Characterisation of immune mediator release during the immediate response to segmental mucosal challenge in the jejunum of patients with food allergy

J Santos, C Bayarri, E Saperas, C Nogueiras, M Antolin, M Mourelle, A Cadahia, J-R Malagelada

Abstract

Background—Food allergy is a common complaint among patients with a broad spectrum of abdominal and extra-abdominal symptoms that must be distinguished from other more common non-immunological food intolerances.

Aims—To investigate whether human intestinal hypersensitivity reactions are associated with detectable release of inflammatory mediators from activated cells, which may serve as a biological marker of true allergic reactions.

Patients/Methods—In eight patients with food allergy and seven healthy volunteers, a closed-segment perfusion technique was used to investigate the effects of jejunal food challenge on luminal release of tryptase, histamine, prostaglandin D2, eosinophil cationic protein, peroxidase activity, and water flux.

Results—Intraluminal administration of food antigens induced a rapid increase in intestinal release of tryptase, histamine, prostaglandin D2, and peroxidase activity (p<0.05 v basal period) but not eosinophil cationic protein. The increased release of these mediators was associated with a notable water secretory response.

Conclusions—These results suggest that human intestinal hypersensitivity reactions are characterised by prompt activation of mast cells and other immune cells, with notable immediate secretion of water and inflammatory mediators into the intestinal lumen. Analysis of the profile of markers released into the jejunum after food provocation may be useful for the objective diagnosis of food allergy.

Keywords: food allergy; intestinal perfusion; mast cells

Adverse reactions to food are a common complaint among adult patients with a broad spectrum of abdominal and extra-abdominal symptoms.¹ ² Food allergy is an immune mediated adverse reaction to food or food additives, which because of its potentially fatal consequences must be distinguished from other more common non-immunological food intolerances.¹ ³ Most allergic reactions to food involve IgE mediated hypersensitivity responses resulting from cross linking of specific IgE, bound to high affinity receptors on the surface of mast cells, to common food epitopes.¹ Animal studies have conclusively shown that mast cells are key effectors in IgE dependent gastrointestinal hypersensitivity reactions.⁵ In humans, evidence of mast cell involvement in such reactions is indirect. Morphological and biochemical studies have shown that mucosal food challenge in sensitised individuals is associated with reduced gut mucosal mast cell numbers or histamine concentrations and mast cell degranulation.⁶ ⁷ In addition, allergic reactions at various mucosal sites are now recognised to occur in association with eosinophil, neutrophil, and mononuclear cell infiltration, which may further contribute to the pathogenesis of allergic diseases.⁸ ¹¹

At present, the diagnosis of food allergy is based on a suggestive clinical history and laboratory testing to detect the presence of food specific IgE antibodies, but confirmation requires a positive oral provocation double blind placebo control food challenge.³ As double blind placebo control food challenge is cumbersome and often relies on subjective symptoms, the diagnosis of allergic gut disease may remain elusive. We reasoned that intestinal hypersensitivity reactions should be associated with detectable luminal release of inflammatory mediators from activated cells. Documentation of in vivo release of these substances may serve as a biological marker of true allergic reactions which can reliably differentiate them from other non-immunological adverse reactions to food. Thus, in this study we investigated the effects of food challenge on luminal release of tryptase, histamine, prostaglandin D2 (PGD2), eosinophil cationic protein (ECP), peroxidase (PO) activity, and intestinal water transport in a balloon isolated jejunal segment.

Patients and methods

SUBJECTS

Seven healthy volunteers with no previous history of gastrointestinal disease and eight patients with suspected food allergy and predominant gastrointestinal symptoms were studied. The study protocol was approved by the institutional review board of the Hospital Vall d’Hebron. Written informed consent was obtained from each participant.

Abbreviations used in this paper: ECP, eosinophil cationic protein; PO, peroxidase; PG, prostaglandin; PEG, polyethylene glycol.
Candidates for the study were initially evaluated by allergy specialists and underwent a full medical history, physical examination, and a battery of prick skin tests (Laboratorios Leti SA, Barcelona, Spain) for 28 common foodstuffs, latex, and inhalants. Histamine and saline were used as positive and negative controls respectively. Prick skin test reactions were read 20 minutes after a challenge. Wheal diameters were measured at right angles. A net wheal equal or superior to that of histamine and 3 mm larger than saline was considered positive. Total serum IgE (CAP system; Pharmacia, Uppsala, Sweden) was determined in every participant, and, in addition, patients were tested for food specific IgE in serum (CAP system RAST FEIA; Pharmacia) if skin tests were positive or if involvement was suspected from clinical criteria.

