

ORIGINAL ARTICLE

In vitro model for IgE mediated food allergy

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Abstract

Background. In intestinal food allergy, the non-specificity of gastrointestinal symptoms and the limited access to the reacting organ are the reasons for the limited understanding of the pathophysiology of this disease and the difficulties in establishing an appropriate diagnosis in the individual patient. **Objective.** To develop an *in vitro* model reproducing pathophysiological mechanisms of IgE mediated food allergy. **Methods.** Distal duodenum biopsies of nine patients with food allergy and 10 control subjects were cultured for 3 h with medium alone and with 1 mg/ml of peptic-tryptic digest of wheat gliadin, wheat albumins, and apple proteins. Each biopsy was used for conventional histological examination and for immunohistochemical detection of IgE-positive cells. We have also analyzed the expression of tight junction proteins, occludin, claudin-1, and ZO-1 by immunoconfocal microscopy. Histamine and tryptase release were measured in the culture medium and collected at 0, 30 min, and 3 h of culture using an enzyme and radio immunoassay, respectively. **Results.** Exposure of small intestinal biopsy specimens of patients with food allergy to food allergens led to a significative increase of IgE-positive cells with a significative increase of histamine and tryptase release and an altered expression of tight junction proteins. No differences were found in intestinal biopsies of controls, cultured with or without food antigens. **Conclusions.** Small intestinal organ culture is a functional model of food allergy and could be considered as an *in vitro* oral food challenge, with evident reduction of costs and risks for the patients.

Key Words: Food allergy, histamine, IgE, organ culture, tight junctions, tryptase

Introduction

Food allergy affects about 2–4% of adults and 6% of children [1–3] and occurs mainly in susceptible individuals by IgE mediated hypersensitivity response, resulting from cross linking of specific IgE bound to high affinity receptors on the surface of mast cells, to common food epitopes [4].

In intestinal food allergy, the non-specificity of gastrointestinal symptoms (e.g. diarrhea, abdominal

pain or nausea) and the limited access to the reacting organ are the reason of limited understanding of the pathophysiology of this disease and the difficulties in establishing an appropriate diagnosis in many patients [5,6]. Diagnostic tests like skin prick test (SPT) or measurement of specific IgE antibodies in peripheral blood do not offer an effective tool for the clinical diagnosis of food hypersensitivity. In fact, skin tests with conventional total extracts present low specificity [7] and low allergenic potency [8].

Moreover, the use of fresh foods shows low reproducibility problems due to variations in the allergenic content of the different species [9]. Nowadays, double-blind, placebo-controlled food challenge (DBPCFC) is considered the gold standard, although it does not discriminate between food allergy and non-immune-mediated food hypersensitivity [10]. In addition, DBPCFC is considered time-consuming, costly and sometimes too risky to be used in daily practice [11]. Therefore, an *in vitro* test that could accurately diagnose food allergy would be of great value.

Since the pioneering work of Browning and Trier [12], organ culture of intestinal explants has become an established method in studies on various aspects of metabolism of the small intestine. An organ culture system has the potential to be a more efficient and easy way to measure *in situ* intestinal antibody IgE production and mast cells mediator release with retention of gut structure and normal epithelial cell function for periods up to 24 h or even 48 h.

Therefore, the purpose of the present study was to develop a method to evaluate mucosal behavior in patients with food allergy. These considerations have prompted us to examine the use of an organ culture system of small intestinal biopsies of patients with food allergy as a model of food allergy since food hypersensitivity reactions are associated with luminal intestinal release of inflammatory mediators from activated mast cells.

Methods

Patient recruitment and clinical features of patients

Patients were selected from an outpatient clinic of the Gastroenterology Unit in Padua University amongst subjects who reported gastrointestinal symptoms (diarrhea, abdominal pain, bloating, vomiting) after ingestion of foods containing wheat and apple. Candidates for the study were initially evaluated by allergy specialists and underwent a full medical history, physical examination, and a series of prick skin tests (Bencard, SmithKlineBeecham, Munich, Germany) for common foods, latex, and inhalants. The patients enrolled in this study were selected on the basis of the following criteria: (1) wheat and apple were the foods suspected to cause the gastrointestinal symptoms according to patients' histories; (2) the symptoms disappeared with a foodstuff-free diet and appeared again immediately after the re-introduction of wheat and apple based foods, as proved by open oral challenge; (3) positive SPTs and RAST test to gluten, albumin, and apple; and (4) absence of any

documented gastrointestinal disease, including celiac disease, excluded by serological testing and intestinal biopsies. All patients were measured for total IgA, to exclude IgA deficiency, and IgA anti-tTGase antibodies. To further exclude celiac disease, histological abnormalities of small bowel mucosal architecture were evaluated using Marsh modified classification [13]. Moreover, the patients underwent an intestinal permeability test in order to exclude the possibility that any intestinal damage was present. Additional examinations, including endoscopy and histology of the upper and lower gastrointestinal tract, were conducted. Patients with macroscopic alterations of the mucosa or with histological sign of acute inflammation were excluded from the study, as well as those suffering from previous anaphylactic reactions, pregnant women, those affected by mastocytosis, severe cardiopulmonary disease, current infections, endocrine diseases, and those who did not follow the study protocol. The study was approved by the Regional Ethics Committee. Written informed consent was obtained from each participant in the study.

