controlled hospital setting under professional observation as earlier described in detail (33, 35). The results of the first 10 positive open milk challenges were confirmed by double blind placebo controlled challenge as previously described (31). Subsequently, all challenges were open. None of the mothers were on diet without milk or egg during pregnancy or lactation. However, in 9 exclusively breast-fed infants elimination of CMP was performed via withdrawal of the mother's intake of cow milk, and milk challenge through renewed maternal intake of cow milk (33). In formula fed infants a cow milk-based formula, Allomin[®], processed by spray-drying and containing 15 g protein/l with a casein/whey ratio of 4/6, was used for challenge. The following volumes of Allomin were administered: initially 5 ml followed at 2-h intervals with 10, 20, 40, 80 ml. If no symptoms had occurred after 8 h (after a total cumulated dose of 155 ml Allomin) the child was sent home with increasing amounts (up to 160/meal) to be taken at home until customary daily intake of milk was tolerated. In the case of late reactions the patient was reinvestigated in hospital as soon as possible, and at a suspected reaction to CMP the last dose was repeated once. Before and after challenge and at any adverse reaction the patients were examined and symptoms and signs were noted. At home possible symptoms were recorded in a diary by the parents,

and all patients were reinvestigated 3 weeks after the start of the challenge.

Clinical and immunological classification of patients

As earlier described (9) 117/1749 infants (6.7%) showed cutaneous, gastrointestinal or respiratory symptoms suggestive of CMPA. Of these 39/117 (33%) fulfilled the diagnostic criteria for CMPA giving a 1-year incidence of 39/1749 (2.2%). 64% showed cutaneous symptoms, 59% gastrointestinal symptoms, and 33% respiratory symptoms. On the basis of the results of SPT and AL-RAST to CMP (SPT $\geq 2+$ and/or AL-RAST class ≥ 2) 21 infants were classified as having IgE-mediated CMPA (CMA) and 18 infants as having non-IgE-mediated CMPA/cow milk protein intolerance (CMI) (Table 1). Further, according to the time of clinical reaction after milk challenge (within 1 h after intake of up to a total of 155 ml Allomin (corresponding to 2.3 g milk protein), 14/21 infants with IgE-mediated CMPA (CMA) and 5/18 infants with non-IgE-mediated CMPA (CMI) were classified as "immediate reactors", and the remaining 7/21 and 13/18 as "late reactors" (Table 1).

Growth

At the time of diagnosis (at the first milk challenge) median age was 20 weeks (range 8–48 weeks), median body weight 7000 g (range 4738 g–9700 g). At the challenge at the age of 12 months (± 4 weeks) median body weight was 10 kg (range 7.4–13.5). All 39 infants had a normal growth.

Dietary intervention

Once the diagnosis of CMPA had been confirmed, milk-free diet was continued until a new milk challenge was performed at the age of 12 months. All infants with confirmed CMPA had Nutramigen as a milk substitute and it was recommended to avoid introduction of solid foods until the age of 6 months, and highly allergenic foods such as citrus fruit, egg, fish, tomato, strawberries, chocolate, nuts, berries, peas and beans until the age of 12 months. Advice on milk-free diet and sufficient nutrition was given by a specialist dietician.

Laboratory investigations

Cord blood (CB) serum samples were collected routinely in the study population during 1985. From the 39 infants with CMPA serum samples were taken 3 weeks after each milk challenge, and at the age of 12 months also immediately before milk challenge. Schedule for the determinations of serum-IgE and antibody levels in 39 infants with CMPA is depicted in Table 2. Total-IgE levels were analysed by Pharmacia (Phadebas IgE PRIST).

Controls

Schedule for the determination of serum IgE and antibody levels in 33 controls is shown in Table 2. Control sera for analysis of specific IgE to bovine milk protein (CRIE-IgE) and IgG₄ RAST to bovine milk protein were taken from 8 infants without CMPA (milk challenge negative). Control sera for analysis of IgE and IgG subclass antibodies were

Table 1. Clinical and immunological classification and clinical course in 39 infants with cow milk protein allergy (CMPA)

	21 IgE-mediated (CMA)		18 non IgE-mediated (CMI)	
	14 "immediate reactors"	7 "late reactors"	5 "immediate reactors"	13 "late reactors"
Still intolerant to CMP at 12 months	10	2	2	3
Tolerant to CMP at 12 months	4	5	3	10

Table 2. Schedule for the determination of IgE and antibody levels in 39 infants with and 33 controls without cow milk allergy

	0	6	12	18 months
IgE	x o ^a		x	
CRIE-IgE	x	x o ^b	xx	
AL-RAST to CMP		x	xx	
IgG anti OA-AB	x o ^a	x	xx	o ^a
IgG anti BLG-AB	x o ^a	x	xx	o ^a
IgG _{1,2,3,4} anti-BLG-AB	x o ^a	x	xx	o ^a
IgG ₄ RAST to CMP (whey fraction)	x	x o ^b	xx	

x=infants with cow milk allergy, o=controls. CRIE-IgE: specific IgE to bovine se-albumin, immunoglobulin, lactoferrin, alpha-lactalbumin and BLG. a: n 25. b: n 8. For further explanations see text.

collected from 25 healthy infants followed from birth (cord blood) until the age of 18 months (venous blood sample). None of these 25 control infants developed signs or symptoms suggestive of CMPA.

