Double blind, placebo controlled food reactions do not correlate to IgE allergy in the diagnosis of staple food related gastrointestinal symptoms

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Abstract

Background—The mechanisms for adverse reactions to foods in the gastrointestinal tract are poorly understood. There is conflicting evidence in the literature on the role for IgE mediated allergy in gastrointestinal reactions to staple foods.

Aim—The aim was therefore to study the role of IgE mediated allergy in a group of patients with a history of gastrointestinal symptoms related to staple foods (cows' milk, hens' egg, wheat and rye flour) verified in double blind placebo controlled challenges (DBPCFC).

Patients—Fifteen patients with DBPCFC, identified by screening of 96 consecutive patients referred to our allergy clinic for investigation of suspected gastrointestinal symptoms due to staple foods.

Methods—The screening included diaries as well as elimination diets and open and blinded food challenges. The frequency of atopy were compared between the double blind positive and double blind negative patients.

Results—The positive DBPCFC in the 15 patients included eight patients with milk intolerance, four with wheat flour, two with egg, and one with rye flour. There was no indications of an allergic pathogenesis in all 15 patients with positive DBPCFC, as the skin prick test and radioallergosorbent test were negative for the relevant allergens. The frequency of atopy was four of 21 (19%) in the double blind negative group and three of 15 (20%) in the double blind positive group.

Conclusion—In adult patients with staple food induced gastrointestinal symptoms, objectively verified by DBPCFC, there were no indications of IgE mediated allergy to the relevant foods suggesting other mechanisms in adults than in children. Future studies may include measures of local events in the shock organs in relation to food intake, for instance utilising inflammatory markers in jejunal fluids.

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A history of food related gastrointestinal symptoms is often inconsistent with the results of in vivo or in vitro tests and is difficult to evaluate. As a consequence patients may be

given a too restrictive diet without a sound basis and with a risk of malnutrition as a consequence.¹⁻³ Diagnosis and treatment depend on identification and elimination of provoking antigens. To firmly establish the diagnosis with a rational therapy it is necessary to apply a properly controlled challenge after remission has been obtained on an elimination diet.

Double blind, placebo controlled food challenge (DBPCFC) has been reported as the only conclusive way to establish the presence of adverse reactions to foods in children⁴ as well as adults.⁵ Bock *et al*⁶ have produced a manual for DBPCFC and recommended its use as an office procedure for most patients. Because of absence of definitions of positive and negative reactions and objective criteria to evaluate these reactions and the use of varying number of placebo challenges versus active challenges earlier studies^{4,5} with DBPCFC technique have been difficult to interpret. Therefore, the results from blind challenges performed in accordance with Bock *et al*⁶ should be used to objectively confirm those of open challenges. However, even in cases with reactions to staple foods verified by DBPCFC according to Bock *et al*, underlying mechanisms are unclear. There are conflicting data on the role of IgE mediated allergy in such cases.^{4,5} The aim of this study was therefore to investigate the presence of IgE mediated allergy in patients in whom sensitivity to staple foods was verified with DBPCFC.

Methods

PATIENTS

Ninety six consecutive adult patients, with a history of food related gastrointestinal symptoms and presumed intolerance to at least one staple food, were included in the study. They were referred to the Allergy Centre for allergological investigation. The mean age of the 18 men and 78 women was 40 years, range 17-71. A thorough history using a questionnaire with particular attention to the kind and amount of food necessary to elicit a reaction, reproducibility of symptoms and presence of other presumed atopic diseases, was taken. Eight of these patients chose to interrupt further investigation for different reasons including pregnancy, lack of time, living far from the hospital. Thus, 88 patients remained for further investigation.

All patients with double blind verified milk intolerance were tested for lactose intolerance. This was achieved by an oral dose of 50 g

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lactose, which had to produce a blood glucose of 1.2 mmol or more to be considered as normal. An increase of blood glucose less than 1.2 mmol indicated lactose intolerance.