Patients who underwent small intestinal biopsy as a part of routine investigation and who had no previous or current history of gastrointestinal disease were enrolled in the study as controls. Patients were instructed to eliminate foodstuffs involved in positive reactions for two weeks before the *in vitro* food challenge test with improvement of clinical symptoms and abolition of acute episodes during the elimination diet in all cases. Acetylsalicylates, non-steroidal anti-inflammatory drugs, steroids, immunosuppressants, antihistamines, anticholinergics, and mast cell and eosinophil stabilizers (ketotifen, nedocromil, and cromolyn) were prohibited for at least two weeks before the study. Each subject underwent specific IgE detection and SPTs.

Specific IgE detection and skin prick tests

The detection of specific IgE to offending foods was performed for sera of all subjects with the ImmunoCAP-system (CAP-System-FEIA, Pharmacia Diagnostic, Uppsala, Sweden). The results were expressed as CAP scores from class 0 to 6, according to the manufacturer's instructions. SPTs were performed using commercial allergens to the common inhalants (Lofarma, Milan, Italy) and a wheat flour in-house extract prepared by suspending 1 g of flour in 5 ml of saline buffer. Histamine phosphate at 10 mg/ml and glycerol saline solution were used as positive and negative controls, respectively. The SPT was read after 15 min; the wheal reactions were

outlined with a marker and transferred to a paper with transparent tape. The wheal size was measured using the formula: $(D + d)/2$ (D is the maximum diameter and d its perpendicular diameter) and a positive SPT was defined as a wheal size ≥ 3 mm [14].

Food antigen preparation

We selected three allergens: wheat gluten, wheat albumins, and apple proteins.

The gluten proteins were extracted from whole meal bread wheat flour (*Triticum aestivum*, variety Spada) by stirring for 1 h 20 g of flour in 100 ml of 70% aqueous ethanol. After centrifugation, the supernatant containing the gliadin fraction and part of the glutenins was exhaustively dialyzed against 0.05 M acetic acid and then freeze-dried. In order to overcome the poor solubility of wheat gliadins in buffered solutions, we prepared a peptic-tryptic digest of these proteins (PT gliadins) called Fraction III according to the method of Frazer et al. [15].

Albumins were extracted dissolving 1 g of wheat flour (*Triticum aestivum*, Spada variety) in 10 ml 0.5 M NaCl for 2 h at room temperature. Insoluble material was removed by centrifugation at 12,000 rpm for 10 min. The supernatant was collected, lyophilized, and freeze-dried at -20°C . Apple proteins were extracted dissolving 10 g of apple pulp in 40 ml of cool acetone at -80°C overnight. After centrifugation at 12,000 rpm for 10 min, apple extract was washed with cool acetone for 10 min and centrifuged again at 12,000 rpm for 10 min. The pellet was dissolved in acetone/diethyl ether (1:1, v/v). The suspension was centrifuged at 12,000 rpm for 10 min and the supernatant was freeze-dried at -20°C .

In vitro food challenge test

Five biopsy specimens from the duodenum were obtained during gastroduodenal endoscopy and placed immediately in ice-chilled saline solution, and then processed for *ex vivo* organ culture.

One specimen was immediately snap frozen and stored at -80°C to record initial histology. The remaining ones were cultured as previously described [16].

In brief, small intestinal biopsy specimens were cultured for 3 h, one with medium alone, one with *Triticum aestivum* PT gliadins (1 mg/ml), one with *Triticum aestivum* albumins (1 mg/ml), and one with apple proteins (1 mg/ml). At 30 min of culture, 400 μl of supernatant were removed for determination of histamine and tryptase concentrations, stored at -80°C , and replaced. After incubation, the biopsy specimens were immediately snap

frozen and stored at -80°C . The culture supernatants were collected and stored at -80°C .

Determination of histamine and tryptase release

Histamine and tryptase release were measured in the culture medium, and collected at 0, 30 min, and 3 h of culture using an enzyme immunoassay (Immunotech SA, Luminy, France) for histamine detection and a radioimmunoassay (UniCAP Tryptase, Pharmacia Upjohn) for tryptase determination.

