

Local allergic reaction in food-hypersensitive adults despite a lack of systemic food-specific IgE

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Background: Objective tools are lacking for the diagnosis of local gastrointestinal inflammatory reactions in skin prick test (SPT)–negative and serum IgE antibody (s-IgE Ab)–negative patients with suspected food allergy.

Objective: The purpose of this investigation was to evaluate the presence of eosinophils, T cells, local IgE-bearing cells, IL-4, and IFN- γ in small intestinal biopsy specimens from adult SPT-negative/s-IgE Ab–negative patients with food allergy during symptomatic and nonsymptomatic periods.

Methods: Fourteen patients with food allergy–related gastrointestinal symptoms confirmed by double-blinded, placebo-controlled food challenge (DBPCFC) were investigated. Eleven of the patients were SPT-negative and s-IgE Ab–negative. Sex- and age-matched healthy volunteers were used as controls. Duodenal biopsies were studied with immunostaining through use of a panel of mouse monoclonal antibodies specific for eosinophils, CD3, CD4, CD8, IgE, IL-4, and IFN- γ .

Results: Significant increases in numbers of MBP⁺ eosinophils, IgE-bearing cells, and T cells were found in the duodenal mucosa of the patients when they were symptomatic in comparison with when they were asymptomatic and in

comparison with healthy controls. Numbers of IL-4⁺ cells were increased and numbers of IFN- γ ⁺ cells were reduced in the patients when they were symptomatic in comparison with when they were asymptomatic and in comparison with the controls. There were no differences in total s-IgE levels between any of the groups.

Conclusion: A significant correlation was found between the appearance of symptoms of food hypersensitivity and the duodenal presence of IgE-bearing cells, activated eosinophils, and T cells in patients with negative SPT results and negative s-IgE Ab to the offending food. We suggest that a localized IgE-mediated response caused the gastrointestinal symptoms seen in these patients. (*J Allergy Clin Immunol* 2002;109:879-87.)

Key words: Duodenal mucosa, food hypersensitivity, eosinophils, eosinophil major basic protein, immunoglobulin E, T lymphocytes

Immune-mediated hypersensitivity to food proteins affects 1% to 2% of adults and is responsible for a variety of symptoms involving the gastrointestinal (GI) tract, the respiratory tract, and the skin.¹⁻³ No single laboratory test or other procedure, such as skin prick testing, can offer an effective diagnostic tool for food allergy. The double-blinded, placebo-controlled food challenge (DBPCFC) has been accepted as the gold standard for diagnosing food hypersensitivity, but the DBPCFC does not discriminate between allergy and non-immune-mediated intolerance.⁴⁻⁷ In adults in particular, the significance of GI allergy is still questioned.⁷

In the present study and in our previous work, we found that food-related GI hypersensitivity in adults with positive DBPCFC results often showed negative skin prick test (SPT) results and undetectable serum IgE antibody (s-IgE Ab) to the relevant food antigens.^{4,8,9} The presence of s-IgE Ab to specific foods might suggest an IgE-mediated allergic disorder; on the other hand, negative SPT results and a lack of s-IgE Ab, together with positive DBPCFC results, do not exclude IgE-mediated food allergy.⁶⁻¹⁰ To further investigate the mechanisms behind such reactions, we performed an immunohistochemical characterization of local cellular infiltration and cytokine production in the duodenal mucosa during

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Abbreviations used

BLP: Basal lamina propria
 DBPCFC: Double-blinded, placebo-controlled food challenge
 GI: Gastrointestinal
 MBP: Major basic protein
 MoAbs: Mouse monoclonal antibodies
 s-IgE Ab: Serum IgE antibody
 SPT: Skin prick test

symptomatic and asymptomatic periods in patients with food allergy to evaluate the possibility of a localized immunologic response limited to the intestinal mucosa.

METHODS

Study population

Fourteen patients with food allergy and 14 age- and sex-matched healthy volunteers were recruited by the Asthma and Allergy Research Group, Sahlgrenska University Hospital, Göteborg, Sweden. Eleven of the patients had no signs of IgE-mediated hypersensitivity to the relevant food antigens when examined with SPT or analyzed in serum by means of the Pharmacia CAP system (Pharmacia Diagnostics AB, Uppsala, Sweden); the other 3 patients were positive in both SPT and s-IgE Ab to the challenged food and were used as reference patients. The mean age of the negative patients (3 male and 8 female) was 48 years (range, 23-65 years); the mean age of the positive reference patients (3 female) was 39 years (range, 19-49 years).

