

Intragastral provocation under endoscopic control (IPEC) in food allergy: mast cell and histamine changes in gastric mucosa

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(Received 30 August 1983; accepted for publication 20 July 1984)

Summary

In fourteen patients with food allergy, intragastral provocation under endoscopical control (IPEC) was performed. In all patients positive immediate-type reactions of the gastric mucosa were observed consisting of oedema, erythema and petechial bleeding. Microscopically, mast cell degranulation was observed and measured by mast cell counts using the *o*-phthaldialdehyde technique. Concomitantly, tissue histamine content in gastric mucosa decreased significantly after allergen provocation, while there was no change in normal volunteers. Plasma histamine concentration increased in most patients; the increases were most evident in four patients showing mild systemic reactions (urticaria and bronchospasm). The technique described might prove to be useful in establishing the diagnosis in doubtful cases of food allergy.

Introduction

Immediate-type allergic reactions after ingestion of certain foods are not uncommon. In many cases diagnosis is not a problem since the patient is well aware of the type of food causing his problems. In a wide variety of patients, however, many different foods or food ingredients are suspected of eliciting incompatibility reactions. It is in these cases that it is difficult to detect the relevant allergen (May & Bock, 1978; Speer, 1979; Soothill, 1980; Ring, 1981). Positive skin test reactions are often found to most substances tested without any conclusive causal relationship in the patient's history. Serum food-specific IgE antibodies can be detected by the radioallergosorbent test (RAST); histamine release (from peripheral basophil leucocytes) can be measured after stimulation with the suspected food allergens (Shelley, 1964; Rowe & Rowe, 1972; Gerrard, 1980). These *in vitro* tests often show poor correlations so that a provocation test is necessary to establish the diagnosis. Elimination diet schedules are sometimes helpful but often difficult to evaluate (Rowe & Rowe, 1972; Soifer & Hirsch, 1975). In the classical provocation test in food allergy the patient is given the suspected agent (or

placebo) in a capsule and the general reaction is evaluated according to objective (for example, skin changes, blood pressure, pulse rate) and subjective symptoms (for example, nausea). In rare cases leucocyte and platelet counts have been shown to be helpful (May & Bock, 1978; Speer, 1979). The reaction of the gastrointestinal tract to the oral provocation can be followed by X-ray examination (Werner, 1960) showing gastric mucosal changes and increased persistaltic movement. We studied fourteen patients with known and well-documented food allergy, challenged intragastrically under endoscopic control, and we recorded the visible mucosal changes together with quantitative investigations on the mast cell counts and histamine content of biopsy specimens as well as plasma. The aim was to find out if a direct provocation of the mucosa of the stomach under endoscopic control might be helpful in the diagnosis of food allergy, whilst avoiding severe systemic reactions as they occur after a classical oral provocation test.

Patients and methods

Patients

Fourteen patients (seven male, seven female, age 16–45 years) attending the Dermatology Department of the Ludwig-Maximilians University, Munich, with a history of suspected food allergy (at least three recurring episodes) were investigated. The results were compared to those of ten healthy non-atopic volunteers. Informed consent was obtained from subjects or their parents prior to investigation.

Methods

Skin-prick test were performed on the lower forearm of the patients. If negative, intradermal tests were done. Reactions were read after 20 min., 24 and 48 h; negative (NaCl) and positive (histamine) controls were included.

IgE antibodies. Total serum IgE was measured with the paper radio-immunosorbent test (PRIST), specific antibodies of IgE class against food allergens were measured using RAST (Pharmacia Diagnostics, Uppsala).

Precipitating antibodies. The sera of the patients were investigated for precipitating antibodies against food allergens using Ouchterlony's technique.

Intragastral provocation under endoscopic control. Under endoscopic control (Olympus GIF III), 1 ml of allergen extract dilution or solvent was applied to the mucosa of the dorsal part of the corpus ventriculi. The mucosal reaction was visually monitored by two observers and graded according to its intensity (Table 1).