IgG antibodies to dietary proteins

IgG antibodies to OA (IgG anti-OA-AB) and to BLG (IgG anti-BLG-AB) were measured by enzyme linked immunoassays (ELISA) as described in detail previously (29). The assays were performed in microplates (Nunc, Roskilde, Denmark) and included a biotin-avidin amplification step. Calibration was achieved with a high-titered human serum for each assay and the results expressed in U/l. All determinations were done in duplicate and assay variation was assessed by 3 control sera selected to represent high, medium and low antibody levels included in each run and assay ($n=20$). The coefficient of variation (CV%) within assays varied from 9–11% for the IgG-OA-AB and 7–17% for the IgG-BLG-AB. The CV% between assays varied from 17–27% for the IgG-OA-AB and from 18–24% for the IgG-BLG-AB.

IgG subclass antibodies to dietary proteins

The IgG subclass antibodies to BLG were measured with ELISA as described previously (28). Monoclonal mouse antibodies against IgG₁ (clone-no. NL-16), IgG₂ (M73013), IgG₃ (ZG4) and IgG₄ (RJ4) were purchased as ascitic fluids from Oxoid (Bedford, UK). Microplate wells were coated with BLG at 9 mg/l of 0.1 bicarbonate buffer, pH 9.6. Separate wells were coated with rabbit F(ab')₂ anti-antibody and normal rabbit F(ab')₂ at 2.5 mg/l bicarbonate buffer. Non-specific binding was blocked with 0.1%

human serum albumin (Behring, Marburg, Germany). After washings, diluted sera and myeloma standards (a gift from WHO, Professor F. Skvaril, Berne, Switzerland) were incubated overnight. The plates were washed and incubated with monoclonal antibodies, followed by alkaline phosphatase-coupled anti-mouse antibody (Orion, Helsinki, Finland) and substrate.

From the myeloma standards (OD 405 nm-values from normal rabbit F(ab')₂-coated wells subtracted) the amount of antibody bound in $\mu\text{g/l}$ was estimated. The detection limit varied from 0.08–0.25 $\mu\text{g/l}$. All determinations were made in duplicate and 2 control sera selected to represent high and low antibody levels were included in each plate. The within-run CV% were 4–7% and the between-day CV% 10–26%.

IgG₄ RAST to bovine milk protein

IgG₄ RAST to bovine milk protein was performed as described in (25). In brief, sera were affinity purified against bovine whey 0582 (prepared as described in (22)), containing soluble milk protein coupled to Sepharose 4B prior to the incubation with bovine whey 0582 coupled to paper discs. Bound cow milk-specific IgG₄ was detected with monoclonal anti-IgG₄ (Unipath, Bedford, UK) labelled with 125I.

Crossed radio-immuno-electrophoresis

Specific IgE to bovine milk proteins: se-albumin, immunoglobulin, lactoferrin, alpha-lactalbumin and BLG were measured by crossed radioimmuno-electrophoresis (CRIE), as described in (22). Semi-quantitative CRIE scores were background corrected by the subtraction of scores obtained by applying a 1000 donor normal serum-control pool. The CRIE score of this 1000 donor pool was similar to that of 8 control infants proven to be milk challenge negative.

Skin test procedure

SPT to CMP was performed as earlier described in detail (35). The results were given as mean diameters ($D1 + d2/2$), from which the mean diameters of the negative reference were deducted. Further, a weal half the size of the positive reference was described 1+, a weal of the same size as the histamine reference as 2+ and a weal double the size 3+.

Statistics

The Mann-Whitney test was used to assess differences between the groups. The Wilcoxon/Pratt rank

sum test was used to compare paired samples, and Spearman's R-test to evaluate correlation between variables. The chi-square test was used to assess differences between categories.

The study was approved by the Human Research Ethics Committee of the counties of Fyn and Vejle, and parental informed consent was obtained for each infant.

Results

The development in the levels of IgG-anti OA-AB is shown in Fig. 1. A decrease is seen from birth (cord blood) to the age of 6 months, followed by an increase to the age of 12 months. The only significant differences between groups are higher IgG anti-OA-AB levels in infants with IgE-mediated cow milk allergy (CMA group) after milk challenge at 6 months and at 12 months compared with infants with non-IgE-mediated cow milk allergy (CMI group) (Mann-Whitney test $p = 0.04$ and 0.038 , respectively).

For IgG anti-BLG-AB (Fig. 2) an increase is found from birth (cord blood) to 6 months, followed

by decrease to 12 months, and a significant increase after milk challenge at 12 months in both the CMA and CMI group (Wilcoxon/Pratt test; $p = 0.001$ and $p = 0.0322$, respectively). At that time a trend towards a higher level in the CMA-group was found (Mann-Whitney test $p = 0.073$). In controls an increase was found from birth (cord blood) until 18 months. No differences appeared between the levels in controls and infants with CMPA (at birth, and at 12 months versus 18 months).