The consecutive adult patients were verified as positive to staple foods (cow's milk, hen's egg, wheat flour, and soy). The screening included diaries as well as elimination diets and open and blinded challenges. DBPCFCs were performed according to whether the expected symptoms were objective or subjective: 2 or 6 challenges were performed on separate days.¹¹ To be evaluated as positive, the expected symptoms had to appear at the active challenges and not with placebo. The suspected food was concealed (fresh) in a cold drink made by a dietician, and the amount of food was individualized according to the reactivity of the patient. The active/placebo recipes were made in such a way that they resembled each other. In the days following the challenges, the patients completed symptom protocol forms. When all of the challenges were completed, the forms were assessed and the code was broken.

Nine patients with negative SPT/negative s-IgE Ab results and 2 patients with positive SPT/positive s-IgE Ab results had early-phase reactions (5 to 60 minutes), and 2 patients with negative SPT/negative s-IgE Ab results and 1 patient with positive SPT/positive s-IgE Ab results showed delayed reactions (≥ 24 hours) after the onset of active challenges. These clinical manifestations persisted for 3 to 48 hours in all patients. Every patient had 1 or more GI symptoms, such as diarrhea, flatulence, nausea, abdominal distension, and pain; some patients also showed symptoms of oral mucosal blebs, asthma, eczema, and vomiting after active challenges. Seven patients were positive in SPT and/or s-IgE Ab to other foods and/or inhalant allergens, 6 patients had had lifelong food hypersensitivities, 11 patients had had allergic manifestations in early childhood,

and 7 patients had atopic family histories. Clinical and demographic characteristics of the subjects are shown in Tables I and II.

Fourteen age- and sex-matched healthy volunteers with a mean age of 46 years (range, 24-68 years) who had (1) no histories of food allergy or inhalant allergy, (2) no GI symptoms, and (3) negative Phadiatop test results (Pharmacia Diagnostics AB) were used as controls. The Phadiatop is a common qualitative screening test for s-IgE Abs against different foods and aeroallergens.

Skin prick testing

The SPTs were performed and the reactions graded according to the recommendations of the Standardization Committee of the Northern Society of Allergology.¹² Wheal reactions corresponding to the 10-mg/mL histamine reference were recorded as +++; wheals one half this size were recorded as ++; wheals one fourth the size of the reference were recorded as +. Patients with skin reactions of 3 mm greater than the PBS control were defined as positive.

Food challenge

Duodenal biopsy samples were obtained from patients with food hypersensitivity both when they developed GI symptoms after the open food challenge¹³ and 2 to 3 months later, when they were almost free of symptoms—ie, when any symptoms that arose were mild and stable because of an elimination diet. During the open food challenges the patients were encouraged to eat the offending food during a period of 7 days. On day 8, when the GI symptoms were well developed and similar to those that appeared after DBPCFC, the biopsy specimens and serum samples were taken. During both periods the patients were encouraged to complete symptom protocol forms for 2 weeks, beginning with the 7-day washout period, when all foods that could cause symptoms were carefully avoided. In both described periods, medications that could influence the results were avoided.

Biopsies and serum preparation

Endoscopic biopsy specimens from the proximal part of the duodenum were taken with a Fujinon EG 200-SP instrument (Fujinon Inc, Wayne, NY). The tissue specimens were immediately embedded in OCT compound (Tissue-Tek Miles, Inc, Elkhart, Ind), frozen in isopentane, and precooled with liquid nitrogen; finally, they were transferred into pure liquid nitrogen. Biopsy specimens and sera were kept at -70°C until analyzed. Informed consent was obtained from each of the participants, and the Ethics Committee of the Medical Faculty, University of Gothenburg, approved the study protocol.

Antibodies used for immunostaining

The following mouse monoclonal antibodies (MoAbs) were used: antihuman eosinophil major basic protein (MBP; 15381A IgG1 κ , PharMingen, San Diego, Calif); anti-CD3 (UCHT1, IgG1 κ); anti-CD4 (MT 310, IgG1 κ); anti-CD8 (DK 25, IgG1 κ); and anti-IgE (CIA-E-7.12, IgG1 κ) (all from DAKO, Glostrup, Denmark); anti-IL-4 (MP4-25D2, rat IgG1, PharMingen); and anti-IFN- γ (7-B6-1, mouse IgG1, Mabtech AB, Stockholm, Sweden).

