

IgE levels in faecal extracts of patients with food allergy

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IgE levels in faecal extracts (Copro-IgE levels) were investigated in food allergy (FA) patients before and after the challenge test administration of food allergens. IgE levels were measured by time-resolved fluoroimmunoassay. In addition, the effects of administration of oral sodium cromoglycate (SCG) on the Copro-IgE levels were studied. Copro-IgE levels in patients with FA, who were placed on an elimination diet, did not differ from those of healthy children. After a challenge test immediate symptoms of urticaria and wheezing were observed in all FA patients. Copro-IgE levels in each patient increased markedly within 24 h of the challenge test. Moreover, FA patients treated orally with SCG showed neither the increase in Copro-IgE levels nor any remarkable symptoms after the challenge. Our results suggest that the increased Copro-IgE levels may be a specific consequence of the local immune response to food allergen stimulation in the gut mucosa.

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Key words: challenge test; faecal extract; fluoroimmunoassay; food allergy; IgE; secretory IgA; sodium cromoglycate.

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Ingested foods can provoke allergic symptoms of urticaria and wheezing in susceptible individuals, and are an important cause of allergic disease in infancy and young children (2, 5, 15, 18, 25). In most cases IgE-mediated hypersensitivity reactions are responsible for the development of food allergy (FA). However, the local immunity of the gut mucosa in FA is poorly understood. A few studies have reported on the local immunologic response to food allergen in the gut mucosa, which serves as a portal of entry for food allergens. IgE-bearing mast cells are commonly found in the intestinal mucosa of patients with FA, and IgE levels in intestinal secretions and faeces have been demonstrated by paper radioimmunosorbent test (PRIST) in some patients with FA (1, 9, 10, 12, 16).

The purpose of this study was to evaluate the IgE response to food allergen challenge in the gut mucosa in patients with IgE-mediated FA. IgE levels in faecal extracts (Copro-IgE levels) were investigated in FA patients before and after challenge test administration of food allergens, by time-resolved fluoroimmunoassay. This method was developed to measure IgE levels at a lower concentration range than that measured by PRIST. In addition, the effects of administration of oral sodium cromoglycate (SCG) on the Copro-IgE levels were studied.

Material and methods

The study comprised 11 patients with FA, 8 boys and 3 girls, aged from 5 months to 2 years. All patients developed urticaria and wheezing in response to oral challenge with chicken's egg, confirmed by double-blind challenge. Total serum IgE levels were measured by radioimmunosorbent test (Pharmacia Diagnostics AB, Uppsala, Sweden), and serum specific IgE was titrated with the Phadebas RAST (Pharmacia Diagnostics AB). Total serum IgE levels of the patients were raised above normal limits, and the specific IgE RAST to chicken's egg white was positive (range 2+ to 4+). Besides allergy to egg, there were positive IgE RAST to both cow milk and soy beans in three cases and to cow milk in 6 of the 11 patients. Parents of all patients gave verbal informed consent for participation of their children in this study.

Double-blind placebo controlled food challenges were performed in a manner similar to the method previously described by Bock et al. with slight modifications (3). Before the study, parents were instructed to totally eliminate food allergens confirmed by RAST from the children's diet for a period of 2 weeks. Antihistamines, corticosteroids or β -agonists were discontinued before admission. All patients were admitted to hospital for the study period, which lasted for 3 days. On each day small samples (1–2 g) of faeces passed from each child were collected in

plastic containers and frozen within 2 h of production. On day 1 after collection of the initial faecal specimens each patient was challenged with 10 g raw egg white or placebo mixed in juice to hide the taste. After the challenge the medical and nursing staff recorded the occurrence of any symptoms from the skin, respiratory tract, eyes, nose and gastrointestinal tract. On days 2 and 3 patients reverted to their elimination diet.

In 3 of 11 patients with FA the effects of oral administration of SCG were studied. After the first challenge test the three subjects were treated for 30 days with oral SCG at 150 mg/day in three divided doses. After 30 days a second challenge test was carried out. Administration of oral SCG was continued after challenge.

Nonatopic healthy children with no atopic family history, aged from 5 months to 1 year (mean, 1 year and 1 month), served as controls.

Extraction of faeces

Sampling of faeces was done following the method of Kolmannskog et al. (11–13). One-gram samples of frozen faeces were suspended in 10 ml phosphate-buffered saline, pH 7.2, for 30 min, and then centrifuged at 4°C for 30 min at 10 000 rpm. The resulting supernatants were used for determination of IgE levels. Blood was not detected in any of the samples tested with tetramethylbenzidine 0.1%.