Table 1. Evaluation of mucosal reaction after intragastral provocation under endoscopic control

Grade	Symptoms
I	Mild erythema (pink)
II	Marked erythema plus swelling
III	Dark red erythema plus petechial bleedings

Biopsy specimens. With a 5 mm forceps biopsy, specimens were taken from the fundus, corpus and antrum prior to allergen provocation. One and 5 min. after allergen application additional biopsies were taken from the corpus mucosa in the allergen-treated area. Biopsy specimens were divided into three samples for histamine determination, toluidine-blue staining for quantification of mast cell counts according to Mohri *et al.* (1978), and for evaluation of histamine-containing mast cells using the *o*-phthaldialdehyde technique, as described by Ehinger, Owman & Sporrang (1968) and Reimann *et al.* (1981). For mast cell counts the specimen was frozen immediately at 70°C and stained with toluidine-blue and treated with *o*-phthaldialdehyde evaporation. For histamine determination specimens were weighed, frozen and homogenized in perchloric acid.

Histamine determination. Tissue histamine levels were determined using a fluorimetric technique according to Lorenz *et al.* (1972). Prior to, as well as 3 min. after allergen challenge, plasma histamine concentrations were determined again using the Lorenz' technique.

Allergen extracts. For skin tests and for intragastral provocation, allergen extracts from Bencard Allergy Service (Johann Wülfing, Neuß, Germany) were used. The allergens used for intragastral provocation included milk, fish, walnut, Crustacea, vegetables (celery, peas, beans) and banana. The same extracts were applied to the gastric mucosa of the healthy volunteers.

Statistical evaluation. If not mentioned otherwise, mean values and standard error of the mean were determined. The statistical significance between differences was evaluated using the parameter-free Wilcoxon test.

Results

Clinical data

The clinical data of our patients, are shown together with the results of skin tests, total serum IgE and RAST (Table 2). The predominant symptom of food allergy in most of our patients was urticaria; five patients had suffered anaphylactic shock. A positive history of other atopic diseases (hay fever, extrinsic asthma) was found in six of the fourteen patients, a positive family history of atopic diseases in nine patients. Seven patients showed increased serum IgE values. In eight patients skin tests were positive; equally in a further eight patients positive RAST results were found.

Precipitating antibodies against food allergens were not detectable in any patient.

Intragastral provocation under endoscopic control (IPEC)

In all of the patients investigated, visible changes of the gastric mucosa followed the application of allergen extract. The reactions were of varying intensity (Table 3) ranging from mild oedema and erythema to massive swelling and petechial bleeding. In four patients mild systemic anaphylactic reactions (urticaria, bronchospasm, diarrhoea) were observed which were easily reversible by symptomatic treatment. In almost all patients, increases in plasma histamine concentrations were observed; these were particularly evident in those patients showing systemic reactions. There was no visible mucosal reaction after application of allergen extracts in normal volunteers.

In most cases the macroscopic reaction of the gastric mucosa started between 20 sec. and 1 min. after allergen application and persisted for approximately 5 min. (grade

Table 2. Clinical and *in vitro* data of fourteen patients with food allergy

Patient	Symptoms	Other atopic disease	Family history of atopy	Suspected allergen	Skin test	RAST	IgE (kU/l)
1	Anaphylactic shock, diarrhoea	R, A	+	Celery	0	n.d.	621
2	Urticaria, bronchospasm	R	+	Pea	+	1	34
3	Urticaria	R	+	Milk	+	1	1010
4	Urticaria	—	—	Fish	0	2	120
5	Urticaria	—	—	Milk	0	0	88
6	Urticaria	—	+	Beans	0	0	33
7	Urticaria	—	—	Celerey	+	n.d.	98
8	Urticaria, dyspnoea	—	+	Banana	+	n.d.	440
9	Anaphylactic shock	R, E	+	Egg	+	3	180
10	Urticaria, angioedema	—	—	Crab	+	1	150
11	Urticaria	—	—	Pea	0	0	48
12	Urticaria	R, E	+	Walnut	+	2	96
13	Anaphylactic shock, urticaria	A, E	+	Walnut	+	4	180
14	Urticaria	—	+	Milk	0	1	52