Immunoperoxidase staining protocol

Cryostat sections (7 μm) were fixed in ice-cold acetone (30 seconds in 50% acetone followed by 5 minutes in 100% acetone) and then air-dried for 30 minutes. Endogenous peroxidase activity was blocked by incubation for 20 minutes at room temperature in a solution of 1 U/L glucose oxidase (Sigma, St Louis, Mo), 10 mmol/L glucose, and 1 mmol/L NaN_3 preheated for 15 minutes at 37°C . For

TABLE I. Clinical features of 14 DBPCFC-positive patients

Case	Age/sex	DBPCFC positive	SPT/serum IgE antibodies to tested food	Time to onset of symptom (h)	Duration of symptom (h)	Symptom after challenge	SPT inhalants/food
1	65/F	Milk	—/—	1	12-24	AD, D, N	+/+
2	65/F	Milk	—/—	5 min	12-24	AD, AP, A, N, D	—/—
3	61/F	Milk	—/—	5 min	12-24	AD, D, OMB	—/—
4	60/F	Wheat	—/—	5 min	24-48	AP, D, OMB	—/—
5	59/F	Milk	—/—	1	12-24	AD, AP, D, F	+/+
6	54/F	Wheat	—/—	1	12-24	AP, D, OMB	—/—
7*	49/F	Wheat	++/2	30 min	12-24	AD, AP, D, OMB	+++ / +++
8	41/F	Milk	—/—	24	24-48	F, N	—/—
9*	40/F	Wheat	++/4	24	24-48	AD, AP, D, F, N	+++ / +++
10	35/F	Soy†	—/—	1	12-48	AD, D, E, F, N	++++ / ++++++
11	32/M	Egg	—/—	20 min	3-6	AP, D	++/—
12	32/M	Wheat	—/—	24	24-48	AP, D	—/—
13	23/M	Egg	—/—	1	12-24	AP, D/O	—/—
14*	19/F	Soy	++/2	30 min	12-24	AP, OMB, V	+++ / +++++

A, Asthmatic symptoms; AD, abdominal distension; AP, abdominal pain; D, diarrhea; E, eczema; F, flatulence; N, nausea; O, obstipation; OMB, oral mucosa blebs, pain and irritation at tongue and palate; V, Vomiting.

*Reference patients.

†Also DBPCFC-positive to milk and egg.

All patients intolerant to milk were negative in a lactose test.

TABLE II. Clinical history and atopy-related features in 14 DBPCFC-positive patients with histories of food-related gastrointestinal symptoms

Case	Lifelong food hypersensitivity	Foods reacted to as a child	Asthma	Rhinitis/conjunctivitis	Eczema	Atopic family
1	+	Egg, chocolate, pear: AP, D until teenaged; then milk products	—	+ sb	+ sb	0
2	—	—	+ sb	+ sb	+ sb	—
3	+	Milk products: AP, D, F, V, 5 ya	—	—	—	—
4	—	—	—	—	+5-9 ya	+
5	—	—	—	+1-2 ya	+1-2 ya	+
6	—	—	—	—	—	+
7	—	—	—	—	+7-10 ya	+
8	+	Milk products: AP, F, N, teenaged	—	—	+10 ya	—
9	—	—	—	—	—	—
10	—	—	+1 ya	+ sb	+ sb	+
11	—	—	—	+3 ya	+3-10 ya	—
12	+	Egg, chocolate: E, 5 ya	—	—	+ sb	—
13	+	Egg: AP, D, 1 ya	+3 ya	+ sb	—	—
14	+	Peanut, nuts, fruits, soy: A, AP, V, 2 ya	—	—	+ sb	+

ya, Years of age at initial onset of symptoms; sb, since birth. (For the meanings of the other abbreviations, see footnotes to Table I.)

immunostaining, the slides were incubated overnight in a humid chamber at 4°C with MoAbs directed against MBP (2.5 µg/mL), CD3 (2.9 µg/mL), CD4 (5 µg/mL), CD8 (1 µg/mL), and IgE (2.9 µg/mL) diluted in PBS-Tween (0.05%) containing 2% BSA. Sections used as negative controls were incubated with isotype-matched

irrelevant MoAbs (mouse IgG1 κ, DAKO). After washings with PBS (3 × 5 minutes), the sections were incubated for 1 hour at room temperature with a biotinylated F(ab')₂ rabbit antimouse immunoglobulin (DAKO) diluted 1:400 in PBS-Tween plus 2% BSA plus 2% normal human serum. Thereafter, the slides were incubated with