Immunohistochemistry

Cryostat sections (5 μm) of each sample were fixed in cold acetone for 10 min and then air-dried for 30 min. After blocking the background staining with normal goat serum, tissue sections were incubated for 1 h at 37°C with mAbs anti-human IgE (1:50; Dako) and mAbs anti-human tryptase (1:200; Dako), diluted in PBS-Tween (0.05%) containing 2% BSA. Reactions were viewed using an ABC detection kit (Vectastain, Vector Lab., USA) with 3,3'-diaminobenzidine (Dako, UK) as chromogen, and then sections were counterstained with Mayer's hematoxylin.

Small intestinal mucosa mast cells and IgE-positive cells were counted in at least four different areas and examined blindly by a second observer under optical microscopy under $\times 400$ magnification. Immunohistological analysis of IgE-positive cells was then carried out by optical microscopy using a computer assisted image analyzer.

The IgE immunoreactivity was evaluated by the "CIRES" workstation (Zeiss, Jena, Germany) image analysis system consisting of a conventional light microscope (Axiocope model, Zeiss, Jena, Germany) connected to a 3 CCD color video camera (KY-F55BE, JVC, Japan). The images were captured by a frame grabber (Kontron, Eching, Germany) and then analyzed. During all measurement sessions, illumination was kept constant at fixed value and the stray light effect reduced by Koler's illumination setting. Ten non-overlapping frames of the small intestinal mucosa were captured randomly from the biopsy sections that were stained with mAbs anti-human IgE, at magnification $\times 20$. A quantitative evaluation of the relative number of IgE containing cells was determined by determining the difference between the percent positive picture in the control tissue incubated with normal rabbit serum (background) and in the sample treated with rabbit anti-IgE. The results were expressed as a mean of relative positive area (positive area over total) in each field, as previously described [17].

Immunofluorescence and confocal microscopy

Since mast cell degranulation increases paracellular permeability [18], we have also analyzed the expression of tight junction proteins, occludin, claudin-1, and ZO-1 by immunofluorescence microscopy in small intestinal biopsies of controls and allergic patients, cultured for 3 h, with or without food antigens.

Cryostat sections (5 μm) of frozen tissues were mounted on polylysine-coated slides, fixed in 4% paraformaldehyde, and permeabilized with 0.01% Triton-X. Rabbit primary antibody, ZO-1, occludin, and claudin-1 (Zymed Laboratories, CA, USA) were diluted 1:500. Incubation with antibodies was performed as previously described [19]. Specimens were examined with a laser scanning confocal microscope (LSM 510, Carl Zeiss, Inc., Thornwood, NY, USA) equipped with an argon laser and a helium-neon laser for double fluorescence at 488 nm and 543 nm.

For morphometric analysis, all aspects of tissue processing, immunolabeling, and imaging were strictly standardized.

Digital images were collected by adjusting the sensitivity of the photomultiplier according to the fluorescence intensity of the positive control section from non-treated samples in each immunolabeling run, and the fluorescence intensities in the positive control sections were adjusted within the 4096-level scale. Section planes were obtained using $\times 60$ objective lens and a zoom setting of 1.5 (800×600 pixels corresponds to $45,000 \mu\text{m}^2$) under identical conditions, i.e. laser power = 35 mW, pinhole size = 100 μm , ND filter = 20%, and scanning speed = 23.1 s/frame. Photomultiplier voltage was adjusted from 640 V to 680 V according to the intensity of the positive control image of the series to standardize the condition. For morphometric analysis of tight junction expression, a single optical plane was selected and four images of villi and four images of crypts were obtained from each section. Altogether, 10 sections were used for each timepoint. The images were converted into 256 grayscale to quantify the fluorescence intensity of tight junctions immunofluorescent staining and analyzed using the Scion image program (Scion Corporation, Frederick, MD, USA). The data for morphometric analysis of tight junction expression were expressed as both the total grayscale value of tight junction fluorescence per cell and the number of tight junction-positive pixels per cell.

Statistical analysis

Morphological and immunohistochemical measurements were all taken blindly by a single operator in

order to reduce interobserver error. The Mann-Whitney U-test and the Friedman ANOVA sign test were used to make statistical calculations. The level for significance was set at $p < 0.05$.