Table 3. Intra-gastral provocation under endoscopic control: local mucosal changes, systemic reactions and plasma histamine concentrations

Patient	Local reaction grade	Systemic reaction	Plasma histamine (ng/ml)	
			Before	After 3 min
1	III	Diarrhoea, urticaria	0.2	1.9
2	III	Bronchospasm	0.1	0.6
3	I	0	0.2	0.4
4	I	0	0.1	0.3
5	I	0	0.2	0.2
6	II	0	0.1	0.4
7	I	0	0.1	0.3
8	I	0	0.2	0.2
9	II	Urticaria	0.3	0.8
10	III	0	0.1	0.3
11	I	0	0.2	0.4
12	I	0	0.2	0.5
13	I	Urticaria	0.3	1.7
14	I	0	0.2	0.2

I reactions). In three patients (grade III) there was still a visible reaction after 10 min. when the endoscope was withdrawn.

Tissue mast cell counts

Mast cell counts in the gastric mucosa of food allergic patients prior to challenge were significantly higher than in normals (Fig. 1). After challenge with allergen, mast cell counts, as measured by toluidine-blue, showed no change. There was, however, a significant decrease in histamine containing cells as determined by the *o*-phthaldialdehyde reaction (Fig. 2).

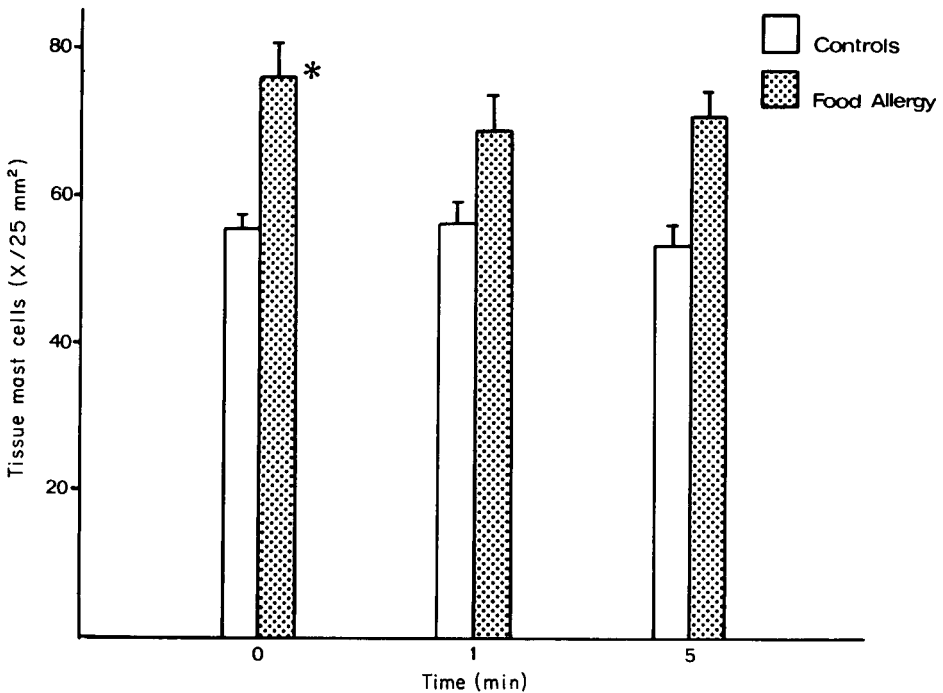


Fig. 1. Tissue mast cell counts (toluidine-blue) of corpus mucosa at different time intervals after intragastral provocation in patients with food allergy ($n=14$) and normal controls ($n=10$).

There was no change in mast cell counts in either technique after provocation with food allergen extracts in normal volunteers (Figs 1 & 2).