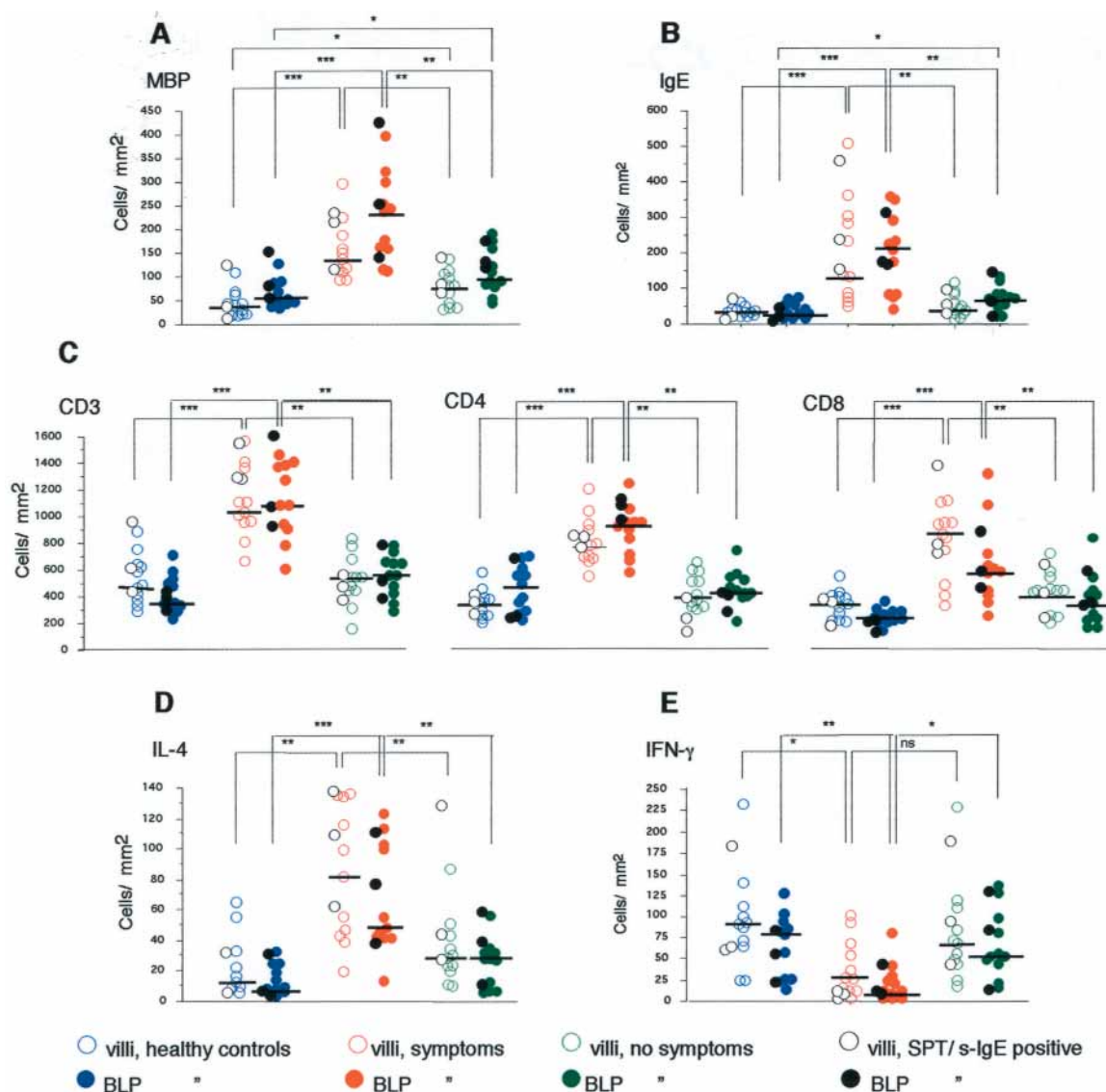


FIG 1. Numbers of MBP⁺ eosinophils (A), IgE-stained cells (B), CD3⁺, CD4⁺, and CD8⁺ T cells (C), IL-4-stained cells (D), and IFN- γ -stained cells (E) in duodenal biopsy specimens taken from healthy controls and SPT-negative/s-IgE Ab-negative patients with and without symptoms ($n = 11$). Results are expressed as individual data points, and the medians are indicated. Three patients with positive SPT results/positive s-IgE Ab and their healthy controls are included as references (black dots).

* $P < .05$.