Measurement of IgE levels

IgE levels in faecal extracts were measured by time-resolved fluoroimmunoassay DELFIA Total IgE assay (Pharmacia Diagnostics AB, Uppsala, Sweden), as previously reported by us (8). The DELFIA Total IgE assay is a solid phase, two-site fluoroimmunoassay in which two monoclonal antibodies (derived from mice) are directed against two separate antigenic determinants on the IgE molecules.

Briefly, 200 µl of duplicates of IgE standards in 0.01, 0.05, 0.1, 0.5 and 1.0 U/ml dilutions, together with the assay buffer and the 200-µl extracts of faeces, was pipetted into the bottom of each well. The contents of the frame were incubated with slow shaking for 1 h at room temperature. After incubation, each strip was flushed and washed with plate-wash. Next, 200 µl of diluted anti-IgE-europium tracer solution was added to each well after further flushing a few times with the tracer solution, and the contents of the frame were incubated with slow shaking for 1 h at room temperature. After the second incubation, each strip was again flushed and washed with plate-wash. Then, 200 µl of enhancement solution was added to each well directly from

the reagent bottle, and the fluorescence of the content of the frame was measured in the time-resolved fluorometer (Pharmacia Diagnostics AB, Uppsala, Sweden).

Regarding the sensitivity of the DELFIA system, the fluorescence of 0.015 U/ml was at least + 2 standard deviation (SD) of the assay buffer. Values were reliable at levels of 0.015 U/ml or more. According to present results, an IgE concentration of 0.015 U/ml was chosen as the cut-off limit.

Measurement of secretory IgA

Secretory IgA (sIgA) levels in the faecal extracts (Copro-sIgA levels) were measured by enzyme immunoassay using a sIgA column set AMANO (Amano Pharmaceutical Co, Aichi, Japan) (24). Samples were incubated for 2 h with β-D-galactosidase-labeled secretory competent antibody, then passed through a 0.1-ml Sepharose 4B column containing human-IgA antibodies. After the column was washed to remove the unbound label, the buffer in the column was washed and replaced by a solution of o-nitrophenyl-β-D-galactoside and incubated at 25°C overnight. The enzyme reaction was stopped by washing the column with sodium carbonate solution, and the absorbance of the elution was measured at 420 nm. The concentration of sIgA can be detected at a minimum level of sensitivity at 3 µg/ml, without interference from free IgA and secretory components.

Measurement of α₁-antitrypsin (α₁AT)

α₁AT levels in faecal extracts (Copro-α₁AT levels) were measured by nephelometric immunoassay using human-α₁AT antibody (Hoechst Japan, Tokyo, Japan). Samples, passed through a membrane filter, were incubated with human-α₁AT antibody at 25°C for 10 s. Then the scatter of the elution is measured at 840 nm by nephelometer analyser (Hoechst Japan, Tokyo, Japan).

Statistical analysis was made using Student's t test and paired t test.

Results

Measurement of Copro-IgE levels

Two samples of faecal extracts were measured five times on different days. Table 1 shows the mean values ± SD and coefficients of variation (CV) for both cases; good reproducibility was obtained, CV being 5.5% and 5.8%, respectively. Simultaneous reproducibility was investigated with regard to three samples of faecal extracts. Faecal extracts were measured eight times. Table 1 shows the mean values ± SD and CV for both cases; good reproducibility was obtained.

Table 1. Reproducibility of Copro-IgE levels

A Reproducibility of Copro-IgE levels measured 5 times on different days

	Copro-IgE levels U/ml	
	Sample A	Sample B
1	0.114	0.056
2	0.129	0.065
3	0.116	0.058
4	0.127	0.062
5	0.124	0.060
Mean±SD	0.122±0.0067	0.060±0.0035
CV	5.5%	5.8%

B Simultaneous reproducibility of Copro-IgE levels measured 8 times

	Copro-IgE levels U/ml		
	Sample C	Sample D	Sample E
1	0.116	0.056	0.031
2	0.125	0.065	0.036
3	0.114	0.058	0.032
4	0.114	0.062	0.030
5	0.126	0.057	0.029
6	0.128	0.060	0.032
7	0.125	0.056	0.034
8	0.114	0.061	0.033
Mean±SD	0.120±0.006	0.059±0.035	0.032±0.0022
CV	5.0%	5.9%	6.9%

SD=standard deviation, CV=coefficients of variation.

ibility was obtained, CV being 5.0%, 5.9% and 6.9%, respectively.