Table 1 gives the characteristics of the study participants. We used rather stringent criteria to select a group of patients in whom the diagnosis of food allergy was highly probable. All eight patients with food allergy who participated in the study gave a history of food related recurrent gastrointestinal symptoms, alone or in combination with other manifestations including eczema, urticaria, asthma, and angioedema. The spectrum of gastrointestinal symptoms included recurrent unexplained abdominal pain, nausea and vomiting, belching and epigastric discomfort, and diarrhoea. Skin prick tests were positive, at least to one of 28 food allergens tested, whereas all controls were negative. Each patient had gastrointestinal or skin manifestations and at least one other organ was always involved.

Table 1 Characteristics of participants in study

<table>
<thead>
<tr>
<th>No food allergy (n=7)</th>
<th>Food allergy (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M/F)</td>
<td>3/4</td>
</tr>
<tr>
<td>Age range (years)</td>
<td>20–26</td>
</tr>
<tr>
<td>Positive food skin prick test*</td>
<td>0/7</td>
</tr>
<tr>
<td>Total serum IgE range (kU/l)</td>
<td>20–95</td>
</tr>
<tr>
<td>Specific serum IgE range (kU/l)</td>
<td>—</td>
</tr>
<tr>
<td>Clinical manifestations†</td>
<td>0/7</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>0/7</td>
</tr>
<tr>
<td>Asthma</td>
<td>0/7</td>
</tr>
<tr>
<td>Skin</td>
<td>0/7</td>
</tr>
<tr>
<td>Angioedema</td>
<td>0/7</td>
</tr>
<tr>
<td>Anaphylaxis</td>
<td>0/7</td>
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</tbody>
</table>

*All eight patients with food allergy had at least one positive skin prick test among the 28 foodstuffs tested, whereas all controls tested negative for all. Each patient had gastrointestinal or skin manifestations and at least one other organ was always involved.

**JEJUNAL PERFUSION METHOD**

The studies took place in a special unit under strict medical supervision where advanced cardiopulmonary resuscitation equipment was always at hand. The study was designed to be interrupted at any time if a participant decided not to continue or if the doctor in charge judged that a severe reaction was occurring. A modified double lumen closed-segment perfusion technique was used to investigate intestinal biochemical responses. After an overnight fast, a multichannel tube was introduced orally and placed in the jejunum under fluoroscopic control. The infusion port was opened 5 cm distally to the angle of Treitz and the drainage port 60 cm distally. Two additional channels were connected to inflatable latex balloons intended to isolate the intervening jejunal segment and placed just oral and caudal to the infusion and drainage ports. Both balloons were filled with 25 to 40 ml of air to just under the perception threshold. An additional gravity drainage port placed just oral to the proximal balloon prevented the accumulation of gastroduodenal and biliopancreatic secretions.

The isolated jejunal segment was perfused with a water solution containing mannitol 180 mmol/l, xylose 100 mmol/l, and polyethylene glycol (PEG) 4000 g/l, as a non-absorbable marker, at a rate of 5 ml/min using a calibrated volumetric pump (IMED 927; Milton Trading Estate, Abingdon, UK). To limit transmural water flow, the perfusate did not contain glucose or electrolytes. The osmolality of the perfusion solution was 280 mmol/kg, the pH was adjusted to 7.8 with NaOH 0.05 N, and the temperature was 37°C. The appearance of the jejunal effluent was constantly reviewed for the presence of yellow content and, in addition, each sample was analysed for food antigens according to ELISA. Phenylmethanesulphonyl fluoride (Sigma Chemical Co, St Louis, Missouri, USA) 50 mg/ml was added to one set of tubes to prevent in vitro prostanoid generation. Phenylmethanesulphonyl fluoride is known to inhibit trypsin activity, except those for trypsinase analysis as phenylmethanesulphonyl fluoride is known to inhibit trypsin activity.