Tissue histamine

Histamine concentrations of different parts of the stomach and duodenum did not show significant differences between food allergic patients and normals, although the values measured in the fundus ventriculi and in the duodenum were slightly higher in atotics (data not presented). After allergen challenge in food-allergic patients the histamine concentration of the corpus mucosa showed a significant decrease from 1 to 5 min. after allergen application (Fig. 3). There was no change in tissue histamine in mucosal biopsies from unchallenged areas.

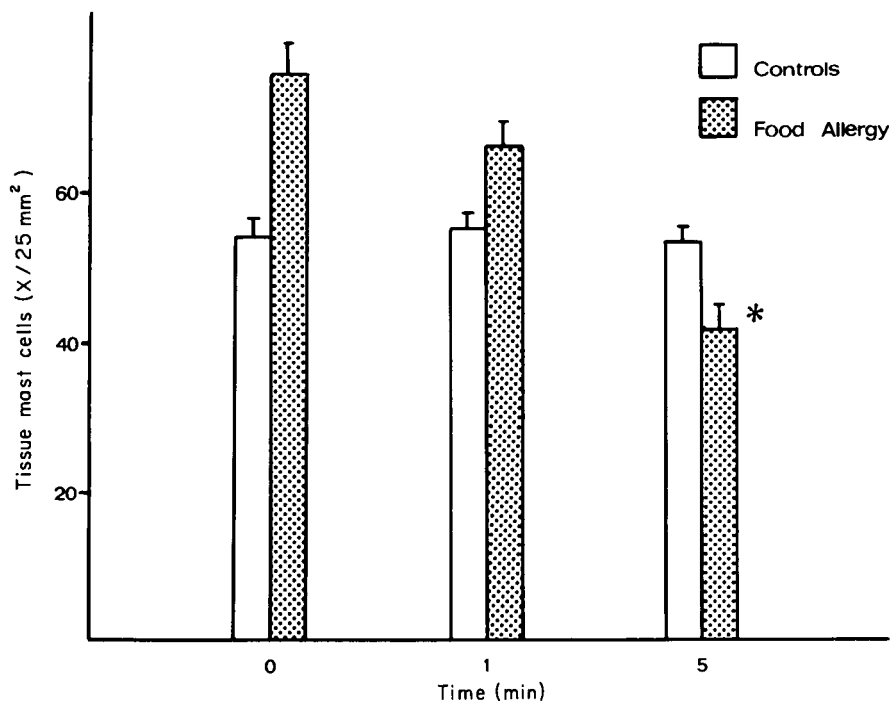


Fig. 2. Tissue mast cell counts (*o*-phthaldialdehyde) of corpus mucosa at different time intervals after intragastral provocation in patients with food allergy ($n = 14$) and normal controls ($n = 10$).