For IgG₁ anti-BLG-AB (Fig. 3) and for IgG₄ anti-BLG-AB (Fig. 4) the same characteristic changes as for IgG anti-BLG-AB were found with the exceptions of a significantly higher level of IgG₁-anti-BLG-AB in the CMA group in comparison with the CMI group after milk challenge at 12 months (Mann-Whitney test $p = 0.020$), and non-significant increase of IgG₁ anti-BLG-AB and IgG₄ anti-BLG-AB in the CMI group after milk challenge at 12 months (Wilcoxon/Pratt test (p (two-tailed) 0.0742 and 0.25 , respectively).

At no time, was any correlation found between the levels of IgG anti-BLG-AB, IgG₁ anti-BLG-AB, IgG₄ anti-BLG-AB and IgG₄ RAST to CMP

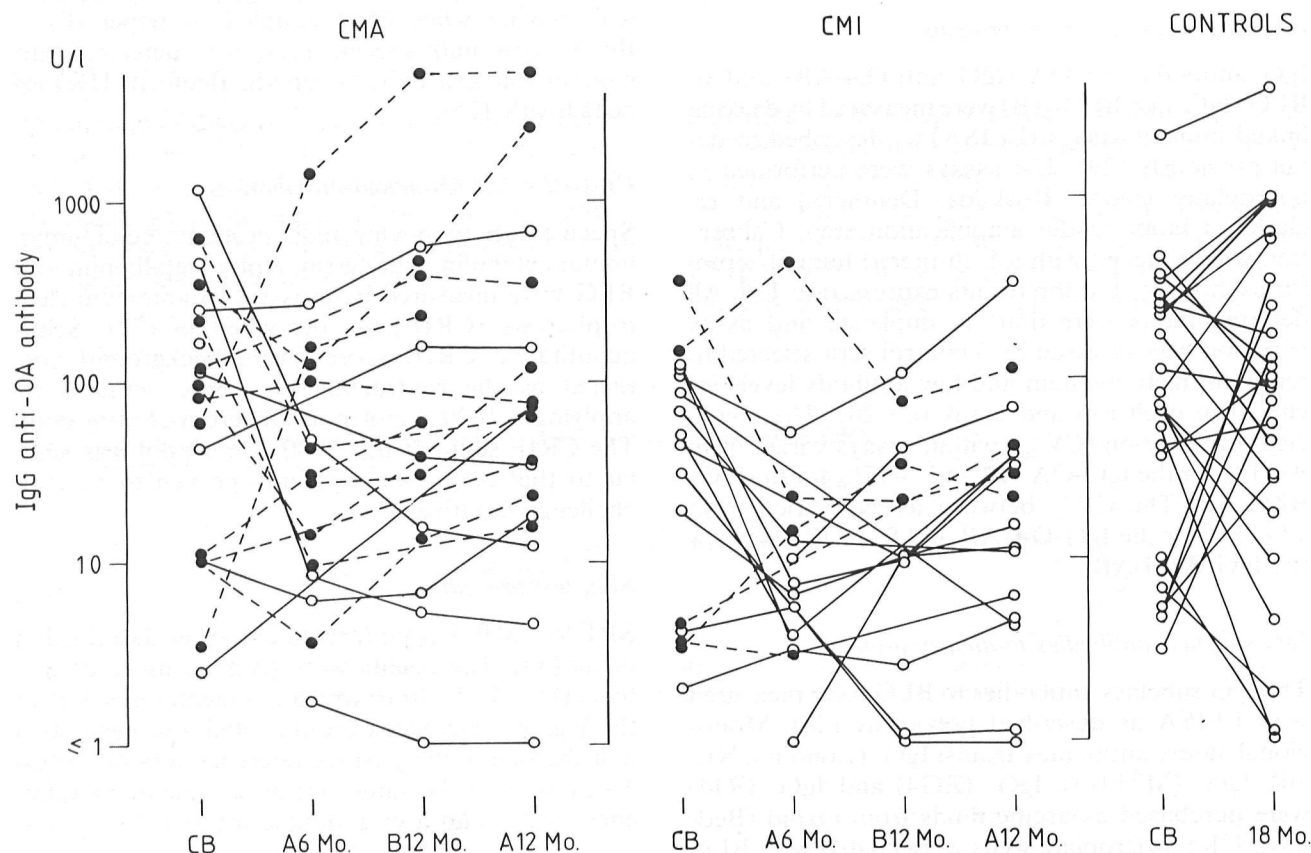


Fig. 1. Levels of IgG anti-ovalbumin antibody in 35/39 infants with cow milk allergy, 19/21 with IgE-mediated (CMA) and 16/18 with non-IgE-mediated (CMI) cow milk allergy, and in 25 healthy infants without cow milk allergy (controls). CB: cord blood; A6 Mo: after milk challenge at 6 months; B12 Mo: before milk challenge at 12 months; A12 Mo: after milk challenge at 12 months; 18 Mo: at 18 months. ○ = infants tolerant to cow milk at 12 months; ● = infants still intolerant to cow milk at 12 months. For further explanation see text.