ALLERGEN EXTRACTS FOR FOOD CHALLENGE

In the food challenge experiments staple foods were selected, such as cows' milk, hens' egg, wheat and rye flour. In the open challenges foods were used in their natural form. In the blind challenges the same foods were used, freeze dried, and pulverised if necessary. Dextrose was used as placebo. Foods as well as placebo were placed in opaque gelatine capsules, size 1, tinted with titanium oxide.

SKIN PRICK TEST (SPT) AND

RADIOALLERGOSORBENT TEST (RAST)

The allergen extracts for SPT were prepared and standardised in the laboratory of the Allergy Centre, Sahlgrenska University Hospital. For the SPT one panel of common alimentary allergens (27 allergens) and another panel of the most common aeroallergens (11 allergens) were used as in an earlier study.⁷ SPT was performed and the reactions were graded according to the recommendations of the Standardisation Committee of Northern Society of Allergology.⁸ In cases with positive open food challenge Phadebas RAST was determined (Pharmacia Diagnostics AB Uppsala, Sweden) adopting the Phadebas RAST scoring system. Atopy was defined as positive SPT ($\geq ++$) for common inhalant allergens.

PROCEDURE FOR FOOD CHALLENGE

Food diary

All patients were instructed to register in a diary abdominal (for example, colic, diarrhoea) and other symptoms, according to a scale from 0 to 3, as well as their intake of foods and drugs during 14 days. They were advised to eat a diet without any restrictions and it was emphasised that they should include foods that they used to avoid but not foods suspected of giving severe reactions. The diary was examined by a dietitian and a physician, dividing the patients into two groups. In group 1 there was a suspected connection between intake of specific foods and symptoms. Group 2 included patients with chronic abdominal symptoms and patients with no obvious relation between foods and symptoms in the diary.

Elimination diet

The purpose of the elimination diet was to make the patients as free as possible from symptoms. The patients in group 1 were given an individual elimination diet excluding suspected foods for one to two weeks. The patients in group 1 who did not recover on their individual elimination diet were introduced to the strict elimination diet following the same procedure as group 2, whereas those

with unchanged symptoms in group 2 were not evaluated further. The patients in group 2 were given a strict elimination diet for one to two weeks consisting of water, potatoes, rice, meat (lamb, elk, veal), sugar and salt and rarely causing intolerance symptoms.

Open oral food challenges

The patients in group 1 were challenged with those foods that were suspected at amounts decided from the diary. The patients kept a diary for 48 hours after the challenge. The patients in group 2, who showed obvious improvement during the strict elimination diet, were challenged after an interval of three to five days with groups of foods (dairy products, cereals, egg, meat, fish, respectively). If symptoms occurred the foods were reintroduced separately. During these periods the patients also kept a diary. For further investigation only dairy products, cereals and egg were chosen and the patients were challenged in the same way as in group 1. The food challenges were always supervised by a physician and a nurse and were carried out in our outpatient clinic. The patients in groups 1 and 2 with no symptoms from the food challenges were advised to reintroduce the previously suspected foods, while those patients who reacted with abdominal symptoms were taken for DBPCFC.

DBPCFC

Each of the suspected foods were investigated with two active and two placebo challenges. Thus, one double blind provocation consisted of two active and two placebo challenges. The amount of food for each blind active challenge was the same as that which elicited symptoms in the open challenge. To evaluate an active blind challenge as positive the symptoms from the open challenge had to be reproduced. That means that the time of onset, the strength, and the duration of symptoms should be closely similar to the open challenge in at least one of the two active challenges. The patients with no symptoms on active blind food challenges, or patients with placebo reactions were told to add the tested foods to the diet.

The open and blind challenges were performed at the outpatient clinic. The patients were advised to keep a diet free from all suspected foods. They had to be almost asymptomatic (symptoms that arose were mild and stable) so that the patients' symptoms elicited by the challenge easily could be distinguished. Before and after open or blind challenges the patients fasted for two hours. After each challenge the patient had to stay at the clinic for a two hour observation period and they were also asked to complete a 48 hour diary recording foods, drugs, and symptoms. Antihistamines and acetylsalicylic acid were withheld during the diary period and for 48 hours before the challenges. The results of positive DBPCFC were compared with the outcome of SPT and RAST.