**INTESTINAL CHALLENGE**

Food antigens were selected according to clinical history and results of skin prick tests. Foodstuffs were lyophilised and the dried powder was vacuum packed in opaque containers until used. Egg ovalbumin (Sigma Chemical Co), one of the most common food allergens in our country, was used as a control antigen in...
healthy volunteers. On a single-blind basis, food allergen (prawn in two of eight, lobster in one of eight, peanut in three of eight, hazelnut in two of eight) or ovalbumin was administered as a bolus (8 g dried food/8 ml 0.9% saline) through the infusion port.

EXPERIMENTAL DESIGN

After the jejunal segment had been rinsed for one hour with the perfusion solution (equilibration period), intestinal effluents were collected at 15 minute intervals over 30 minutes under basal conditions and over 90 minutes after the antigen challenge. Throughout the whole procedure, symptoms and signs were recorded using a standardised questionnaire filled in by the participants. The attending doctor noted any additional signs attributable to the procedure—for example, hypotension.

Four 5 ml blood samples, one immediately before the allergen challenge and thereafter at regular 30 minute intervals, were obtained through an intravenous catheter with a heparin lock placed in the arm. Blood samples were transferred to plastic tubes containing EDTA and immediately placed on ice. Samples were then centrifuged at 1500 \( g \) for 10 minutes at 4°C, and plasma was pipetted into cryotubes which were snap frozen and stored at −80°C until assay.

SAMPLE ANALYSIS

Tryptase concentrations in plasma and intestinal effluent samples were measured by radioimmunoassay (Tryptase RIACT; Pharmacia). Effluent samples were analysed for the presence of histamine by enzyme immunoassay (Immunotech SA, Luminy, France), PGD, by radioimmunoassay (Amersham, Den Bosch, The Netherlands), ECP by radioimmunoassay (Pharmacia), PO activity by enzymatic kinetics, and PEG by turbidimetry.

CALCULATIONS

Net intestinal water flux was calculated by a standardised formula, as follows:

\[
\text{net water flux (ml/min)} = (V \times (\text{PEG}_p/\text{PEG}_a)) - V
\]

where \( V \) is the infusion rate (5 ml/min), \( \text{PEG}_p \) the concentration of PEG in the perfusion solution, and \( \text{PEG}_a \) the concentration of PEG in the jejunal effluent.

Mediator release was calculated using the following formula:

\[
\text{mediator release (units/min)} = (M_a \times (\text{PEG}_p/\text{PEG}_a)) \times V
\]

where \( M_a \) is the mediator concentration in the jejunal aspirate.

PEG recovery in each 15 minute collecting period was obtained from the following equations:

\[
\text{PEG}_a \times (V \times 15 \text{ min}) = \text{PEG infused (A)}
\]

\[
\text{PEG}_a \times V_c = \text{PEG recovered (B)}
\]

\[
\text{PEG recovery (%)} = (B \times 100)/A
\]

Table 2  Comparison of basal jejunal release of mast cell mediators and water flux between healthy controls and patients with food allergy

<table>
<thead>
<tr>
<th></th>
<th>Tryptase (units/30 min)</th>
<th>Histamine (nmol/30 min)</th>
<th>Prostaglandin D(_2) (µg/30 min)</th>
<th>Water flux (ml/30 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>0.46 (0.28–0.54)</td>
<td>5.2 (3.7–7.6)</td>
<td>26.1 (6.7–50.5)</td>
<td>102.1 (27.1)</td>
</tr>
<tr>
<td>Patients</td>
<td>0.39 (0.37–0.51)</td>
<td>5.0 (4.0–6.1)</td>
<td>37.0 (27.4–62.2)</td>
<td>92.0 (41.0)</td>
</tr>
</tbody>
</table>

As tryptase, histamine, and prostaglandin D\(_2\) were not normally distributed they are expressed as median and 25th/75th percentiles. Data for water flux followed a normal distribution and are represented as mean (SD). No statistical differences were found between allergic patients and volunteers for any of these variables at baseline.

Figure 1  Effect of antigen challenge on jejunal release of (A) tryptase, (B) prostaglandin D\(_2\) (PGD\(_2\)), (C) histamine, and (D) water flux. As data for tryptase, histamine, and PGD\(_2\) were not normally distributed, these variables are represented as median and 25th/75th percentiles of six to eight subjects. Water flux data followed a normal distribution and thus each point is represented as mean (SD) from six to eight subjects. Statistical analysis was performed with non-parametric or parametric tests according to the distribution of data. *\( p<0.05 \), **\( p<0.001 \) v time matched controls.
where \( V_t \) is the total volume collected in each 15 minute period.

