

Immunoglobulin E and Immunoglobulin G4 Antibodies to Cow's Milk in Children with Cow's Milk Allergy

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The presence of cow's milk specific antibodies of immunoglobulin E and G4 classes were studied in 47 children with a positive clinical history of cow's milk allergy. The children were challenged with cow's milk orally. The clinical diagnosis was verified by immediate reactions in 25 patients while 22 had late reactions or were provocation test negative in spite of the clinical history. There was no relation between levels of cow's milk specific IgG4 antibodies and provocation test results, i.e. neither with immediate or late reactions. Total IgE was elevated above +1 SD for age in 31 of 41 tested patients. Of these, 29 had immediate type reactions to cow's milk, wheat flour and/or egg white, while only two of 10 children with IgE of less than +1 SD had a demonstrable allergy to any of these foods. The sensitivity of the total IgE determinations for the diagnosis of food allergy was 94% and the specificity was 80%. Specific IgE antibodies to cow's milk were demonstrated in 11 of 14 children with immediate reactions and in three of 15 who were provocation test negative or had only late reactions. This means a sensitivity of 79% and a specificity of 80%. At least one of the four patients with specific IgE but negative provocation test results had earlier shown an immediate reaction when challenged with cow's milk, indicating that the specific IgE antibodies were not truly "false" positive reactions but a consequence of previous allergy. Our results confirm an association between elevated total serum IgE and food allergy and an association between positive RAST to cow's milk and positive provocations in young children. We did not find any evidence for specific IgG4 antibodies playing a role in these patients.

Key words: children; cow's milk allergy; IgE antibodies; IgG4 antibodies.

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CLINICAL ASPECTS

In young children with suspected food allergy elevated serum IgE supports the diagnosis. In this study the sensitivity of the test was 94%. Similarly, demonstration of specific IgE antibodies to cow's milk supports a diagnosis of cow's milk allergy. However, lack of demonstrable specific IgE antibodies does not exclude the diagnosis. Determination of specific IgG4 antibodies remains a research tool of limited clinical significance.

Recent studies have indicated that short-term sensitizing IgG antibodies (STS-A) possess biological properties similar to IgE, i.e. capacity to bind to mast cells and cause degranulation of them upon contact with antigen (9). Although the IgG subclass containing STS-A has not

been identified conclusively, similarities in biological activity between STS-A and IgG4 have been demonstrated (9). Thus, the IgG4 antibodies may represent a class of reaginic antibodies. Other investigators, however, have not been able to confirm this (12).

In infants and young children immediate type hypersensitivity to foods is common. In many children with food allergy no specific IgE antibodies can be detected although a number of investigators have reported an association between immediate hypersensitivity and the presence of serum IgE antibodies (2). In these children, hypothetically, food allergy could be related to the presence of IgG4 antibodies. We therefore decided to study the incidence of both IgE and IgG4 antibodies specific to milk in sera from children with a clinical history suggesting allergy to cow's milk. The results were compared with results of provocation tests.

MATERIAL AND METHODS

Patients

Two groups of patients were studied. Group I consisted of 29 children investigated at the Hospital for Allergic Diseases in Helsinki because of suspected food – usually milk – allergy. Of the children, 27 were less than 3 years old (nine were infants), one was 5 years and one was 8 years of age. The children were initially challenged with small quantities of milk applied on the skin, then on the lips, and finally orally in increasing quantities, which approached those normally consumed. Challenges were performed in the hospital, and in most cases a negative provocation test required 2–3 days. The challenge was usually terminated, when a positive reaction was observed. Positive reactions included worsening of eczema, urticarial reactions, watery nasal discharge, wheezing and bronchial secretion, vomiting and diarrhoea within 4 h. Fourteen of the children reacted to cow's milk and 13 did not. In two cases late reactions were seen more than 24 h after the challenge. The provocation tests were evaluated without knowledge of specific IgG4 assay results and in most cases without knowledge of the results of IgE determinations.

Group II consisted of 18 children, investigated at the Department of Pediatrics, East Hospital, Gothenburg, because of suspected

cow's milk allergy. Of the children 15 were less than 12 months old, one was 13 months and two were 27 and 28 months respectively. All the children were challenged orally in the hospital with cow's milk as described above; 10 showed a positive immediate reaction in the skin (within 1 h), one showed both an immediate reaction in the skin and a late reaction in the gastrointestinal tract and two patients had a late gastrointestinal reaction only. Five of the patients did not react to the provocation test. However, three of them had reacted previously on provocation with cow's milk.

