

Infants and children with cow milk allergy/intolerance

Investigation of the uptake of cow milk protein and activation of the complement system

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Seventeen children with challenge-verified cow milk allergy/intolerance (CMAI), age 3-78 months, median 12 months, were re-challenged with cow milk in increasing doses. All subjects developed symptoms, such as bronchospasm, rhinitis, diarrhoea, erythema or eczema. Blood samples were taken before and up to 24 h after the start of the challenge. The cow milk protein β -lactoglobulin (BLG) was determined in serum with ELISA (lower detection limit 0.3 μ g/l). BLG was detectable in five children at low levels (below 2 μ g/l). Analysis of the size distribution of the BLG by size exclusion chromatography indicated immunoreactive material as small aggregates. Plasma samples were analysed by electroimmunoassay for complement factor split product C3d, which was not demonstrable above background values in any of the cases. CMAI in infants and children may not be related to systemic activation of the complement system and may be elicited without considerable amounts of immunoreactive BLG in the circulation.

Key words: cow milk allergy; cow milk protein intolerance; β -lactoglobulin uptake; complement factor split product C3d; ELISA; electroimmunoassay.

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Cow milk allergy/intolerance (CMAI) is a common disorder in infants and children, in particular in the first few years of life (2, 8). A proportion of the patients present with evidence of IgE-mediated reactions, such as positive skin prick tests or radioallergosorbent tests. However, the pathogenesis is unclear in the subjects without signs of IgE-mediated reactions.

Excessive uptake of cow milk antigens has been suggested as a causative factor in CMAI (20), but only few studies have directly addressed the question in man (3). As to the pathogenesis of CMAI, activation of the complement system was indicated in a previous study (13) mainly by measurement of total complement factor C3. However, a study with food-allergic children, who underwent double-blind challenges did not demonstrate significant changes in the levels of complement factors (14).

Deposition of C3 was observed in the duodenal mucosa (18), but also this observation was not confirmed (16). In view of this lack of precise information the purposes of the present study were to quantitate the uptake of the cow milk protein β -lactoglobulin (BLG) and to determine the level of complement activation by the measurement of complement factor split product C3d in infants and children with CMAI.

PATIENTS AND METHODS

Seventeen infants and children with CMAI based on generally accepted diagnostic criteria (8) participated (Table I). The diagnosis of CMAI was confirmed by milk elimination and challenge within 4 weeks prior to the study. The elimination and challenge procedure has been described in detail elsewhere (9). At the chal-

lenge was given up to 160 ml cow milk formula (Allomin) or 66 ml cow milk, corresponding to approx. 2.3 g of protein. Ten infants and children (age range 3–78 months) with immediate clinical reactions (symptoms < 1 h) to cow milk took part in the study (Group I). Subsequently, seven infants and children (age range 7–39 months) with late clinical reactions (symptoms > 1 h) were studied (blood sample obtained only 24 h after the start of the challenge). Serum-IgE and RAST determinations were made by standard procedures. All procedures were performed only after informed consent from the parents.

At the day of the study the participants appeared at the out-patient clinic in the morning. A physician (A.H. or S.H.) was present during the challenge and precautions for the occurrence of anaphylactic reactions were fully taken. The challenge started with 10 ml of cow milk or formula, followed by 20 ml at 1 h, 40 ml at 2 h and so on, up to 160 ml or until symptoms appeared. The parents were allowed to take the infant or child home in the afternoon. The parents were told to report any symptoms during the following days, and if symptoms appeared the child was reassessed at the clinic.

For Group I blood samples were taken from an indwelling cannula before the start of the challenge, at 30 min, 60 min, 120 min and 180 min. If symptoms occurred no further samples were taken. The blood was prepared as plasma with 10 mM/l EDTA and as serum. If the cannula could not be maintained no attempts were made to replace it, as this was considered a further strain to the patients and thus unethical.

The seven children in Group II with late reactions had a blood sample taken 24 h after the start of the challenge.

The quantitative determination for BLG in serum was performed as described in detail previously (6). The lower detection limit of the ELISA was approximately 0.3 µg/l serum. The assay variation (CV%) was within assay 10% and between assay 22% at 10 µg/l, and 11% and 37% at 1 µg/l, respectively ($n = 20$).

High performance liquid gel permeation chromatography (HPLC) was performed with a Toyo Soda TSK SW 3000 G column, which

was combined with ELISA as previously described (6). As MW markers were used thyroglobulin (Tg, 660 kD), IgG (150 kD) and myoglobin (17 kD).