Intestinal samples with detectable trypsin activity or a PEG recovery index outside the 90–110% range and studies with two or more periods with unacceptable PEG recovery were discarded.

All the formulae used have been previously validated in similar studies in our laboratory.15

STATISTICAL ANALYSIS
A normality test (Shapiro-Wilk W test) was used to determine whether the data followed a Gaussian distribution. Most of the data, except those for water flux, failed this test. Accordingly, these data were analysed for statistical significance of differences with non-parametric tests. In particular, in allergic patients, the Wilcoxon signed rank test (paired data) was used to compare basal output of mediators with that 30 minutes after the challenge. Selected comparisons between allergic and control participants at every 15 minute interval were performed with the Kruskal-Wallis test followed by the Dunn test. Results for water flux were compared by Dunnett and Fisher’s exact test after a significant one way analysis of variance and by paired Student’s \( t \) test. Data are expressed as median and 25th/75th percentiles except those for water flux which are expressed as mean (SD). \( p<0.05 \) was considered significant.

Results

EFFECT OF ANTIGEN CHALLENGE ON JEJUNAL RELEASE OF TRYPTASE, HISTAMINE, PGD\(_2\), AND WATER FLUX
Baseline luminal release of tryptase, histamine, PGD\(_2\), and water flux was similar in healthy volunteers and patients with food allergy (table 2). In healthy volunteers, intraluminal administration of ovalbumin did not modify luminal release of these mediators, whereas jejunal water flux gradually decreased throughout the study (30 minutes: 82.4 (32.5) ml; 60 minutes: 64.8 (15.9); 90 minutes: 52.3 (25.4)) (fig 1).

In patients with food allergy, intraluminal food challenge elicited gastrointestinal symptoms and skin manifestations in each patient and sharply increased the luminal release of tryptase (0.68 (0.61–0.92) units/30 min), histamine (23.1 (16.1–29.4) nmol/30 min), PGD\(_2\) (55.4 (49.8–67.2) \( \mu \)g/30 min), and jejunal water flux (150 (53.2) ml/30 min) in the first 30 minute period after the challenge (\( p<0.05 \)). PO activity gradually returned to basal values by the end of the study (fig 2).

EFFECT OF ANTIGEN CHALLENGE ON PLASMA CONCENTRATIONS OF TRYPTASE
Plasma concentrations of tryptase in healthy volunteers (0.56 (0.5–0.76) units/l) were similar to those of patients with food allergy (0.74 (0.53–1.12) units/l), and were not significantly modified during the 90 minutes after antigen challenge (data not shown).

Discussion
In this study we have characterised biochemical events associated with human intestinal hypersensitivity reactions. The results of our perfusion studies indicate that allergen challenge of an isolated jejunal segment significantly increases luminal release of tryptase, histamine, PGD\(_2\), and PO activity in patients with food allergy as compared with healthy volunteers. The burst release of these mediators into the jejunal lumen was detectable in the first 15 minute period after allergen challenge and, except for PGD\(_2\) and PO activity, was maintained throughout the study.

Increased concentrations of histamine, PGD\(_2\), and the specific mast cell mediator tryptase in various biological fluids have been previously shown in nasal, bronchoalveolar,
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increased basal concentrations of ECP in faeces, tears, and nasal and intestinal territories. 

We also found that increased luminal release of tryptase after food provocation was not associated with significant changes in its serum concentrations. This finding seems to be in contradiction with previous studies showing an increase in circulating concentrations of tryptase after oral food challenge. However, a significant increase in serum tryptase concentrations was only observed at about four hours after food challenge. Likewise, peak concentrations of plasma tryptase after experimentally induced systemic anaphylaxis occurred one to two hours after the challenge, suggesting a slow rate of diffusion away from the tissue of origin. We believe that the short time course of our study may have precluded the detection of increased circulating concentrations of tryptase because of the delayed diffusion of this molecule from its intestinal source.

Allergic diseases at various mucosal sites, including the gastrointestinal tract, have been shown to occur in association with a characteristic pattern of eosinophil granulocyte rich inflammation, which may further contribute to the pathophysiology of allergic diseases. 