Determination of IgE antibodies

Total serum IgE was determined employing the Phadebas IgE PRIST® (Pharmacia Diagnostics). Specific IgE antibodies to cow's milk were determined by the Phadebas RAST® using commercially available milk allergen discs (group I). For analysis of the sera from the patients in group II special allergen discs were prepared by conjugating the proteins of cow's milk and a commercially available cow's milk based formula (Baby Semp 1, Semper AB, Stockholm) to CNBr-activated paper discs as described by Ceska & Lundkvist (4). A pepsin digested β -lactoglobulin preparation (kindly provided by Dr Bo Lönnerdal, University of Uppsala, Uppsala, Sweden) was conjugated to CNBr activated Sepharose (Pharmacia Fine Chemicals, Uppsala, Sweden) according to the manufacturer's manual. As tracer we used ^{125} I-anti IgE (Pharmacia Diagnostics).

Determination of specific IgG4 antibodies

Specific IgG4 antibodies were determined using a radioallergosorbent test (RAST) technique. Radio-labelled specific anti-human-IgG4 antibodies kindly provided by Dr Terry Merrett (Benenden Chest Hospital, Benenden Cranbrook, England) were used as tracer. These specific sheep anti-human-IgG4 antibodies were originally prepared and isolated by the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam (1).

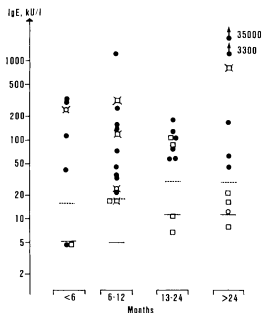


Fig. 1. Total S-IgE in 41 children with suspected food allergy. Patients with immediate-type reactions after challenge with cow's milk and/or wheat flour are indicated by closed circles ($n = 25$) and patients with late reactions to these food items by open circles ($n = 3$). Open squares indicate negative provocation test results ($n = 13$). Of the patients with negative provocation results or with delayed reactions six were skin test positive to egg white (indicated by stripes on the open symbols). Mean values for different age groups are indicated by bars and +1 SD by dotted lines (ref. 10).

The allergen discs used in the assays were Phadebas RAST[®] cow's milk discs (patient group I) or the specially prepared discs described above (patient group II). The test was performed as described by Dr T. Merrett (personal communication), i.e. samples were diluted 1:10 in a solution of 50% foetal calf serum in buffer. Fifty μ l of these diluted samples were then incubated with allergen discs for 1 h on a shaker at room temperature. After washing, the discs were incubated over night with 100 μ l ¹²⁵I-anti-IgG4. After renewed washings the radioactivity bound to the discs was determined. The results were expressed as per cent bound of total activity added (%T). The mean binding to milk discs obtained with sera from 14 normal healthy persons was 12.8%T (SD 4.3).

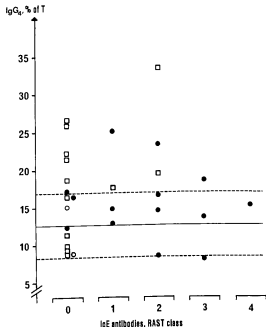


Fig. 2. Cow's milk specific IgG4 (% bound to discs of total radioactivity added) and IgE antibodies (RAST-class) in 29 children (group I) with suspected food allergy. Patients with immediate type reactions are indicated by closed circles ($n = 14$) and patients with late reactions by open circles ($n = 2$). Open squares indicate negative provocation test results ($n = 13$). The results of 14 healthy controls (mean $\pm$ SD) are indicated by full and broken lines.

In the assay performed as above there was no evidence of influence on the results by heterophilic antibodies as verified by inhibition experiments with sheep and pig serum. The presence of foetal calf serum did not block the binding of antibodies to cow's milk proteins, since the results were similar in control experiments using pig serum.

RESULTS

Total IgE was determined in 41 of the 47 patients (Fig. 1). Twenty-nine of 31 sera with elevated S-IgE (i.e. above +1 SD for age) (8, 10) were obtained from children who had immediate type reactions when challenged with cow's milk or wheat flour or were skin prick test positive to egg white. Of the 10 sera with a

Table 1

Relation between Phadebas RAST® and oral provocation test results in 47 children with clinical history of cow's milk allergy

RAST test	Provocation test result			Total
	Immediate	Delayed	Negative	
Positive	17	0	4	21
Negative	8	4	14	26
Total	25	4	18	47

total S-IgE of less than +1 SD only two were obtained from children with one or more of these reactions.

Specific IgE antibodies to cow's milk were demonstrated (RAST score 1–4) in 14 patients in group I; 11 of them also had positive immediate reactions when challenged (Fig. 2). Of the 15 patients lacking demonstrable specific IgE antibodies to milk three had an immediate reaction upon challenge, while two had delayed reactions and 10 were negative.

In the second group of patients, specific IgE antibodies to cow's milk pepsin-digested β -lactoglobulin and milk-based formula were sought for. Such antibodies were found in six of 11 patients with immediate reactions, and in one of the five patients who were negative on provocation. This patient who was provocation test negative at 11 months of age when the specific IgE antibodies were determined had previously reacted to cow's milk when challenged at 6 months of age. The two patients with late reactions also lacked demonstrable specific IgE antibodies. The relation between presence of specific IgE antibodies and provocation test results in the two groups are summarized in Table 1.