** $P < .005$.

*** $P < .001$.

avidin-conjugated peroxidase (ABC-complex, DAKO) for 30 minutes. The peroxidase staining was revealed with amino-ethyl-carbazole (Sigma); this was followed by a light counterstaining with Mayer's hematoxylin and mounting in Aquamount (BDH Laborato-

ry, Poole, United Kingdom). For IL-4 (10 $\mu\text{g/mL}$) and IFN- γ (2 $\mu\text{g/mL}$) staining, the sections were pre-fixed with 2% formaldehyde (Sigma) for 8 minutes at 4°C and thereafter treated as described above. The slides were coded and evaluated in a random order.

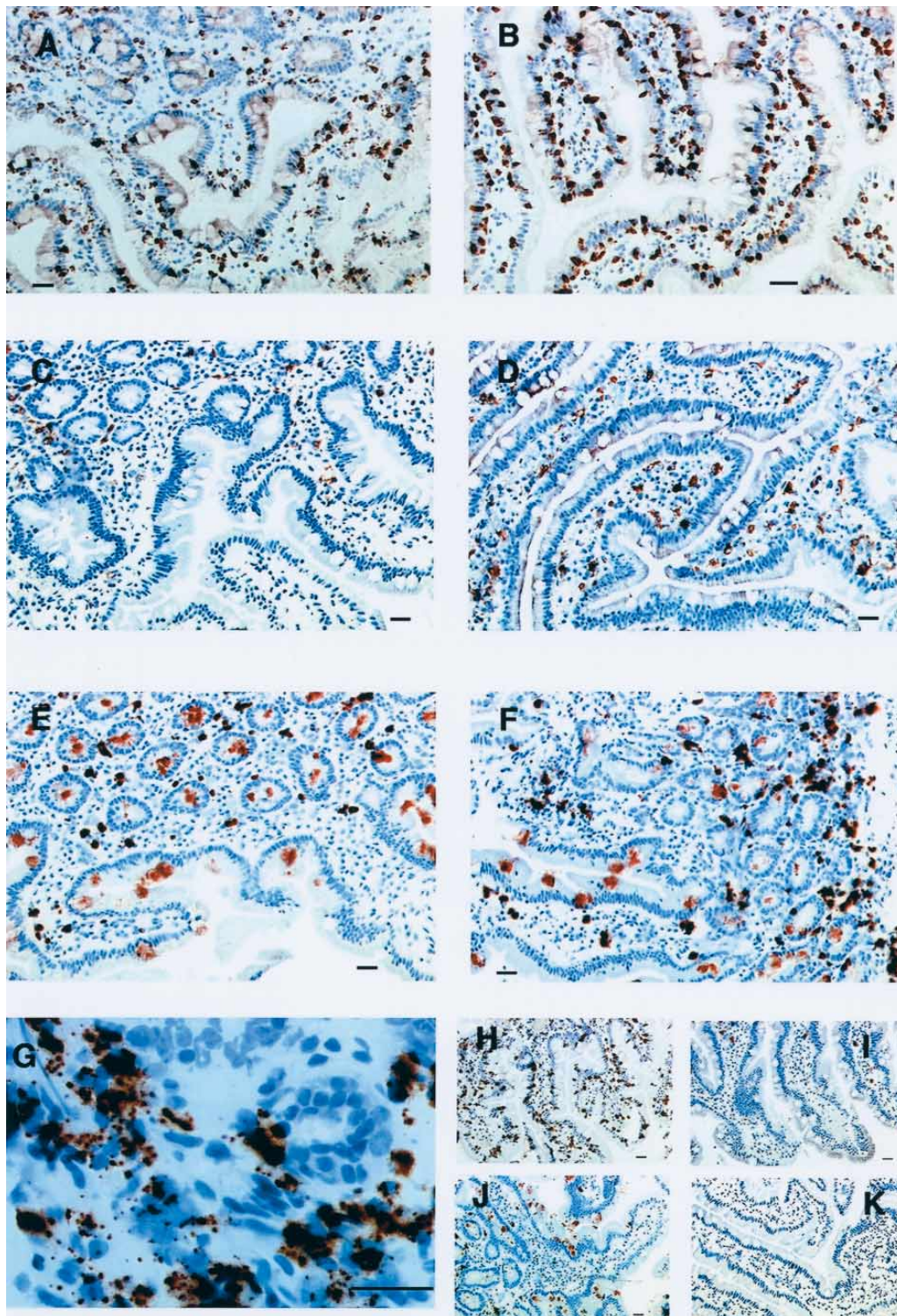


FIG 2. Immunostaining of duodenal biopsies from food-allergic patients and controls. *Red-brown color* indicates positive staining; *blue color* indicates counterstaining of nuclei. *Black bars* indicate 100 μ m. **A**, CD3⁺ T cells in a nonchallenged patient without symptoms. **B**, Marked increase of CD3⁺ T cells in a challenged patient with symptoms. **C**, Presence of IgE-stained cells in a nonchallenged patient without symptoms. **D**, Enhanced presence of IgE-stained cells in a challenged patient with symptoms. **E**, MBP-stained eosinophils in a nonchallenged patient without symptoms; note nonspecific staining of mucus in goblet cells. **F**, Marked increase of MBP-stained eosinophils in a challenged patient with symptoms. **G**, MBP-stained eosinophils (as in Fig 1, *F*) at higher magnification; note MBP-containing granules free in the tissue. **H**, CD3⁺ T cells in a control. **I**, IgE-stained cells in a control. **J**, MBP-stained eosinophils in a control. **K**, Isotype control staining.

Positively stained cells were counted through use of a computer-supported image analysis system (Leica Q500MC, Cambridge, United Kingdom). All positive cells in at least 4 fields of the mucosa were counted, the villi being separated from the basal lamina propria (BLP) regions. The positive cells were recorded as the numbers of stained cells per square millimeter of tissue.

Determination of total IgE, IL-4, and IFN- γ in serum

Total s-IgE, food-specific s-IgE Ab, and the controls' serum were measured by means of the Pharmacia CAP system, and serum levels of IL-4 and IFN- γ were measured by means of ELISA (Opt EIA, PharMingen) according to the manufacturer's instructions.

Statistical analysis

The values were reported as individual data points and median values. The statistical analyses in the SPT-negative/s-IgE Ab-negative patients were carried out through use of Statview (SAS Institute Inc, Cary, NC) on a Macintosh computer (Apple, Cupertino, Calif). The Mann-Whitney *U* test for nonpaired data and the Wilcoxon signed rank test for paired data were applied. Correlation analysis was made with the Fisher test. The level for significance was set at $P < .05$.