Results*Patients*

A total of 19 subjects were enrolled in this study. A diagnosis of food allergy, based on allergologic history, skin prick, and RAST tests to wheat gluten, wheat albumins, and apple, was established in nine patients (five male; mean age 41 ± 12). Gastrointestinal symptoms after ingestion of offending foods were diarrhea (66%), abdominal pain (33%), bloating (11%), vomiting (11%), and facial edema (11%). Histological analysis of intestinal biopsies in allergic patients showed the following features: no abnormal findings in five, mild chorion edema with moderate eosinophilic infiltrate in two, edema in the Lamina Propria in one, and an extensive cell infiltrates, which included eosinophilic and mast cells in Lamina Propria in one patient. Moreover, all the allergic patients were characterized by the presence of mild gastritis.

Ten subjects (four male; mean age 43 ± 19) who reported a negative allergologic history and investigations were enrolled as controls. Histological diagnosis of control subjects revealed normal duodenal histology in four and mild chronic duodenitis in six patients. All these patients had mild gastritis.

All the patients and controls had levels of total plasma IgA within the normal range and negativity of anti-tTGase antibodies.

Histamine release by small intestine mucosa

In patients with food allergy, a significant increase in histamine production was detected in culture media of mucosa explants treated with food allergens after 30 min ($16.64 \pm 10.02 \mu\text{mol/l}$ vs. $1.49 \pm 1.3 \mu\text{mol/l}$; $p < 0.001$) and 3 h ($9.71 \pm 5.05 \mu\text{mol/l}$ vs. $2.75 \pm 2.79 \mu\text{mol/l}$; $p < 0.001$) of culture, with respect to release at the same time in control subjects. Histamine release was also significantly modified during 3 h culture with food antigens ($16.64 \mu\text{mol/l} \pm 10.02 \mu\text{mol/l}$ vs. $9.71 \mu\text{mol/l} \pm 5.05 \mu\text{mol/l}$; $p = 0.004$) (Table I). The increase is independent of the type of allergen (Figure 1).

On the contrary, the release of histamine in culture media without antigens after 30 min was similar in healthy volunteers and patients with food allergy and

Table I. Histamine release in patients with food allergy and controls after challenge with food allergens.

Patient group	N	Medium (30 min)	Food allergens (30 min)	Medium (3 h)	Food allergens (3 h)
Food allergic patients	9	6.375 $\mu\text{mol/l} \pm 3.17 \mu\text{mol/l}$	16.64 $\mu\text{mol/l} \pm 10.02 \mu\text{mol/l}^*$	3.73 $\mu\text{mol/l} \pm 2.80 \mu\text{mol/l}$	9.71 $\mu\text{mol/l} \pm 5.05 \mu\text{mol/l}^{§**}$
Controls	10	2.45 $\mu\text{mol/l} \pm 2.9 \mu\text{mol/l}$	1.49 $\mu\text{mol/l} \pm 1.3 \mu\text{mol/l}$	2.85 $\mu\text{mol/l} \pm 4.39 \mu\text{mol/l}$	2.75 $\mu\text{mol/l} \pm 2.79 \mu\text{mol/l}$

Data are expressed as mean $\pm$ standard deviation.

* $p < 0.001$ (16.64 $\mu\text{mol/l} \pm 10.02 \mu\text{mol/l}$ vs. 1.49 $\mu\text{mol/l} \pm 1.3 \mu\text{mol/l}$); ** $p < 0.001$ (9.71 $\mu\text{mol/l} \pm 5.05 \mu\text{mol/l}$ vs. 2.75 $\mu\text{mol/l} \pm 2.79 \mu\text{mol/l}$); § $p = 0.004$ (9.71 $\mu\text{mol/l} \pm 5.05 \mu\text{mol/l}$ vs. 3.73 $\mu\text{mol/l} \pm 2.80 \mu\text{mol/l}$).

was not significantly modified during 3 h of culture (Table I).

No differences in histamine levels were found when intestinal mucosa from controls was cultured in the presence of medium with or without food antigens (Table I).

Tryptase release by small intestinal mucosa

Exposing intestinal biopsies from allergic patients to food antigens induced a significant increase in tryptase release at 3 h of culture with food antigens with respect to culture with only medium (12.24 $\mu\text{g/ml} \pm 8.84 \mu\text{g/ml}$ vs. 4.335 $\mu\text{g/ml} \pm 3.31 \mu\text{g/ml}$; $p = 0.046$) and also with respect to release at the same time in control subjects (12.24 $\mu\text{g/ml} \pm 8.84 \mu\text{g/ml}$ vs. 6.06 $\mu\text{g/ml} \pm 4.34 \mu\text{g/ml}$; $p = 0.046$) (Table II).

No differences in tryptase levels were found when intestinal mucosa from controls was cultured in the presence of medium with or without food antigens (Table II).