FLOW CHART FOR PATIENTS SELECTED IN THE STUDY

Eighty eight patients started to register symptoms and intake of foods and drugs (Figure). Of these patients six were not motivated to complete the two weeks' registration and four patients had no symptoms during the period.

Elimination diet

Seventy eight patients remained for elimination diet. Fifty four patients were put on an individual elimination diet and 24 patients were given a strict elimination diet. Eight of 78 patients did not improve on the strict elimination diet and three patients left the study (one

patient moved from the city, another could not tolerate the diet, and the third did not give any reason).

Open oral food challenges

Sixty seven patients were challenged openly. Forty four patients were challenged with one food, 15 patients with two foods, seven patients with three foods, and one patient with four foods. The 99 challenges included 51 with milk, 22 with egg, 19 with wheat, and seven with rye flour. Forty one patients reacted with gastrointestinal complaints similar to their registration from the diary. Of these 41 patients 33 had symptoms caused by one food and eight had symptoms caused by two foods. The 49 positive challenges included 25 with milk, seven with egg, 15 with wheat, and two with rye flour. The remaining 50 food challenges in the other 26 patients were evaluated as negative. These 50 negative challenges included 26 with milk, 15 with egg, four with wheat, and five with rye flour. Ten patients were convinced of symptoms after repetitive intake of foods (milk, six, egg, two, wheat, one, rye, one). Seventeen patients were able to introduce milk, 10 egg, two wheat, and four rye flour. Despite negative challenges some patients continued to avoid milk (three patients), egg (three patients), and wheat (one patient).

The study was approved by the ethics committee of the Medical Faculty of the University of Göteborg.

Results

DBPCFC

Of the 41 patients with positive open food challenges, 36 agreed to participate in DBPCFC. Of these 36 patients 31 had symptoms from one food and five had symptoms from two foods. The 41 food challenges included 21 with milk, six with egg, 13 with wheat, and one with rye flour.

PATIENTS WITH NEGATIVE DBPCFC (n=21)

Twenty one patients were judged to have negative DBPCFC (26 negative challenges). Thirteen of these patients were able to introduce 15 of the 26 foods (milk, nine, wheat, three, egg, three). Three patients had confirmed symptoms after repetitive intake of foods (milk, two, egg, one, and wheat, one). Five patients continued to be on a strict diet despite negative DBPCFC (wheat, five, milk, two).

PATIENTS WITH POSITIVE DBPCFC (n=15)

Fifteen patients reacted with gastrointestinal symptoms similar to their registration from the diary (Table I). The 15 positive challenges included eight with milk, two with egg, four with wheat, and one with rye flour. All eight patients with positive DBPCFC for milk tolerated lactose and SPT and RAST were negative

Flow chart for 96 patients with history of food related gastrointestinal symptoms included in the study.

TABLE I Symptoms related to milk, egg, wheat, and rye in 15 DBPCFC positive patients with a history of food induced gastrointestinal symptoms

Patient	Age/sex	Food	Open challenges	DBPCFC
1	56/F	Milk	D	D
2	50/F	Milk	D	D
3	59/F	Milk	D, AP, N	D, N
4	52/F	Milk	OMB, D	OMB, D
5	56/F	Milk	D, AP, AD	D, AP, AD
6	25/F	Milk	AP, AD, N, F	AP, N, F
7	46/F	Milk	AP, AD, F	AP, AD, F
8	23/F	Milk	D, AP	D, AP
9	41/F	Wheat	D, AP, F	D, AP, AD, F
10	40/F	Wheat	C, R, OMB, AP, D	C, R, AP, D
11	34/F	Wheat	AP, F	AP, F
12	46/M	Wheat	D, AP, AD	D, AP, AD
13	28/F	Rye	AP, AD, F	AP, AD, D
14	50/M	Egg	N, AP, D	N, AP, D
15	47/F	Egg	AP, D	AP, D

AD=abdominal distension, AP=abdominal pain, C=conjunctivitis, D=diarrhoea, F=flatulence, N=nausea, OMB=oral mucosa blebs, R=rhinitis.