In the present study, luminal concentrations of ECP before food provocation were higher in patients with food allergy than in healthy controls, indicating local hyperplasia of the eosinophil population, an activated population, or a mixed pattern. Increased basal release of ECP has been previously detected in intestinal fluid from patients with coeliac or Crohn’s disease, in whom eosinophil populations are notably expanded. Moreover, our finding agrees with those of previous studies showing increased basal concentrations of ECP in faeces from paediatric and adult patients with food allergy, and suggests that infiltration of the jejunal wall by eosinophil granulocytes is a hallmark of human food allergy.

We have also shown that segmental food provocation did not acutely modify luminal ECP concentrations. Previous findings strongly support the suggestion that human eosinophils become activated and degranulate upon both specific (immune challenge) and non-specific (nerve firing, histamine, PGD₂) stimulation in nasal and intestinal territories. In our study, the absence of ECP increase after antigen challenge may be due to late phase release (more than six hours later) of this mediator during hypersensitivity reactions, as has been shown in faeces, tears, and nasal and bronchial fluids. In contrast, non-atopic patients with intolerance to milk or patients with coeliac disease exhibited early increases in intestinal ECP release (20 minutes) after intraluminal antigen provocation. However, the degranulation of eosinophils in these cases may have involved mechanisms other than IgE mediated hypersensitivity, such as IgA or IgG antibodies, which are also potent activating stimuli for the release of ECP.

Our results show that antigen challenge was associated with a notable increase in luminal PO. The classic guaiacol peroxidation assay used in this study does not distinguish between myeloperoxidase and eosinophil peroxidase activities, and it is usually assumed that the PO activity is that of myeloperoxidase and reflects neutrophil influx. In contrast with allergic reactions in skin or airways, where neutrophils are absent in the acute phase and neutrophil specific PO did not increase in bronchoalveolar lavage fluid of asthmatic patients, Wershil et al have shown that IgE mediated reactions are associated with rapid (two hours) neutrophil infiltration of the gastric wall. On the other hand, eosinophil granulocytes are prominent in allergic inflammation, have high peroxidase content and activity, and expand and degranulate rapidly after allergen challenge. Furthermore, eosinophil peroxidase release is not inconsistent with our finding of no increased ECP release after allergen challenge, as not all eosinophil granule proteins are simultaneously released. The differential and selective release of eosinophil peroxidase and ECP has been reported upon IgE, IgA, and IgG mediated activation of eosinophils. Therefore, under the present conditions, the origin of PO release in our human model cannot be established. Immunohistochemical studies and the use of other specific neutrophil or eosinophil mediators would be necessary to address this point.

Animal models of anaphylaxis have shown that intestinal hypersensitivity reactions notably increase water and electrolyte secretion and epithelial permeability to macromolecules. A similar epithelial secretory response has been shown in IgE mediated hypersensitivity in normal human intestine in vitro. In the present study, the increased luminal release of immune mediators was associated with an appreciable water secretory response, and agrees with the results obtained by Knotson et al in atopic patients. Differences in the magnitude of intestinal secretory responses observed in these two studies may be mainly related to the length of the perfused segment and the composition of the perfusion solution, which, in our case, contained mannitol but not glucose or electrolytes to minimise transmural water flux movement and to induce lower rates of water absorption than other commonly used perfusion solutions.

We did not test the response of allergic patients to a control antigen (negative according to our diagnostic protocol). Therefore we cannot exclude the possibility that some of the biochemical responses that we observed were due to unspecific activation of immune cells. However, the highly selected profile of this group of patients, the positive clinical response of all of them to exclusion diets, the detailed selection of the probe antigen (which in each case elicited the highest responses in skin and secretory IgE tests), and the reappearance of symptomatology during the challenge make it...
unlikely that the release of mediators determined here was due to non-specific activation of mast cells and eosinophils.

In conclusion, using a segmental perfusion system, we have shown that immediate intestinal hypersensitivity reactions to luminal antigens are characterised by increased luminal release of tryptase, histamine, PGD₂, and PO activity but not ECP and a considerable water secretory response. These results suggest that prompt activation of mast cells and other immune cells contributes to the immediate functional disturbances associated with such allergic reactions. Analysis of the profile of markers released into the jejunum after food provocation may be useful for the objective diagnosis of food allergy.

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