Morphometric analysis

Percent tissue area labeled by IgE-positive cells in the biopsy specimens from the food-allergic patients was higher than of the control group (2.18% $\pm 1.41\%$ tissue area vs. 0.21% $\pm 0.21\%$ tissue area), when incubated with medium only, but the difference was at the threshold of statistical significance ($p = 0.052$, Table III).

When biopsies were incubated with food allergens, percent tissue area labeled by IgE-positive cells was significantly elevated in the biopsy specimens of allergic patients when compared with biopsy specimens of controls (2.485% $\pm 1.19\%$ tissue area vs. 0.34% $\pm 0.31\%$ tissue, $p < 0.001$) (Figure 2). The increase was not correlated with the type of antigen (Figure 3, Table III).

The number of duodenal IgE-positive cells, determined with optical microscopy, correlated significantly with the determination by image analysis (Pearson $r = 0.91$; R squared = 0.84, $p = 0.028$). Moreover, a significant increase ($p = 0.002$) of the number of mast cells (26 ± 8 per 10 visual fields) was observed in the duodenal mucosa of patients with food allergy, whereas in the control group, mast cells were virtually absent (3 ± 3.5 per 10 visual fields).

We did not observe any inflammatory cell infiltration in the duodenal mucosa of allergic patients or in control subjects.

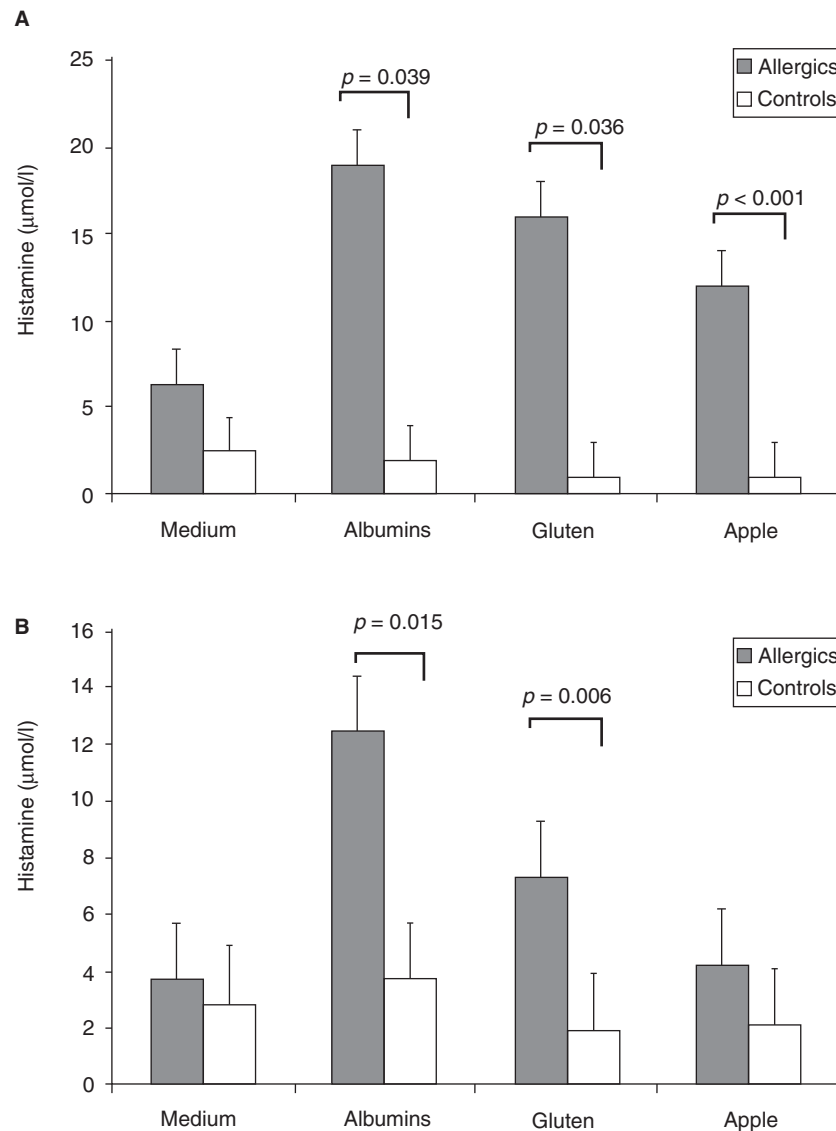


Figure 1. Effect of food antigen challenge on small intestinal mucosa release of histamine after 30 min (A) and 3 h (B) of culture, according to different antigen exposure.

Modification of tight junction proteins expression after food allergen challenge

After culture without antigens, in control subjects and in allergic patients, claudin-1, ZO-1, and occludin were expressed in linear fashion along the whole surface of the epithelial cells from the crypts to the villi with a typical reticular pattern (Figures 4 and 5).