TABLE II Clinical features in the 15 DBPCFC positive patients with a history of food induced gastrointestinal symptoms

Patient	Food	SPT/RAST for tested food	SPT		Time of onset (hours)	Duration of symptoms (hours)
			Inhalant	Food		
1	Milk	-/-	+	-	2-3	24-36
2	Milk	-/-	+	+	<2	24-36
3	Milk	-/-	+	+	2-3	12-24
4	Milk	-/-	-	-	<2	12-24
5	Milk	-/-	+	+	2-3	24-48
6	Milk	-/-	-	-	<2	12-24
7	Milk	-/-	-	-	3-4	24-36
8	Milk	-/-	-	+	<2	12-24
9	Wheat	-/-	-	-	5-6	24-36
10	Wheat	-/-	-	-	<2	12-24
11	Wheat	-/-	-	-	3-4	12-24
12	Wheat	-/-	+++	-	4-6	24-36
13	Rye	-/-	+++	-	3-4	12-24
14	Egg	-/-	-	-	2-3	24-36
15	Egg	-/-	+++	-	2-3	24-36

for milk (Table II). However, four of eight patients with positive DBPCFC had positive SPT for inhalant allergens and four patients had positive SPT for other foods. The four patients with positive DBPCFC for wheat had negative SPT and RAST for wheat and one of them had positive SPT for inhalant allergens (*artemisia vulgaris*). The patient with positive DBPCFC for rye flour had negative SPT and RAST for this allergen but had positive SPT for inhalant allergens (*artemisia vulgaris*). The two patients with positive DBPCFC for egg had negative SPT and RAST for this allergen and negative SPT for the other foods. One of the patients was positive for inhalant allergens and one was negative. Of the eight patients with positive challenges for milk four reacted with gastrointestinal symptoms within two hours. The two patients with egg intolerance reacted after two hours. One of the four patients with wheat intolerance reacted within two hours. The patient with intolerance for rye flour reacted later than two hours after food intake (Table III).

The frequency of atopy was four of 21 (19%) in the double blind negative group and three of 15 (20%) in the double blind positive group.

Discussion

This is the first study, to our knowledge, investigating consecutive adult patients with a history of food related gastrointestinal symptoms to evaluate the connection between IgE

mediated allergy to staple foods (cows' milk, hens' egg, wheat, and rye) and gastrointestinal symptoms by means of elimination diet, as well as open and blinded challenges. Only Farah *et al*⁹ have performed a comparable study, but not concentrating on staple foods. In this study only 15 of 36 patients (42%) originally producing an open positive challenge were positive in DBPCFC. Thus, only a small portion of patients believed to be allergic/intolerant to a food really reacted to that food when challenged under controlled conditions, which is in agreement with previous studies.⁴⁻¹¹ It has been claimed that only a proportion of food induced gastrointestinal symptoms are caused by reagenic antibodies.¹² In our study, like that of Farah *et al*⁹ all double blind verified patients with food induced gastrointestinal symptoms lacked detectable specific IgE antibodies to the offending foods. In our study atopy was not overrepresented among the patients with positive DBPCFC. These results are not in agreement with Sampson, working with children,¹³ and Atkins, investigating adults.¹⁴ IgE mediated allergy to staple foods seems to be more common among children.¹³ Atkins, in a few adults, showed IgE mediated allergy to staple foods, which the patients reacted to in DBPCFC. This seems to be a rare phenomenon in adults and other mechanisms behind verified reactions to staple foods must be sought.