RESULTS

Frequencies of eosinophils in the duodenal mucosa

MBP-positive eosinophils were detected in the villi and in the BLP but not in the epithelium or in the apical parts of the villi in the asymptomatic patients (Fig 2, *E*) and in the controls (Fig 2, *J*). In the patients, we observed after food challenges an increased frequency of eosinophils and a more pronounced mucosal infiltration throughout the villi and BLP (Fig 2, *F*), often accompanied by eosinophil degranulation, as evidenced by the presence of MBP-stained granules that were free in the tissue, as seen in Fig 2, *G*. The frequency of eosinophils in the patients during their symptomatic period was significantly higher (Fig 1, *A*) than that during the asymptomatic period ($P = .003$, both in the villi and the BLP) and significantly higher than that in the healthy controls ($P = .0001$ for both sites). The increased number of eosinophils seen during the asymptomatic period reached significance ($P = .03$ in the villi and $P = .02$ in the BLP) in comparison with what was seen in the healthy controls.

Serum IgE and duodenal IgE-positive cells

The median total serum IgE level in the patients during the symptomatic period did not differ (31 kU/L [range, 10-331 kU/L]) from that during the asymptomatic period (29 kU/L [range, 9-277 kU/L]), and it was indistinguishable from that of the healthy controls (17 kU/L [range, 5-235 kU/L]). There was a small increase in the symptomatic period in comparison with the asymptomatic period ($P < .01$).

As seen in Fig 1, *B* and Fig 2, *D*, the local IgE⁺ cells were abundantly present in the duodenal mucosa during

the symptomatic period in comparison with the samples taken during the asymptomatic period ($P = .003$ in both sites) and in comparison with the healthy controls ($P < .0001$ in the villi and $P = .0002$ in the BLP). During the asymptomatic period, the counts in the villi did not differ between the patients and the healthy controls, but there were still slightly higher numbers of IgE⁺ cells in the BLP ($P = .02$). The patients with early reactions did not differ from those with late-phase reactions in the number of mucosal IgE⁺ cells. The intensity of the IgE staining was always higher in the patients (Fig 2, *C*, *D*, and *I*), and the morphologic analysis revealed that most of the IgE⁺ cells were mast cells, only a few being IgE⁺ eosinophils. In the patients with the most abundant IgE staining, the pattern of staining suggested IgE-bearing dendritic cells (not shown), and in one of these patients we identified densely stained IgE⁺ cells with the localization and morphology of plasma cells. There was no overall correlation between the total levels of s-IgE and the numbers of mucosal IgE⁺ cells.