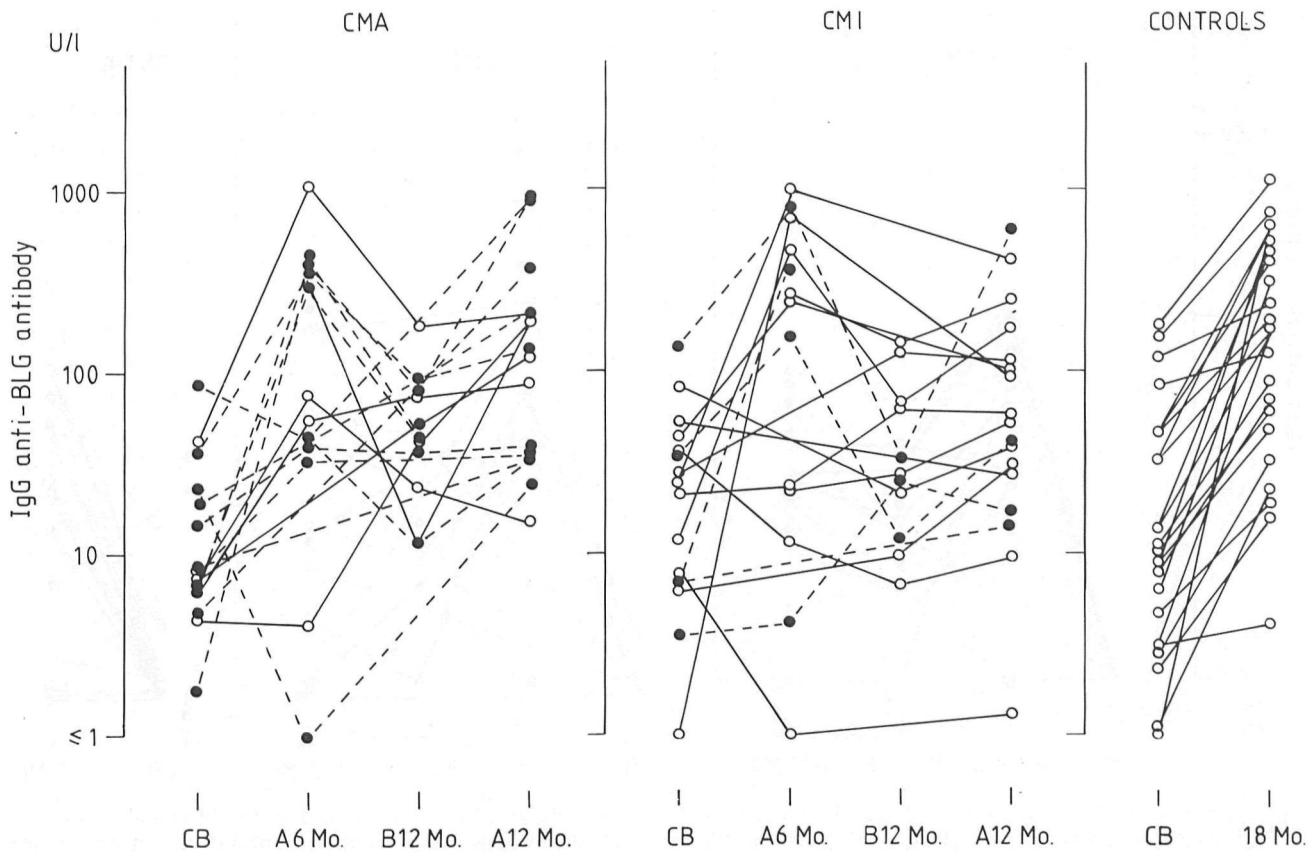


Fig. 2. Levels of IgG anti-BLG antibody in 35/39 infants with cow milk allergy, 19/21 with IgE-mediated (CMA) and 16/18 with non-IgE-mediated (CMI) cow milk allergy, and in 25 healthy infants without cow milk allergy (controls). For further explanations see Fig. 1.

(Table 3) in cord blood and subsequent levels of these values irrespective of the type of cow milk allergy (CMA or CMI) and irrespective of whether or not tolerance to milk was achieved. Besides, in cord blood no correlation was found between the levels of IgG₄ anti-BLG-AB and the scores of IgE-CRIE to BLG.

In the CMA group there was a trend towards higher levels of IgG anti-BLG-AB after milk chal-

lenge at 12 months in the children with persistent CMA (challenge positive) versus those having achieved tolerance to CMP (Mann-Whitney test $p = 0.12$), and for IgG₁ anti-BLG-AB the same trend was found. Further, at 12 months, the whole group of milk-challenge positive (irrespective of whether CMA or CMI) had significantly higher values of IgG₁ anti-BLG-AB compared with the group of milk tolerant children (Mann-Whitney $p = 0.036$).