C3d was quantified in EDTA-plasma by double-zone rocket immunoelectrophoresis as described previously (4). Quantification was made in arbitrary units (AU/ml) with reference to a heat-converted serum standard. The between-days assay variation (CV%) was below 4%.

RESULTS

Clinical details. The infants and children who participated in the study all reacted to cow milk at the challenge (Table 1). Only five of the 17 children had a positive prick test or RAST to milk or a high serum level of IgE as signs of atopy. Generally, each child showed more than one clinical sign of CMAI after the challenge. The symptoms and signs consisted of gastrointestinal symptoms (11/17) such as vomiting, colic or diarrhoea, eczema or urticaria (10/17), rhinitis (5/17) and wheeze (4/17). In Group I 4/10 participants had an intake of more than 160 ml of cow milk formula before symptoms occurred. All the subjects in Group II had more than 160 ml of formula before symptoms.

BLG was determined at detectable levels in only two patients in Group I (Fig. 1). One infant (No. 2) showed BLG level of 2 µg/l after 10 ml of cow milk. At this time the indwelling cannula could not be maintained. One child (No. 6) had a low level of BLG in the blood, when symptoms appeared. In Group II demonstrable BLG-levels at low levels up to 2 µg/l were observed in 3/7 subjects.

Estimation of the BLG size distribution. HPLC in combination with ELISA was performed in the patients in Group I. Background levels of high molecular weight BLG were observed before and after the challenge in all the samples. The two subjects with detectable BLG with ELISA alone disclosed an increase in macromolecular immunoreactive material after the challenge.

Subject No.8, who was negative in the ELISA for BLG, also showed (Fig.2) a rise in high-MW immunoreactive material, indicating BLG-containing immune aggregates of an intermediate size (below 700 kD).

C3d-determinations. Measurement of complement factor split product C3d did not reveal any indication of complement activation. All samples contained levels of C3d below 30 arbitrary units (AU/ml), adult normal range 0–40 AU/ml (data not shown). No differences were observed before and after the challenge.

DISCUSSION

The objectives of this study were to investigate the uptake of dietary protein and possible activation of the complement system in infants and children with CMAI. The patient group included a majority of subjects with non-IgE-mediated reactions to cow milk protein, i.e. intolerance. All the participants previously experienced at least one positive clinical reaction, and they all reacted to cow milk at the present

challenge. Low or non-detectable levels of BLG were observed and no indication of increased C3d production was obtained. The size of the BLG observed corresponded to small immune aggregates (Fig.2).

No clinical control group was included in the present study due to practical constraints. We had expected to see a number of negative challenges, which might have formed a negative control group, but all challenges in the present series were positive presumably because only patients with a positive challenge within the last 4 weeks were selected. Due to the nature of the results this does not invalidate the present study.

In a previous investigation of slightly older children with coeliac disease and corresponding controls (7) we observed BLG levels up to 100 µg/l after the intake of 10 ml of cow milk per kg body weight, thus considerably higher levels of dietary protein in the blood. The levels in the coeliac children did not differ when compared with non-coeliacs, but for ⁴/₅ were higher after gluten challenge than on a gluten-free diet. In a study of older children with atopic dermatitis

Table 1

Clinical data and signs of atopy (serum IgE, RAST and skin prick test for cow milk) in 17 infants and children with cow milk allergy or intolerance (CMAI) at challenge with cow milk's formula

Patient No.	Age (months)	Intake (ml)	Symptoms	S-IgE (kU/l)	RAST (0–4)	Skin prick test (±)
Group I (consecutive samples)						
1	3	> 160	V,C,E	< 2	1	–
2	3	> 160	V,E	< 2	1	–
3	5	> 160	W,R	2	0	–
4	5	80	V	ND	1	–
5	17	20	E,D	2	0	–
6	78	10	U,E,W,R	3620	4	+
7	12	80	E,D	2	0	
8	28	> 160	E,D	20	0	
9	19	80	V,D	< 2	0	–
10	4	80	C,D	< 2	0	–
Group II (only 24 h sample)						
11	14	> 160	E,R	15	3	–
12	14	> 160	W,U,E	428	4	+
13	24	> 160	W,R,V	9	1	–
14	19	> 160	E,R	11	1	–
15	39	> 160	V,C	20	0	–
16	13	> 160	E,R	15	2	–
17	7	> 160	C	2	2	–

Abbreviation: V: vomiting, C: infantile colic, D: diarrhoea, W: wheeze, R: rhinitis, U: urticaria, E: eczema, ND: not done.