Allergic reactions to foods and other allergens may be explained by the presence of profilin, which constitutes a novel family of plant pan allergens.¹⁵ Profilin therefore provides an explanation as to why certain allergic patients display type I allergic reactions with various pollen and even food from distantly related plants. However, how commonly such cross reactions may occur is still not confirmed. There are still no indications that profilin could explain gastrointestinal symptoms related to vegetables rich in profilin. In our study only two of five patients, intolerant to wheat and rye flour, had positive SPT for plant allergens.

Allergy-like mechanisms with no detectable specific IgE in skin or serum might be of importance in some food related gastrointestinal symptoms. In certain patients with adverse reactions to foods, lacking specific serum IgE antibodies, there is evidence for local presence of IgE on mast cells in intestinal biopsy specimens, suggesting one explanation why the presence of circulating or skin bound IgE antibodies alone may not relate well to the occurrence of intestinal allergy (Bengtsson *et al*).⁷ These IgE antibodies may be transported into the intestinal mucosa on mast cells as suggested by Brandtzaeg.¹⁶ Kolmannskog¹⁷ has described the presence of IgE in faeces from patients with negative SPT and RAST and Reimann¹⁸ has found release of tissue histamine with intragastric provocation under endoscopic control in patients with negative SPT and RAST for the tested allergens. However, mediators from mast cells can also be released seemingly independent of any IgE triggering of the cells.¹⁹

TABLE III Clinical atopy related features in the 15 DBPCFC positive patients with a history of food related gastrointestinal symptoms

Patient	Atopic family	Asthma	Rhinitis/ conjunctivitis	Eczema	Urticaria
1	-	+	+	-	-
2	+	+	+	-	-
3	-	+	+	-	-
4	-	+	-	-	-
5	+	+	+	-	-
6	+	-	+	-	-
7	+	+	+	-	-
8	-	-	+	+	-
9	-	-	-	-	-
10	-	-	+	-	-
11	-	+	+	+	-
12	+	+	+	+	-
13	+	-	+	+	-
14	+	-	-	+	-
15	-	+	+	+	-

Recently a good correlation was detected between the capacity of patients' sera to provide antibody mediated cellular cytotoxicity (ADCC) against β lactoglobulin coated red cells and the occurrence of cows' milk allergy in children with immediate, as well as delayed onset of gastrointestinal symptoms.²⁰ Thus, ADCC might be yet another pathogenic mechanism in food allergy. Other types of immune reactions to food proteins, which may be associated with gastrointestinal reactions, are circulating immune complexes.²¹ In addition, there are many T cells within the intestinal mucosa and immunohistopathology, applied to biopsy specimens of intestinal mucosa taken before and after food reintroduction, suggest the presence of T cell mediated immune reactivity to foods within the gut.²²

Patients with positive DBPCFC for milk in this study, we studied after intestinal challenges by a jejunal technique and compared with the reaction in healthy controls. Details of these studies have been described elsewhere.^{23 24} Milk challenges induced significantly increased levels of inflammatory markers such as hyaluronan, eosinophil cationic protein, histamin, and albumin compared with prestimulation values, whereas no such increase were seen in the control subjects. A possible mechanism could be a local intestinal allergy.

In irritable bowel syndrome patients a history of adverse reactions to foods is common. In one study of 101 outpatients with irritable bowel syndrome 67% reported that the symptoms of indigestion were aggravated by food, making a diet necessary.²⁵ Alun Jones *et al*²⁶ suggested that food intolerance plays an important part in irritable bowel syndrome, while others²⁷ found evidence of food intolerance in only three of 27 patients (15%) with irritable bowel syndrome as measured by a double blind technique.