In control subjects, after challenge with food allergens, immunofluorescence staining of tight junction proteins showed an intact tight junctional network and immunofluorescence signal intensity similar to that obtained in culture with only medium (Figure 4). On the contrary in allergic patients, after exposure to food allergens, the tight junction fluorescence appeared reduced at the cell-cell regions and was

characterized by breaks in the continuous rings (Figure 5).

Discussion

In this work, we used an organ culture model to study the pathophysiological mechanisms of IgE mediated food allergy. Numerous studies validated the potential usefulness of organ culture system for the evaluation of small intestinal mucosa immunity, in particular in celiac disease, as recently reported by Picarelli et al. [20,21]. In these studies, despite the authors use of a modified organ culture method (Batch method) [22] in which the cultures were prolonged to 48 h to obtain the maximum

Table II. Tryptase release in patients with food allergy and controls after challenge with food allergens.

Patient group	N	Medium (30 min)	Tryptase (30 min)	Medium (3 h)	Tryptase (3 h)
Food related allergy	9	2.58 $\mu\text{g/ml} \pm 2.04$ $\mu\text{g/ml}$	7.35 $\mu\text{g/ml} \pm 5.32$ $\mu\text{g/ml}$	4.335 $\mu\text{g/ml} \pm 3.31$ $\mu\text{g/ml}$	12.24 $\mu\text{g/ml} \pm 8.84$ $\mu\text{g/ml}$ [§]
Controls	10	3.05 $\mu\text{g/ml} \pm 2.12$ $\mu\text{g/ml}$	3.04 $\mu\text{g/ml} \pm 2.67$ $\mu\text{g/ml}$	6.62 $\mu\text{g/ml} \pm 4.37$ $\mu\text{g/ml}$	6.06 $\mu\text{g/ml} \pm 4.34$ $\mu\text{g/ml}$ *

Data are expressed as mean $\pm$ standard deviation.

* $p = 0.046$ (12.24 $\mu\text{g/ml} \pm 8.84$ $\mu\text{g/ml}$ vs. 6.06 $\mu\text{g/ml} \pm 4.34$ $\mu\text{g/ml}$); $^{\S}p = 0.046$ (12.24 $\mu\text{g/ml} \pm 8.84$ $\mu\text{g/ml}$ vs. 4.335 $\mu\text{g/ml} \pm 3.31$ $\mu\text{g/ml}$).

concentration of anti-endomysial antibodies and anti-tTGase antibodies, we preferred to use the classic organ culture method (3 h) to maintain mucosal architecture and because of the short half-life of histamine in plasma [12,23,24]. Exposure of small intestinal biopsy specimens of patients with food allergy to food allergens led to a significative increase of IgE-positive cells and mast cells with a significative increase of histamine and tryptase release, consistent with previous reports describing the presence of duodenal IgE-containing cells in patients with cow's milk allergy [25,26].

These findings suggest an increase of local IgE production in the intestinal mucosa in patients with food allergy, as reported by Coëffier et al. [27]. An *ex vivo* isolated intestinal mucosa has the potential to measure *in situ* gastrointestinal antibody production, as reported in previous *in vitro* organ culture studies [28,29]. As the mucosal microenvironment is left intact, mucosal cells are provided with the native cytokines needed to mount the antibody response [30]. Moreover, since the whole biopsy sample used in our study is isolated, IgE cannot be derived from plasma exudation or from the recruitment of cells carrying IgE receptors on surface.

To confirm the *in situ* production of IgE and mediators, analysis of mRNA expression would be needed. Recently, Vicario et al. demonstrated a new *in situ* production of IgE after challenge with food antigens from the evidence of IL-4 transcripts and mature IgE mRNA in esophageal mucosa of patients with food allergy [31].

Indeed, we report in small intestinal tissue of allergic patients after food allergens challenge a loss of immunofluorescence and an altered signal distribution of tight junction proteins, suggesting a disruption of epithelial tight junction complex, consistently with an alteration of intestinal barrier function, previously described in allergic patients [32].

Increased intestinal permeability may play an important role in food allergy because it exposes the mucosa to an abnormal challenge of luminal antigens of dietary and bacterial origin. Indeed, increased epithelial permeability was more the consequence, rather than the cause, of food sensitization, although it participates in a self-perpetuating cycle that maintains allergic inflammation. The disruption of epithelial tight junctions that we have shown in small intestinal specimens of allergic patients probably depends on the significative increase of histamine and tryptase secretion that we have found in culture medium after food antigen challenge. In fact, intestinal tissue from patients with food allergy contain elevated numbers of activated T cells, correlated with elevated levels of Th2 cytokines, which activate

Table III. IgE-positive cells quantified by image analysis in the allergic and healthy subjects.