The probabilities for a relation between positive immediate provocation test and specific IgE results for groups I, II and I and II combined were $P = 0.0028$, 0.1013 and 0.0009 respectively (Fisher's test). We also calculated the sensitivity and specificity of the specific IgE antibody assays in both patient groups taking a positive

immediate challenge to reaction to cow's milk as evidence of (immediate type) cow's milk allergy. The sensitivity (percentage of allergic patients with positive Phadebas RAST®) was 68% (17 of 25), and the specificity (percentage of non-sensitive patients with negative cow's milk RAST) was 82% (18 of 22) for the two groups combined (Table 1).

There was no relationship between the presence of milk-specific IgG4 antibodies and the results of provocation tests, nor was there a relationship between the presence of IgG4 and IgE antibodies against milk allergens. Fig. 2 shows the relation between specific IgE and IgG4 antibodies in group I using the Phadebas RAST cow's milk discs. The results were similar in group II, in which the relation was studied using the specially prepared discs with cow's milk based formula (data not shown).

DISCUSSION

Previous studies have demonstrated a relation between elevated S-IgE and clinical allergy to respiratory allergens. This is particularly obvious in children (7, 8, 10). However, the presence of elevated S-IgE determinations in food allergy is less well established. In the present study we found that 29 of 31 patients with allergy to cow's milk, wheat flour or egg white had an S-IgE above +1 SD for age. In contrast only two had such elevated IgE levels of the 10 patients with similar clinical history but who were negative when challenged in hospital. The sensitivity of the total IgE assay in our studies was 94% and the specificity was 80%. This may reflect a rather severe disease in the children, but the results also suggest that determination of total S-IgE can be a useful tool when evidence for food allergy is sought.

The findings support the results reported by Bock et al. on 43 patients with suspected food allergy (2). Of these 15 reacted on challenge with the relevant food item. All but one of these patients had an S-IgE above +1 SD for age and 12 above +2 SD.

There was a good correlation between the presence of specific IgE antibodies against cow's milk and positive immediate challenge

reactions. The sensitivity of the method was 79% using the commercially available cow's milk discs, i.e. a negative RAST did not exclude the possibility of cow's milk allergy. The specificity was 80%, indicating that although some false positive reactions will also be seen, a positive cow's milk RAST in this age group supports a diagnosis of allergy to that food. It should also be pointed out that some "false" positive values may indeed be a consequence of a previous allergy similar to the positive test result in one of our patients who was provocation test negative when the blood sample was obtained but had reacted with an immediate reaction when challenged 5 months earlier.

Several authors have suggested that reaginic antibodies not belonging to the IgE class occur in man (3, 5, 9, 11, 14). Such antibodies could possibly belong to subclass IgG4. If such antibodies play a clinically significant role one would expect to find them in patients with a positive history of immediate type hypersensitivity reactions but with no detectable IgE antibodies against the allergen in question. On these grounds, it has been suggested that IgG4 antibodies could play a role in food allergy. In such cases IgE antibodies frequently cannot be demonstrated (14). We therefore studied the presence of IgG4 antibodies to cow's milk in sera from young children and infants all of whom were selected for suspected cow's milk allergy. The final diagnosis of cow's milk allergy was based on provocation tests.

Although there were some technical problems with the IgG4 antibody assay, particularly high non-specific binding, it was obvious that the serum concentrations of IgG4 antibodies specific to milk did not correlate with the results of the provocation tests. Neither did they correlate with serum levels of milk-specific IgE antibodies. Thus we found no evidence that IgG4 antibodies play a role in children with proven allergy to cow's milk; not even in the nine patients with immediate and five patients with late reactions lacking demonstrable specific IgE antibodies.

Alternatively, it has been suggested that IgG4 could act as a "blocking" antibody, which inhibits allergic reactions in the presence of sensi-

tizing IgE antibodies (5, 6, 14). One of the three patients in group I who had milk-specific IgE antibodies and a negative provocation test result, had the highest level of milk-specific IgG4 antibodies in the study. This could indicate the presence of "blocking antibodies". However, the other two provocation test negative patients in group I with milk-specific IgE antibodies did not have particularly high IgG4 levels.

In conclusion, our results confirm an association between positive RAST to cow's milk and positive provocation test results in young food allergic children. We cannot confirm that IgG4 antibodies play a role as reagins in immediate type hypersensitivity in young children with food allergy. Neither do our results suggest that IgG4 antibodies act as blocking antibodies. Our results do not support suggestions that short-term sensitizing antibodies are of subclass IgG4. The results support the conclusion of Aalberse et al. that IgG4 antibodies are not of importance in this context (13).

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