Table 3. Median and range () IgG₄ RAST values to cow milk protein, 21 infants with IgE-mediated milk allergy (CMA) and 18 with non-IgE-mediated milk allergy (CMI)

	Cord blood <i>n</i> 21	At 6 months <i>n</i> 31	At 12 months	
			Before challenge <i>n</i> 29	After challenge <i>n</i> 36
21 infants with CMA	<i>n</i> 13 8.70 (6.40–19.26)	<i>n</i> 16 8.81 (6.90–13.50)	<i>n</i> 16 9.03 (7.41–13.18)*	<i>n</i> 20 10.32 (7.62–15.00)*
18 infants with CMI	<i>n</i> 8 7.50 (4.5–12.30)	<i>n</i> 15 9.03 (7.56–12.10)	<i>n</i> 13 8.66 (7.87–12.30)	<i>n</i> 16 8.95 (8.04–14.95)
8 control infants without CMA/CMI		<i>n</i> 8 8.50 (6.5–11.90)		

* $p < 0.05$.

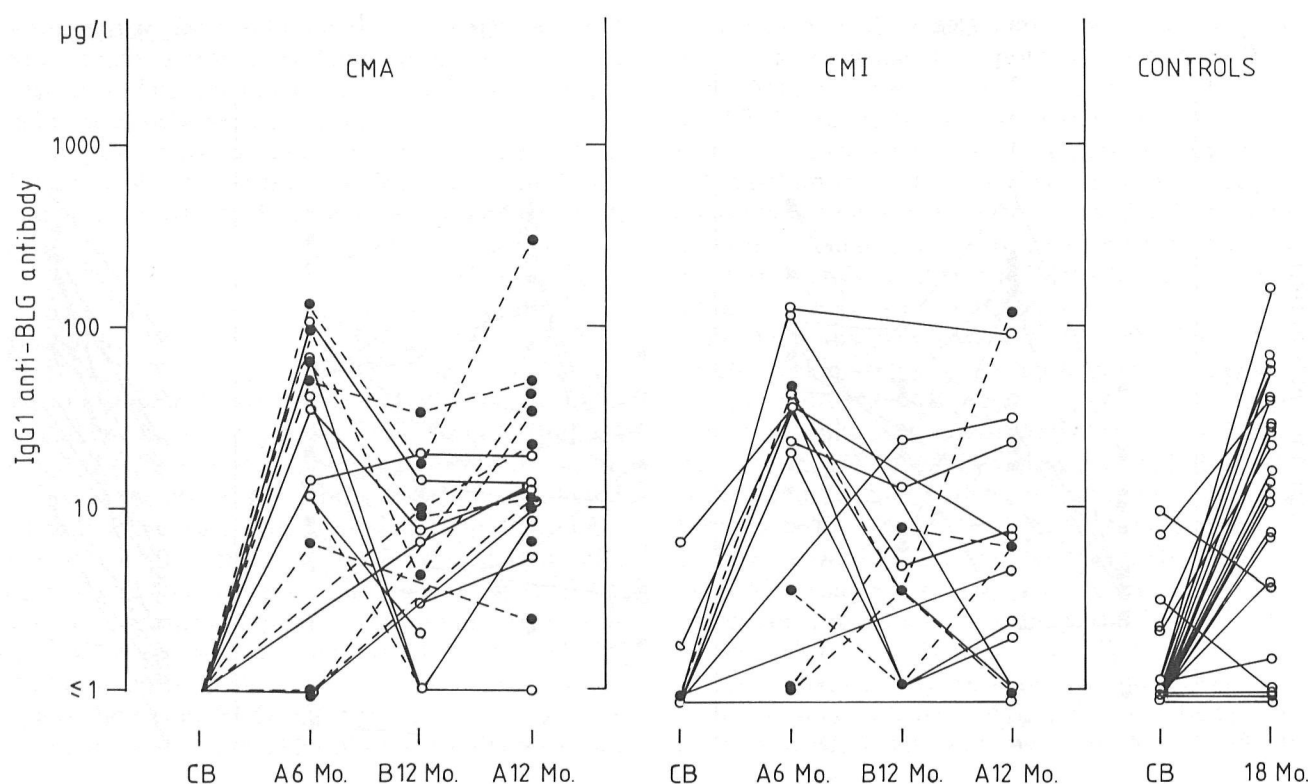


Fig. 3. Levels of IgG₁ anti-BLG antibody in 34/39 infants with cow milk allergy 17/21 with IgE-mediated (CMA) and 17/18 with non-IgE-mediated (CMI) cow milk allergy, and in 25 healthy infants without cow milk allergy (controls). For further explanations see Fig. 1.

Regarding IgG₄ RAST to CM (Table 3) a significant increase was found in the CMA group after milk challenge at 12 months ($p < 0.05$), and the post-challenge levels in the CMA group were significantly higher than the corresponding levels in the CMI group and controls ($p < 0.05$). No other differences were demonstrated between the groups or between the different sampling times.