In our study foods containing incompletely absorbed carbohydrates were common causes of inducers of gastrointestinal symptoms. A causal relation between ingestion of food carbohydrates and chronic gastrointestinal distress has been suspected since the early part of this century.²⁸ In a recent review of functional disorders of the lower gastrointestinal tract a causal relation between ingestion of food carbohydrates and chronic gastrointestinal distress was emphasised.²⁹ It is striking that many of the foods found to cause discomfort in patients with a history of functional bowel disease (for example, wheat, potatoes, onions, chocolate, fruit, milk) contain significant amounts of carbohydrates, which may become incompletely absorbed (for example, starch, oligosaccharides, fructose, sorbitol, and lactose). Nanda *et al*³⁰ have shown that 48% of 189 patients with the irritable bowel syndrome improved with elimination diet for three weeks. The foods most commonly incriminated were carbohydrates like dairy products (40%) and grains (39%). Rumessen *et al*³¹ have shown that pronounced gastrointestinal distress may be provoked by malabsorption of small amounts of fructose, sorbitol, and

fructose-sorbitol mixtures in patients with functional bowel disease. These findings may have direct influence on the dietary guidance given to a large group of patients with functional bowel disease and may make it possible to define separate entities in this disease complex. Investigators of food intolerance seldom mention the possible beneficial effects of a reduction in carbohydrate load on abdominal symptoms. It is tempting to speculate that the mechanism behind the unconfirmed gastrointestinal symptoms in our study depends on incompletely absorbed carbohydrates. Systematic studies of the relation between food intolerance and the magnitude of carbohydrate malabsorption/fermentation are lacking.

Other non-immunological mechanisms behind food related gastrointestinal symptoms have been proposed: (1) Abnormal metabolism of histamine.³² Lessof *et al*³³ have shown low levels of diamine oxidase (histaminase) activity in the jejunal mucosa in patients with a history of urticaria and abdominal symptoms. (2) β casomorphine-7, a naturally occurring opioid peptide from cows' milk, is a direct histamine releaser in humans.³⁴ Considerable amounts of β casomorphine were identified in the small intestine contents after ingestion of cows' milk in certain adult humans.³⁵ (3) MacQueen *et al*³⁶ have shown that an important aspect of intestinal hypersensitivity in rats can be subjected to a classic conditioning. There are anatomic interactions between mucosal mast cells and nerves in the lamina propria.³⁷ Therefore, it is interesting to speculate that the neural substrate by which the cognitive conditioning signal affects the mast cells entails connection and interaction between the central pathways and the nerves of lamina propria.

A blind positive challenge should be closely similar to an open positive challenge, in strength, duration, and time of onset of the reaction. For this we used a diary as well as elimination diets, open and blinded food challenges, and compared the results with SPT and RAST. However, it is important to realise the limitations of DBPCFC.³⁸ One reason for this may be that it is unphysiological: (1) During the challenges foods and placebo may be given in capsules, through a nasogastric tube or disguised in a meal. The nasogastric method renders the evaluation of nausea as a symptom difficult. (2) Withdrawal of food from enzymes in saliva, exposing the gastrointestinal tract to less degraded food constituents, may be of importance for the interpretation of the challenges. In addition, there is no general agreement about the amount of foods to use, how to hide taste, smell and colour, time between separate challenges, increase of dose, and how to deal with patients with a history of food intolerance after days or weeks of regular intake of suspected foods (delayed reactions).

The fact that the duodenal fluid absorption rate changes considerably during the interdigestive motility cycle may influence the outcome of food challenges.³⁹ In humans, this cycle, the migrating myoelectric complex,

usually has a duration of one to three hours. The rhythm seems to be generated by the enteric nervous system in lamina propria, but the rate of migration and the relative duration of the different phases are modulated by extrinsic nerves. These nerves connect the enteric nervous system to the central nervous system through sympathetic and parasympathetic pathways. It is perhaps important to challenge at a certain time in the intestinal motility cycle to interpret the challenge in a correct way.

Future studies should include measure of local events in the shock organs in relation to food intake. For example, studying inflammatory markers by a jejunal perfusion technique might be of value to understand different mechanisms behind adverse reactions to foods, in particular as in this study atopy was found only in three of 15 double blind positive patients and the presence of relevant IgE antibodies could not be shown in any patients. However, the basis for studying the mechanisms behind adverse reactions to foods is double blind verified patients. The advantage of our study design, with diaries, elimination diets, open and blinded challenges, is a considerable reduction of the number of blinded challenges.

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