Challenge test

Table 2 shows clinical details and the observed symptoms in each patient after the challenge. All patients had immediate allergic symptoms, wheezing

Table 2. Clinical data and adverse symptoms to food challenge in patients with food allergy

Patient No.	Age	Sex	Serum IgE (U/ml)	IgE RAST class			Allergic symptom
				CE	CM	SB	
1	5 mo	M	130	3	2	2	U,W,V,S
2	8 mo	M	270	4	1	0	U,W,V,S
3	2 yr 6 mo	M	340	4	2	0	U,W
4	7 mo	F	370	4	2	1	U,W,S
5	9 mo	M	150	4	1	0	U,W,S
6	1 yr	F	220	4	2	2	U,W,S
7	1 yr 5 mo	M	320	2	2	0	U,W,S
8	1 yr 6 mo	M	260	2	2	0	U,W,S
9	1 yr 9 mo	M	160	2	0	0	U,W,S
10	1 yr 11 mo	M	130	2	2	0	U,W,S
11	2 yr 2 mo	F	530	2	0	0	U,W

CE: chicken egg; CM: cow milk; SB: soy bean; U: urticaria, W: Wheezing, V: vomiting, S: soft stool.

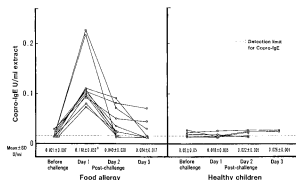


Fig. 1. Copro-IgE levels before and after food allergen challenge test of patients with food allergy and of healthy children. Each post-challenge value was the mean level for each 24-h period after the challenge. SD = standard deviation. * Significant at $P < 0.01$ versus before challenge.

and urticaria within 15 min of administration of chicken's egg. Eight of 11 patients required treatment with bronchodilator and antihistamines. Two patients had vomiting. Although watery diarrhoea did not affect any of the patients following the challenge test, eight patients had a softer stool compared with those before the test.

Fig. 1 shows the changes in Copro-IgE levels before and after administration of food allergens in 11 FA patients and in 5 nonatopic children. Each post-challenge test value was the mean level for each 24-h period after the challenge of each patient. Before the challenge, there was no significant difference between Copro-IgE levels of patients with FA and those of control subjects. In patients with FA, the mean Copro-IgE levels for day 1 after the challenge were significantly higher than those recorded before the test ($P < 0.01$). In contrast, the mean Copro-IgE levels for both day 2 and day 3 after the challenge did not differ significantly from those recorded before the test in FA patients. In control subjects, no

significant differences were found between the Copro-IgE levels before and after the test.

Table 3 shows the Copro-sIgA and the Copro- α_1 AT levels before and after the challenge test in FA patients and controls. The mean levels of post-challenge test were obtained from the maximum of each value found in faecal extracts during the 3 consecutive days after challenge of each patient. Before the test, there was no significant difference between the Copro-sIgA and the Copro- α_1 AT levels of FA patients and those of control subjects. The mean Copro-sIgA levels of FA patients after the test were significantly higher than those recorded before challenge ($P < 0.01$). In control subjects, no significant differences were found between the Copro-sIgA levels before and after challenge. Both in FA patients and controls, there were no significant changes in the Copro- α_1 AT levels before and after challenge.

Effects on administration of SCG

The 3 patients treated with oral SCG (Nos. 1 to 3 in Table 2) had mild itching after the challenge, but none of the symptoms seen after challenge without SCG. Fig. 2 shows the Copro-IgE and the Copro-sIgA levels before and after challenge, with and without oral treatment of SCG. Copro-IgE levels after the challenge with SCG treatment were lower than those without SCG. There were no differences in changes in Copro-sIgA levels with and without SCG treatment.

Table 3. Mean Copro-sIgA and Copro- α_1 AT levels before and after food allergen challenge in FA patients and healthy children

	Copro-sIgA $\mu\text{g/ml}$		Copro- α_1 AT $\mu\text{g/ml}$	
	Before	After	Before	After
Food allergy ($n=11$)	18.0 $\pm$ 8.6	43.5 $\pm$ 16.5*	29.9 $\pm$ 6.9	32.1 $\pm$ 8.5
Healthy control ($n=5$)	24.0 $\pm$ 10.8	28.0 $\pm$ 15.2	28.4 $\pm$ 10.5	30.2 $\pm$ 9.8

Values are expressed as mean $\pm$ SD.