The distribution of elevated specific IgE antibodies (CRIE) to 5 CMP antigens (Table 4) shows a

high frequency of antibodies to bovine se-albumin and BLG, and less often to IgG, alpha-lactalbumin and lactoferrin in the serum samples from the infants with cow milk allergy, irrespective of the time of sampling (even in cord blood) and irrespective of the type of milk allergy (CMA or CMI). The frequency of these elevated CRIE-AB is significantly different from the occurrence in the 8 control infants (0/8). Further, at the age of 6 months a significantly lower frequency of CRIE-AB was found in the CMI group

Table 4. The frequency of specific IgE (CRIE) antibodies to bovine se-albumin, immunoglobulin, lactoferrin, alpha-lactalbumin and BLG in cord blood, at 6 months (after milk challenge), and at 12 months before and after milk challenge in 21 infants with IgE-mediated milk allergy (CMA), 18 infants with non-IgE-mediated milk allergy (CMI) and 8 controls

	Cord blood		At 6 months			At 12 months			
						Before challenge		After challenge	
	CMA n 18	CMI n 15	CMA n 16	CMI n 15	Controls n 8	CMA n 17	CMI n 13	CMA n 20	CMI n 17
Bovine se-alb	12	11	10	4	0	12	9	15	15
IgG	2	5	3	0	0	4	5	8	10
Lactoferrin	0	0	0	1	0	0	0	5	0
Alpha-lactalbumin	0	0	1	1	0	0	0	5	1
BLG	6	6	8	3	0	4	5	9	13
≥ 1 pos	14/18	11/15	13/16*	4/15*	0/8	13/17	10/13	16/20	16/17

* $p < 0.05$.

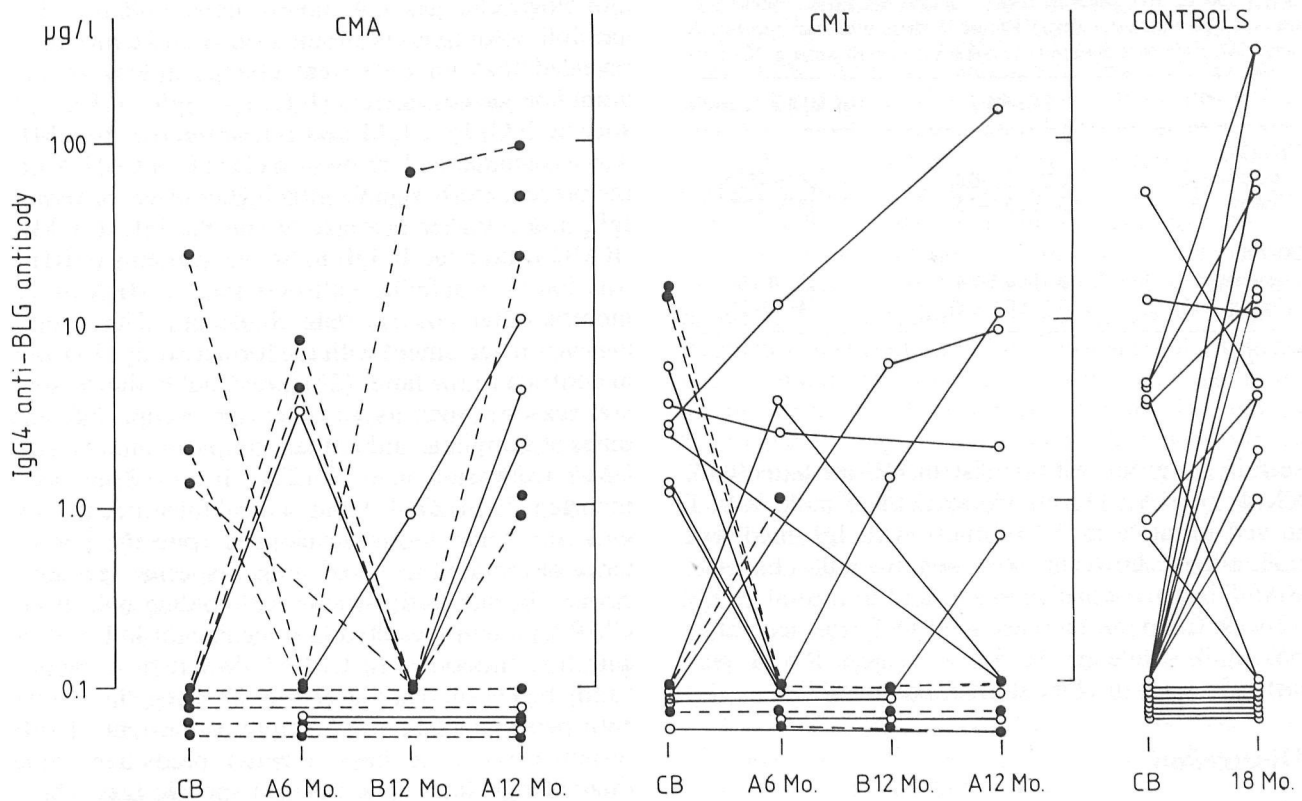


Fig. 4. Levels of IgG₄ anti-BLG antibody in 34/39 infants with cow milk allergy 17/21 with IgE-mediated (CMA) and 17/18 with non-IgE-mediated (CMI) cow milk allergy, and in 25 healthy infants without cow milk allergy (controls). For further explanations see Fig. 1.

compared with the CMA group (4/15 versus 13/16; $p < 0.05$). The total CRIE scores (semi-quantitative) and the relation to type of milk allergy (CMA or CMI) and to subsequent development of tolerance or not to CMP is shown in Table 5. No significant differences are found between CMA and CMI groups or within each group at different sampling times with the exception of a higher CRIE score in infants with

persistent CMA after pos. milk challenge at 12 months.