Patient group	N	Medium	Wheat albumins	Wheat gliadins	Apple
Food related allergy	9	2.18 ± 1.41	3.26 ± 1.35	2.21 ± 0.91	1.62 ± 0.29
Controls	10	0.21 ± 0.21	0.43 ± 0.40	0.24 ± 0.21	0.21 ± 0.21
<i>p</i>		0.052	0.009	0.007	0.000

Data are expressed as mean of relative positive area (positive area over total) in each field.

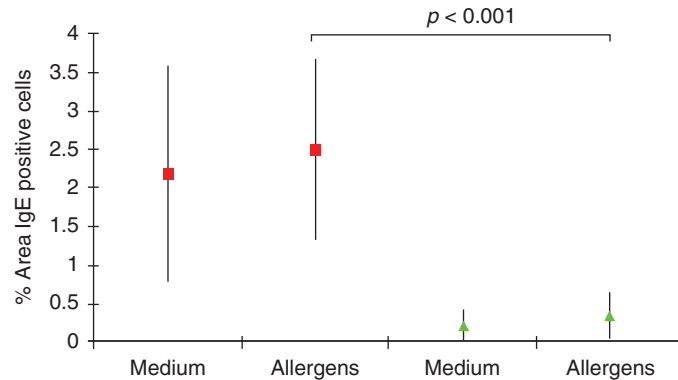


Figure 2. IgE-positive cells quantified by image analysis in the allergic (square) and healthy subjects (triangle) (% IgE-positive cells examined in relation to total tissue area).

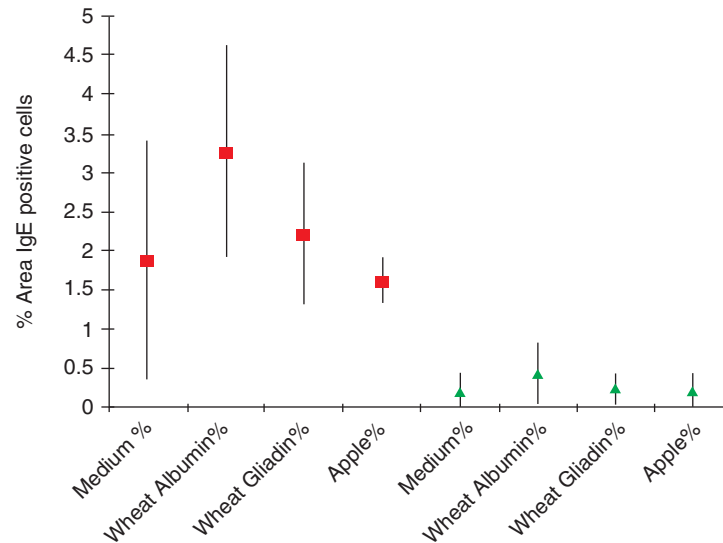


Figure 3. IgE-positive cells quantified by image analysis in the allergic (square) and healthy subjects (triangle) (% IgE-positive cells examined in relation to total tissue area), according to different antigen exposure.

immunological pathways associated with the onset of allergic reactions, including IgE synthesis and mast cell recruitment and activation with production of histamine and triptase [33]. As shown in previous findings, histamine stimulated a rapid and transient increase in the paracellular permeability by inducing myosin light chain phosphorylation [34,35]. MLC phosphorylation causes an increased contractility of the actin cytoskeleton and subsequent redistribution

of occludin and ZO-1 at the tight junctions with a loss of barrier integrity [36] favoring the macromolecule passage and bacterial translocation.

Upon degranulation, mast cells increase paracellular permeability of epithelial cells probably by a tryptase-dependent mechanism induced by activation of PAR2, a protease-activated receptor expressed at the apical and basolateral membrane of the intestinal epithelial cells [37] that affects cytoskeleton