* Significant at $P < 0.01$ versus before challenge.

Copro-sIgA = Copro-secretory IgA, Copro- α_1 AT = Copro- α_1 antitrypsin.

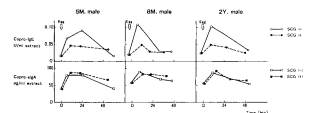


Fig. 2. Changes in Copro-IgE and Copro-sIgA levels before and after challenge in 3 patients with food allergy, with and without treatment with oral sodium cromoglycate. M = months, Y = years, SCG = sodium cromoglycate, Copro-sIgA = Copro-secretory IgA.

Discussion

Kolmannskog et al. have reported that IgE in faecal extracts can be detected by PRIST in some patients with gastrointestinal allergy (11–13). They have shown that the concentrations of IgE in extracts of small samples of dry faeces correlated well with the total daily amounts of IgE. The total daily amounts varied somewhat from day to day, largely within a certain range of concentrations, in each individual case (13, 14). Taking these reports into consideration, we have measured the Copro-IgE levels, in single small samples, of FA patients and control subjects.

The DELFIA system was developed to measure IgE levels of an even lower concentration range than those measured by PRIST. Using a methodology previously described, we are able to measure reliably values as low as 0.015 U/ml of IgE (8). In the present study, we have been able to measure successfully IgE levels in faecal extracts, and have obtained both good reproducibility by measuring on different days, and simultaneous reproducibility of IgE levels in faecal extracts.

In the present study Copro-IgE levels in patients with FA on an elimination diet did not differ from those of healthy children. After a challenge test administration of a known food allergen, immediate symptoms of urticaria and wheezing were observed in all FA patients. Copro-IgE levels in each patient increased markedly within several hours of the challenge test. No symptoms were observed nor was there any elevation of the Copro-IgE levels after egg challenge in control children. These results show that increased Copro-IgE levels and occurrence of allergic symptoms are closely associated in FA patients. In addition, we have investigated α_1 AT levels in faecal extracts before and after challenge. The molecular weight of α_1 AT is 45 000, which is approximately equivalent to the molecular weight of albumin. There were no significant changes in the Copro- α_1 AT levels before and after challenge. It seems unlikely therefore that IgE in the faeces of FA patients is the result of simple diffusion from the blood. Accordingly, it is considered that an increase in Copro-IgE levels represents a specific local immune reaction following food allergen stimulation of the gut mucosa.

Regarding the local immune reaction, it is well known that sIgA acts as a first line of defence against infectious agents and digested allergens in the gut mucosa (6, 7, 9, 23). Kolmannskog et al. have reported that the concentrations of sIgA in faecal extracts were high for many children with gastrointestinal allergy (11). Our patients had the increased Copro-sIgA levels of FA patients within 24 h of administration of food allergens. Increased IgA levels

in nasal secretions during viral infection have been explained by increased local synthesis combined with release of previously formed IgA stored in the local tissues (6, 9). It is not, therefore, unlikely that increased Copro-sIgA levels could be the result of release of previously formed IgA in gut mucosa combined with local synthesis.

It is difficult to determine whether the elevated Copro-IgE in FA patients after food challenge, is released from mast cells or is produced locally by plasma cells in the gut mucosa. It has been reported that there are significantly more IgE-positive cells in the gut mucosa of FA patients (4, 17, 19, 21, 22). Rognum et al. have reported that IgE-positive cells seen in the human intestinal mucosa are mainly mast cells (16). In this present study, the Copro-IgE levels increased within 24 h of challenge, and decreased during day 2. Moreover, FA patients treated with SCG showed neither increased Copro-IgE levels nor any remarkable symptoms after food challenge. It has been reported that the effect of oral SCG is to stabilize gut mast cells and prevent mediator release following food allergen stimulation (20). Treatment with oral SCG did not prevent the observed increase in Copro-sIgA in three food-allergic patients despite preventing the increase in Copro-IgE and their exacerbation of allergic symptoms. It is therefore likely that the elevated Copro-IgE levels in FA patients originate from attached IgE molecules to the surface of gut mast cells following food challenge.

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