Frequencies of CD3⁺, CD4⁺, and CD8⁺ cells in the duodenal mucosa

CD3⁺, CD4⁺, and CD8⁺ T lymphocytes were counted in the villi and BLP of the small intestinal biopsy specimens. Increased frequencies of CD3⁺ (Fig 1, *C* and Fig 2, *B*), CD4⁺, and CD8⁺ T lymphocytes were detected in patients with GI symptoms after food challenge in comparison with the frequencies during the asymptomatic period ($P = .003$ in all comparisons and in both sites) and in comparison with the frequencies in the healthy controls (for CD3⁺, $P = .0002$ in the villi and $P < .0001$ in the BLP; for CD4⁺, $P < .0001$ in the villi and $P = .0003$ in the BLP; for CD8⁺, $P = .0009$ in the villi and $P = .0003$ in the BLP; Fig 1, *C*). There was no significant difference (Fig 1, *C*) between the asymptomatic patients (Fig 2, *A*) and the healthy controls (Fig 2, *H*). The T-cell numbers did not appear to differ during the symptomatic period between the patients with early-phase reactions (10 of 11 patients) and those with late-phase reactions (2 of 11 patients) or between the SPT-negative/s-IgE Ab-negative and SPT-positive/s-IgE Ab-positive reference patients (3 of 14). In general, the CD4⁺ T cells were mainly localized in the core of the villi and in the BLP, whereas the CD8⁺ T cells were mainly residing within or beneath the epithelium. In patients with symptoms, the T cells showed signs of activation with a spiky morphology, but there were no signs of gross morphologic changes such as villus atrophy, crypt hyperplasia, edema, or epithelial damage (Fig 2, *B*).

Detection of IL-4 and IFN- γ in serum and tissue sections

There were no detectable IL-4 and IFN- γ levels in the serum of patients or in the serum of healthy controls (data not shown). In contrast, IL-4⁺ and IFN- γ ⁺ cells were detected in the tissue. These cells appeared to co-

localize with the CD4⁺ T cells. There were significantly higher numbers of IL-4⁺ cells in the villi and in the BLP in the patients during the symptomatic period than during the asymptomatic period ($P = .003$ in both sites); likewise, these numbers were significantly higher than in the healthy controls ($P = .001$ in the villi and $P = .0003$ in the BLP; Fig 1, *D*). The asymptomatic patients did not differ significantly from the healthy controls.

The reverse was seen with the IFN- γ ⁺ cells, the numbers being lower during the symptomatic period than during the asymptomatic period (the difference was not significant for the villi; $P = .03$ in the BLP) and lower in comparison with the healthy controls ($P = .02$ in the villi and $P = .004$ in the BLP; Fig 1, *E*). The number of IFN- γ ⁺ cells did not differ between the asymptomatic patients and the controls.

There was a positive correlation between the numbers of IL-4⁺ cells and IgE⁺ cells in the BLP ($r = 0.814$; $P < .0001$) and a negative correlation between the numbers of IFN- γ ⁺ cells and IgE⁺ cells in the BLP ($r = -0.479$; $P = .004$) in the biopsy specimens from the symptomatic patients (data not shown).

The 3 SPT-positive/s-IgE Ab-positive reference patients and the s-IgE Ab-negative patients showed similar numbers and distributions of IgE⁺ and MBP⁺ T cells and similar cytokine patterns.

DISCUSSION

In the present study, we used immunohistochemistry to characterize putatively disease-mediating cells in the duodenal lamina propria of SPT-negative/s-IgE Ab-negative/DBPCFC-positive adult food-allergic patients. The results suggest that the allergic reaction to food antigens was associated with a local infiltration of inflammatory cells in the human gut wall, with a presence of MBP⁺ eosinophils, CD4⁺ and CD8⁺ T cells, and IgE-carrying cells. It was notable that there were no signs of gross morphologic changes, such as villus atrophy, crypt cell hyperplasia, or epithelial damage in duodenal mucosa, even in the symptomatic patients with abundant eosinophil and T-cell infiltration of the gut mucosa.