Longitudinal values of total IgE (Table 6) and specific IgE to CMP (AL-RAST (Table 7)) are shown in relation to the initial type of milk allergy and later acquisition of tolerance to CMP. No difference in CB-IgE between the groups is found. At 12 months a significantly higher level of total IgE is

Table 5. Frequency and total scores () of IgE antibodies (CRIE) to bovine se-albumin, immunoglobulin, lactoferrin, alpha-lactalbumin and BLG in cord blood, at 6 months (after milk challenge) and at 12 months before and after milk challenge in 21 infants with IgE-mediated milk allergy (CMA) and 18 infants with non IgE-mediated milk allergy (CMI) in relation to development of tolerance to CMP at 12 months

			At 12 months		
			Before challenge <i>n</i> 30	After challenge <i>n</i> 37	
Cord blood <i>n</i> 33			At 6 months <i>n</i> 31		
CMA <i>n</i> 21					
I Intolerant	<i>n</i> 12	10/11 (29)	6/8 (25)	7/8 (22)	11/12 (105)*
II Tolerant	<i>n</i> 9	4/7 (4)	7/8 (23)	6/9 (13)	6/8 (21)*
I+II	<i>n</i> 21	14/18 (33)	13/16 (48)	13/17 (35)	17/20 (126)
CMI <i>n</i> 18					
I Intolerant	<i>n</i> 5	4/5 (16)	2/4 (4)	2/4 (13)	4/4 (26)
II Tolerant	<i>n</i> 13	7/10 (23)	2/11 (10)	8/9 (26)	12/13 (51)
I+II	<i>n</i> 18	11/15 (39)	4/15 (14)	10/13 (39)	16/17 (77)

Intolerant (11/12 CMA+4/4 CMI) had significantly higher total scores after challenge compared with tolerant children (6/8 CMA+12/13 CMI), $p < 0.01$.

Table 6. Total IgE levels, median and range () in cord blood, and at 12 months in 21 infants with IgE-mediated milk allergy (CMA) and 18 infants with non-IgE-mediated milk allergy (CMI) in relation to development of tolerance to cow milk protein at 12 months

	CB-IgE (kU/l)	IgE (kU/l) at 12 months
CMA <i>n</i> 21		
Intolerant <i>n</i> 12	0.2 (<0.1–5.0)	56 (9–459)*
Tolerant <i>n</i> 9	<0.1 (<0.1–0.6)	13 (3–27)*
CMI <i>n</i> 18		
Intolerant <i>n</i> 5	0.3 (<0.1–2.4)	12 (11–25)
Tolerant <i>n</i> 13	0.1 (<0.1–0.6)	4 (<2–31)

* $P < 0.01$.

seen in the group with persistent IgE-mediated milk allergy (CMA). During elimination of milk, RAST turned negative in 7/14 infants with IgE-mediated milk allergy; however, after positive milk challenge RAST became positive again, and in a total of 11/12 with CMA an increase in RAST was seen after pos. milk challenge. In 5/9 a positive RAST persisted in spite of clinical tolerance to CM.

Discussion

We have measured prospectively the development of serum IgE, IgG, IgG subclass and IgE antibodies to dietary cow milk proteins and IgG to ovalbumin from birth (cord blood) to 12 months of age in 39 infants with CMPA, of which 22/39 (56%) developed tolerance to milk. No other previous studies have reported such longitudinal data on cow milk-allergic infants already from birth (cord blood), and related the initial antibody levels to development and later remission of the disease. One previous paper (13) reported the development of serum IgE and specific IgE (RAST) to CMP in 47 infants aged 2 weeks to 20 months at the time of diagnosis and followed up for 6 months to 4 years (mean 28 months), and significantly lower levels of RAST to milk were found in children who developed tolerance to milk. Another study (27), reporting the im-

munologic changes in 47 infants aged 3–66 months, and followed up 6–39 months (median 16 months), revealed that no consistent change in any of the immunologic parameters (IgG, IgA, IgM, IgE), and specific IgG, IgA, IgM and IgE antibodies to CMP was associated with remission (18/47) of CMPA. In the present study significantly higher levels of serum IgE, and a higher increase of specific IgE to CMP (RAST) and specific IgE to bovine proteins (CRIE) was found in infants with persistent CMPA at 12 months (after positive milk challenge). These findings are in agreement with the former study (13), but in contrast to the latter (27), presumably due to several reasons, such as an older age group, different times of sampling, and a small sample of infants with CMA (remission in 6/15 (27)). It has often been reported (5, 14, 27, 47) that clinical tolerance to cow milk and other foods develops despite the persistence of elevated levels of antigen-specific IgE antibodies. In our study strictly withholding milk from CMPA patients resulted in a significant fall in specific IgE antibodies to CMP followed by a significantly higher increase after milk challenge in infants with persistent CMA. Therefore, the extent of IgE response seems to have a better predictive value than a single finding of elevated specific IgE, which may occur also in normal infants without clinical disease (23).