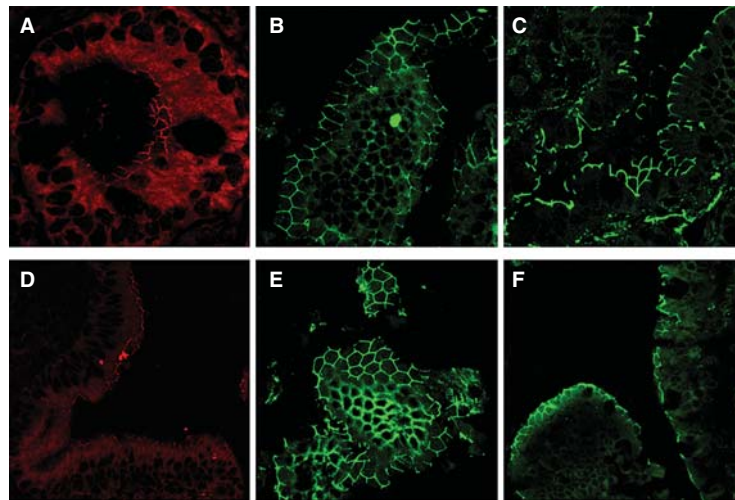


Figure 4. Immunofluorescence antibody staining of occludin (A, D), claudin-1 (B, E), and ZO-1 (C, F) in a small intestinal biopsy of a control subject after 3 h culture without (upper panel) and with food antigens (lower panel).

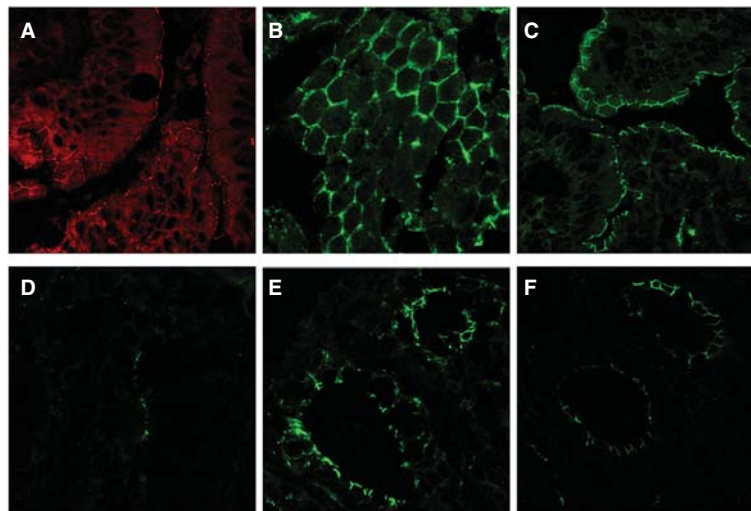


Figure 5. Immunofluorescent antibody staining of occludin (A, D), claudin-1 (B, E), and ZO-1 (C, F) in a small intestinal biopsy of an allergic subject after 3 h culture without (upper panel) and with food antigens (lower panel).

contraction by triggering of MLC phosphorylation with subsequent changes in tight junction permeability. PAR-2-induced phosphorylation of MLC is a short lasting phenomenon (15–120 min) but restoration of permeability linked to re-synthesis-migration of new junction proteins may last more than 12 h during which there is an activation of the resident immune system and attraction of new immunocytes [38]. This finding could explain the presence of tight junction structure alteration 3 h after antigen challenge in our *in vitro* model. Indeed, based on our data, the tight junction alterations could be considered a food allergy marker.

The *in vitro* challenge with offending food in an organ culture system could also be used to assess an IgE immunomediated food allergy.

Elimination diet followed by DBPCFC is considered still the gold standard for the diagnosis of food allergy [39,40]. Therefore, a series of diagnostic tests, as SPT, atopy patch tests, and measurement of IgE concentration against allergens, evaluated by ELISA or Western blotting, have been proposed for the diagnosis of food allergy. The immunochemical methods, suggesting an antigen/antibody interaction, do not necessarily reflect a biological activity such as the capacity to promote mast cell degranulation. Mast

cell activation requires two or more epitopes on the allergen to cross link antigen-specific IgE molecules bound to high-affinity IgE receptors on the mast cell surface. By ELISA, one epitope is sufficient to obtain reactivity, and thus this could lead to false positive results in the identification of relevant allergens that are biologically active. Even if mediator release can also be measured by the basophil assay, this test depends on fresh blood samples because of the absence of suitable long-term human basophil cultures [41,42]. Some recent studies have reported a new *in vitro* mast cell degranulation assay, but its functional degranulation response depends on the type of allergen [43].

Allergists also use skin prick testing to induce type I allergic reactions, but for ethical reasons, large numbers of antigens cannot be tested by this method. Furthermore, skin prick testing does not always correlate with immunochemical analyses [44] and may also be false positive or negative. The determination of allergy mediators in stool samples could be useful in clinical follow-up of patients with food allergy.

The advantage of using this test is that a wide range of foodstuff can be used, including raw material and highly purified natural or recombinant allergens. In fact, single food sources contain a number of allergens that may differ in their physical/chemical characteristics (pepsin stability, heat stability, etc.) and hence in their ability to induce mild or severe symptoms. Unlike other models, this is also a functional model of food allergy and could be considered as an *in vitro* oral food challenge with evident reduction of costs and risks for the patient.

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