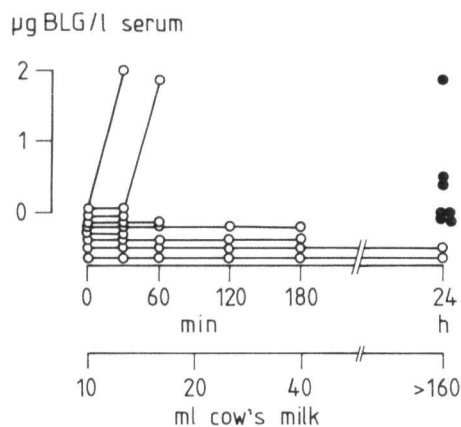


Fig. 1. Measurement of BLG in serum from infants and children with CMAI, after challenge with cow milk or formula. (○) denotes samples from patients followed with consecutive samples (Group I), and (●) patient samples taken 24 h after the start of the challenge (Group II).

Paganelli et al. (15) reported no change in immune complexes containing BLG after milk ingestion, but a rise in antigen-nonspecifically measured immune complexes containing IgA, IgE, IgG and/or complement factor C1q. If similar results were to be found in our patient material changes in the size distribution of BLG-containing aggregates were to be expected, but this was not observed. The observation of low or unmeasurable BLG levels may be a result of a local hypersensitivity reaction in the gut mucosa or possibly in the mesenteric blood circulation. In experimental animals increased passage across the mucosa of "bystander" proteins, but not of the allergen in question, was demonstrated (1, 19), and a similar phenomenon could possibly take place in CMAI, but was not investigated in the present study.

The human milk protein α -lactalbumin was recently introduced as a marker of the macromolecular uptake in infants (11). No antigenic reactions are to be expected against the molecule, which was observed in the blood as well as in the milk of pregnant and lactating women (11). Higher levels of α -lactalbumin were observed after a breast feed in infants with infantile colic than in controls (12). Investigation of the antigen uptake with this probe may be of interest in CMAI.

Activation of the complement system was suggested in an early study of total C3 (13). The appearance of a C3 split product was indicated

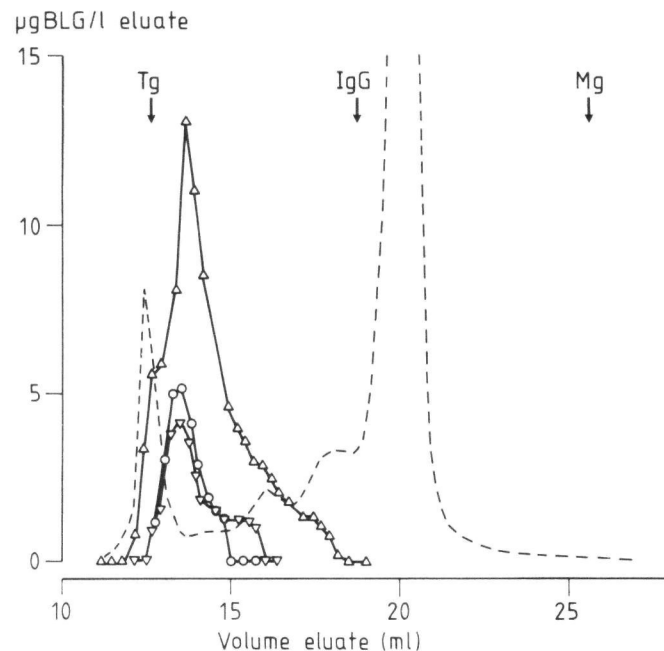


Fig. 2. High performance liquid gel permeation chromatography (HPLC, TSK-SW 3000) in combination with ELISA for BLG determination. Measurement in a child with signs of CMAI more than 12 h after the start of the challenge. (○) denotes analysis before, (▽) 30 min after and (△) 24 h after the start of the challenge. MW-markers include thyroglobulin (Tg, 660 kD), IgG 150 kD) and myoglobin (Mg, 17 kD).

in one case. Also, total C3 levels were reduced in food-allergic children concurrently with the appearance of IgG-ovalbumin immune complexes in serum (10). However, these results were not confirmed in a study of children with various food allergies who underwent a blind food challenge (2). We measured the degradation product C3d, which more precisely indicates complement activation, whereas the levels of total complement factors may fluctuate due to increased production rate or degradation. No indication of complement activation was observed in the present study. However, local complement activation may take place (18), and evidence for local complement activation was recently presented in inflammatory bowel disease (5).

IgE-mediated reactions account for the pathogenesis in only some infants and children with CMAI. A spontaneous release of histamine and histamine-releasing factor was recently demonstrated in patients with atopic dermatitis due to food allergy (17). In patients with food intolerance, as was the majority of infants and children with CMAI in the present study, a further

search for responsible mediators other than those of the complement system seems relevant.

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