A novel finding in this study is that eosinophils are recruited into the duodenal mucosa in patients after challenge with the food antigen inducing their symptoms; in addition, we frequently noted degranulation of these cells, as manifested by the presence of free MBP-stained granular material in the tissue. This was not seen in the asymptomatic patients and the controls. This is in line with previous findings that imply an involvement of eosinophils in the gut of patients with food hypersensitivity by demonstrating elevated levels of eosinophil cationic protein and eosinophil protein X in feces,¹⁴⁻¹⁶ in gut lavage fluid,¹⁷ and in intestinal secretion.⁹ The MBP⁺ eosinophils found in the intestinal mucosa¹⁸ in the present study also stained positive for eosinophil cationic protein (data not shown).

Although the mechanisms of action of MBP in bronchial hypersensitivity have been demonstrated to involve a direct effect on the respiratory epithelium, causing ciliostasis and desquamation of respiratory epithelial cells,¹⁹ the role of MBP in intestinal allergy has not been characterized. MBP can induce histamine release from mast cells and has been suggested to be directly linked to the recruitment, infiltration, and activation of eosinophils at the site of the allergic inflammation.²⁰ In the present study, the local levels of inflammatory cells and mediators in the target tissue correlated well with the clinical symptoms and the degree of GI hyperactivity.

Although the role of IgE-mediated reactions in food allergy is well known, the full significance of local IgE in the GI tract in food-allergic patients has not been established.^{21,22} Some investigators have shown elevated IgE levels in the feces of allergic children,^{23,24} in intestinal juice of food-allergic adults,^{25,26} and in intestinal washings of children with atopic eczema.²⁷ Using isolated jejunal perfusion studies with allergen challenges in SPT-negative/s-IgE Ab-negative patients, we previously showed an immediate release of eosinophil-derived mediators in these patients as well as an increase in baseline IgE-bearing cells.^{4,8,9} This was confirmed in the present study, possibly implying that the hypersensitivity reactions in the gut might be mediated by the increased local presence of IgE-coated mast cells.⁷

Significantly higher frequencies of T lymphocytes were found in the duodenal mucosa of patients when they were symptomatic than when they were asymptomatic; these frequencies were also significantly higher than those in the healthy controls. These results are supported by previous human and animal studies of activation and infiltration of T cells in the intestinal mucosa in response to food antigens.²⁸⁻³⁰ The role of the T-cell infiltration is largely unknown, and it is not established whether these cells take an active part in the inflammatory process or whether they are recruited bystanders with a regulatory function. The capacity of T cells to produce an inflammatory reaction is largely controlled by their repertoire of cytokines.^{31,32} T_H2-derived IL-4 and IL-13 play a critical role not only in the differentiation of T-helper cells to the T_H2 phenotype but also in IgE isotype switching and production and in eosinophil recruitment.^{33,34} Both IL-4 and IL-13 inhibit secretion of IFN- γ , which also leads to enhanced eosinophilia.³⁵ In addition, IFN- γ inhibits B-cell IgE synthesis, thereby downregulating the type I allergic inflammatory response.³⁶ In the present study, we showed an elevated number of IL-4⁺ cells and a lowered number of IFN- γ ⁺ cells in the duodenum after food challenges, indicating an important role for at least some (~10%) of the infiltrating T cells. The serum of the patients did not contain any detectable IL-4 or IFN- γ (data not shown), which is in contrast to other studies that have reported an imbalance of IL-4 and IFN- γ in peripheral blood from both children³⁷ and adults with food allergy.³⁸

Elevated local IL-4 might be important for maintaining local production of IgE. This was further supported by the positive correlation between the numbers of IL-4⁺ and IgE⁺ cells and the negative correlation between the numbers of IFN- γ ⁺ and IgE⁺ cells found in the lamina propria of patients with symptoms.

A recent study showed that CD8⁺ T cells can produce IL-4 and stimulate IgE production in some allergic patients.³⁹ However, the IL-4 staining that we observed was not contained within the epithelial compartment, where the majority of the CD8⁺ cells are situated, and no correlation was found between the number of CD8⁺ T cells and the number of IL-4⁺ cells or IgE⁺ cells.

In conclusion, our study showed that there was a significant correlation between clinical symptoms in adults with allergy confirmed by DBPCFC and the presence of increased numbers of duodenal IgE⁺ cells, eosinophils, and activated T cells, regardless of whether they were early- or late-reacting and regardless of whether they were negative or positive in SPT results and/or s-IgE Ab. The local presence of IL-4-producing cells might fuel a local production of specific IgE, mediating the allergic response seen in these patients.

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