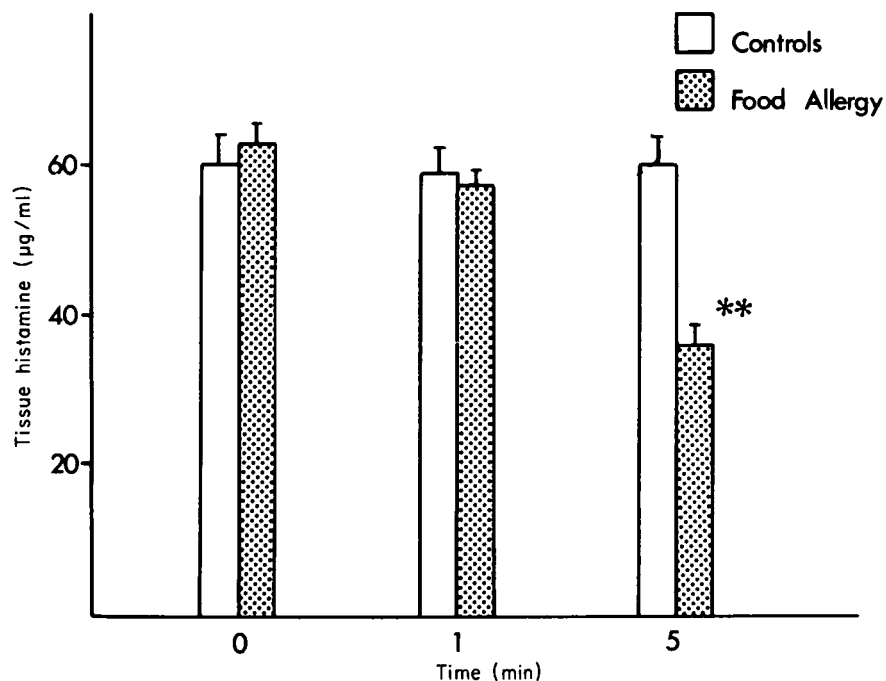


Fig. 3. Tissue histamine content of corpus mucosa at different time intervals after intragastral provocation in patients with food allergy ($n = 14$) and normal controls ($n = 10$). There was a significant ($P < 0.01$) decrease in tissue histamine content 5 min. after allergen challenge in food-allergic patients.

Discussion

The results of this study clearly show that a direct objective evaluation of a provocation test in food allergy is possible by intragastral provocation under endoscopic control (IPEC).

Together with signs of local oedema, erythema and increased vascular permeability of gastric mucosa the histamine content of the tissue decreased while mast cell counts remained unchanged. Control challenges with solvent solution as well as the allergen extract in normal volunteers did not induce similar changes. Mast cell counts and histamine content of gastric mucosa were increased in food-allergic patients compared to healthy controls; this suggests that these parameters may be involved in the pathogenesis of food allergy. Local release of histamine in the gastric mucosa after allergen contact was observed only in allergic patients and verified by both decrease in total histamine content of gastric mucosa as well as decrease in the number of histamine containing (= *o*-phthaldialdehyde-positive) cells. Similar observations have been reported in jejunal biopsies from children intolerant to cow's milk, who showed no increase in total mast cell count per mucosal unit but electronmicroscopical signs of degranulation after oral challenge (Shiner *et al.*, 1975; Kingston, Pearson & Shiner, 1980). Increased jejunal mast cell density was observed in untreated coeliac disease (Shiner & Schmerling, 1972; Kingston *et al.*, 1980).

In our patients the results indicate a local IgE-mediated response involving mast cell degranulation (Austen, 1979). We do not know how long the erythema lasted in each case since the endoscope was in some patients withdrawn before the erythema had disappeared, nor if there occurred 'late-phase mucosal reactions', as they are known from bronchial provocation or skin testing (Solley *et al.*, 1976; Dorsch & Ring, 1981).

It was not the aim of this study to elucidate the mechanism of food allergy. We intended to find a meaningful diagnostic procedure. The patients in our study were selected according to a relatively clear-cut history together with positive skin tests or RAST results in the majority of the cases. The results of IPEC showed a good correlation to history while the skin test and RAST data were sometimes doubtful.

In the classical oral provocation test (food challenge) the patient swallows a certain amount of the suspected allergen which is freeze-dried, powdered, raw or mixed with other foods. Then the clinical symptomatology is registered carefully. However, complaints are often non-specific and subjective, the psychologic influence being considerable. Therefore food challenges have to be performed in a 'blind' or 'double-blind' fashion. The control of laboratory parameters like leucocytes or platelet counts has been recommended to be helpful, but is generally regarded to be a weak parameter of food allergy.

Intragastral provocation under endoscopic control (IPEC) offers the change of an objective evaluation of food challenge. The method is inconvenient for the patient (although probably no more than other diagnostic procedures) but not harmful. It is safer than other ways of food challenge, since a developing reaction is clearly visible and treatment can be stated earlier. Only four of our patients showed mild anaphylactic symptoms; in ten patients the diagnosis of food allergy was established locally without systemic involvement.

Acknowledgements

The technical assistance of Miss Franziska Beier, B. Hammerschmid and Ch. Langer is gratefully acknowledged.

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