Most interesting, in cord blood 25/33 (76%) of infants with later CMPA had specific IgE-AB (CRIE) to bovine milk proteins; however, no difference was found between infants with CMA (14/18) and those with CMI (11/15). At 6 months the frequency of specific IgE-AB was significantly higher in infants with CMA (13/16 (81%), versus 4/15 (27%)). At 12 months higher CRIE scores were found in infants with persistent CMA. These results at 6 and 12 months are in accordance with the RAST to CMP values on the same occasions. The 25/33 (76%) frequency of specific IgE to bovine proteins in cord blood is very high in comparison with previous reports on infants with an atopic heredity (10, 54, 55) giving incidences of 0.4–3.6%.

Table 7. Development in RAST^a to CMP in 21 infants with IgE-mediated milk allergy (CMA) and 18 infants with non-IgE-mediated milk allergy (CMI) in relation to development of tolerance to CMP

	At 6 months Pos RAST CMP After challenge		At 12 months Pos RAST CMP			
			Before challenge		After challenge	
	CMA	CMI	CMA	CMI	CMA	CMI
Pos. challenge	14/21	0/18	5/12	0/5	11/12 ^b	2/5
Neg. challenge	0/21	0/18	5/9	0/13	5/9 ^c	1/13

^a Pos RAST CMP = AL-RAST $\geq$ class 2; ^b 10/11 increase in RAST; ^c 1/5 increase in RAST.

This difference may be due to several factors, e.g. true intrauterine production of specific IgE to bovine protein in this material of newborns (39/1749) with later development of CMPA, "contamination" from maternal blood (materno-fetal), or methodological differences considering our very sensitive, semi-quantitative method (CRIE) versus RAST to whole cow milk protein in previous studies. According to previous reports (22, 56) the CRIE method is able to detect minute amounts of specific IgE to single allergens in human sera, and the sensitivity and the specificity of the CRIE method seem to be comparable with that of RAST (56). Unfortunately, we did not investigate cord blood from controls without later development of CMPA. However, sera from control infants at 6 months of age were without specific IgE to the same bovine proteins (negative CRIE). If prenatal sensitization to CMP is really so frequent, one might have expected clinical reactions at first exposure to cow milk in the sensitized infants, and that did not occur (33). Or, the prenatal sensitization to CMP may be a weak "priming" inducing a low antibody response, which may be "boosted" postnatally (neonatally?) and subsequently followed by development of clinical reactions.

The decreasing levels of IgG anti-OA-AB from birth to 6 months presumably reflect a passive transfer of IgG through the placenta in agreement with previous reports (12, 20, 24, 41). During lactation none of the mothers was on an egg-free diet. Interestingly, significantly higher levels of IgG anti-OA-AB were found in infants with CMA after milk challenge at 6 months (before introduction of egg in the infants' diet) and at 12 months (persistent CMA), indicating a close relationship between IgE and IgG, a relationship previously indicated by other authors (11, 17, 24, 41, 46).

The increasing levels of IgG anti-BLG-AB from birth to 6 months are explained by the fact that all 39 infants with CMPA had been given supplements of cow milk-based formula (33), thus reflecting a normal humoral response to CMP exposure. Likewise, the decrease to 12 months followed by a post milk challenge increase reflect foregoing elimination of cow milk and subsequent milk exposure.

Regarding Ig subclasses to BLG, significantly higher levels of both IgG₁ and IgG₄ were found in infants with CMA at 12 months of age, and significantly higher IgG₁ anti-BLG-AB in infants with persistent CMPA (irrespective of whether CMA or CMI). These findings support the evidence of a relationship between IgE and IgG, indicating a close relationship between IgE and especially IgG₁ and IgG₄ as previously found in infants with a family history of atopy/allergy (41), and regarding IgE and IgG₄ also in non-atopics with helminthiasis (36). Our findings of elevated IgG₄ RAST to CMP (bo-

vine whey fraction) after milk challenge at 12 months in infants with IgE-mediated milk allergy, give support to the supposed relationship between IgE and IgG₄. Our data do not confirm the hypothesis that high IgG levels in cord blood (9) or an increased IgG/IgE ratio (14) may afford protection against CMPA or indicate earlier recovery from CMPA (14).

In conclusion, we found no correlation between cord blood levels of IgG and IgG subclasses to bovine milk proteins and subsequent CMPA, and cord blood IgG was mainly of maternal origin. A high frequency of specific IgE AB to bovine milk proteins in cord blood indicates prenatal sensitization in most infants with later development of clinical reactions to bovine milk protein. A significant decrease of CMP antibodies (both IgE and IgG) after strict elimination of milk and a significant increase of CMP antibodies (both IgE and IgG) after renewed milk exposure merely seems to reflect a normal physiological humoral response to antigen elimination/exposure. However, in infants with persistent cow milk allergy a high antibody response of IgE, specific IgE and IgG subclasses (IgG₁ and IgG₄) to bovine milk protein exposure may indicate a common, possibly genetically governed synthesis of both isotypes in cow milk-allergic individuals.

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