

Indications of 'atopic bowel' in patients with self-reported food hypersensitivity

K. LILLESTØL*,†, L. HELGELAND‡, G. ARSLAN LIED*,†, E. FLORVAAG*,§,¶, J. VALEUR*,†, R. LIND* & A. BERSTAD*,†

*Institute of Medicine, University of Bergen, Bergen, Norway; †Department of Medicine, Haukeland University Hospital, Bergen, Norway; ‡Department of Pathology, Haukeland University Hospital, Bergen, Norway; §Laboratory of Clinical Biochemistry, Haukeland University Hospital, Bergen, Norway; ¶Section for Clinical Allergology, Department of Occupational Medicine, Haukeland University Hospital, Bergen, Norway

Correspondence to:
Dr K. Lillestøl, Institute of Medicine,
Haukeland University Hospital,
N-5021 Bergen, Norway.
E-mail: kristine.lillestol@med.uib.no

Publication data

Submitted 9 January 2010
First decision 25 January 2010
Resubmitted 6 February 2010
Accepted 8 February 2010
Epub Accepted Article 16 February 2010

SUMMARY

Background

An association between atopic disease and gastrointestinal complaints has been suggested.

Aim

To explore the association between atopic disease, gastrointestinal symptoms, and possible gastrointestinal manifestations of atopic disease in patients with self-reported food hypersensitivity.

Methods

Symptoms, skin prick tests, serum markers of allergy and intestinal permeability were recorded in 71 adult patients. Eosinophils, tryptase- and IgE-positive cells were counted in duodenal biopsies.

Results

Sixty-six (93%) patients had irritable bowel syndrome (IBS) and 43 (61%) had atopic disease, predominantly rhinoconjunctivitis. All 43 were sensitized to inhalant allergens, 29 (41%) to food allergens, but food challenges were negative. Serum total IgE and duodenal IgE-positive cell counts were significantly correlated ($P < 0.0001$) and both were significantly higher in atopic than in non-atopic patients ($P < 0.0001$ and $P = 0.003$ respectively). IgE-positive cells appeared to be 'armed' mast cells. Intestinal permeability was significantly elevated in atopic compared with non-atopic patients ($P = 0.02$). Gastrointestinal symptoms and numbers of tryptase-positive mast cells and eosinophils did not differ between groups.

Conclusions

Patients with self-reported food hypersensitivity had a high prevalence of IBS and atopic disease. Atopic patients had increased intestinal permeability and density of IgE-bearing cells compared with non-atopic patients, but gastrointestinal symptoms did not differ between groups.

Aliment Pharmacol Ther 31, 1112–1122

INTRODUCTION

Hypersensitivity to food is commonly reported. Up to 35% of the general population in Western countries think they have food allergy,¹ but self-reported prevalences of food hypersensitivity are generally believed to be overestimated, when compared with studies using objective diagnostic tools.^{1, 2} The discrepancy between self-reported and confirmed food hypersensitivity is particularly large in studies based on double-blind placebo-controlled food challenges (DBPCFC), which is regarded as the gold standard diagnostic method.³ When this method is used, only 1–2% of the adult populations get a diagnosis of confirmed food hypersensitivity.^{4, 5} There is, however, a lack of understanding of the pathophysiological mechanisms of adverse reactions to food, in particular, with respect to reactions which are not 'classical' IgE-mediated immediate hypersensitivity reactions. Hence, current standard diagnostic methods may be of limited value when it comes to other less well-known forms of food hypersensitivity.³

Gastrointestinal symptoms are among the most common complaints attributed to food hypersensitivity.^{6, 7} In our previous studies, we found that the majority presented with nonspecific gastrointestinal symptoms consistent with the criteria for functional gastrointestinal disorders, predominantly irritable bowel syndrome (IBS), while the prevalence of food allergy as confirmed by DBPCFC was low.⁸ However, even if 'true' food allergy is difficult to confirm in such patients⁹ and may play only a minor role in the development of functional gastrointestinal complaints, a role for other allergic conditions in the pathogenesis of gastrointestinal complaints has been suggested. The prevalence of IBS-like symptoms is found to be increased in patients with allergic symptoms such as rhinitis and asthma, when compared with non-allergic patients.^{10, 11} Minor histological abnormalities in the gastrointestinal tract of patients with atopic diseases have also been documented. Some studies have found the numbers of different immunocytes, such as eosinophils,^{12–14} mast cells,¹⁴ and IgE-positive cells,^{15, 16} to be elevated in the small intestinal mucosa of patients with atopic eczema, rhinitis or asthma. Other studies have suggested that patients with atopic diseases have a gut barrier dysfunction as indicated by an increased intestinal permeability.^{17–21}

We wanted to explore whether these previously reported associations between atopic disease, IBS-like

complaints and intestinal inflammatory changes could be of relevance for adult patients with gastrointestinal complaints self-attributed to food hypersensitivity. The aims of the present study were: (i) To determine the prevalence of atopic disease in patients with gastrointestinal complaints who consecutively went through allergological, dietary and gastroenterological examinations for possible food hypersensitivity and (ii) To compare gastrointestinal symptoms, intestinal permeability and counts of allergy-related cells in the duodenal mucosa (IgE-positive cells, eosinophils and mast cells) between atopic and non-atopic patients with self-reported food hypersensitivity. Moreover, we wanted to study the relationships between allergy-related cells in the duodenal mucosa and corresponding serum markers. In addition to levels of total IgE, we measured eosinophil cationic protein (ECP) as an indicator of eosinophil activation²² and tryptase as an indicator of mast cell activation.²³

For this study, atopic disease was defined as having at least one positive skin prick test and/or elevated levels (≥ 0.35 kUa/L) of serum specific IgE antibodies to at least one allergen, as well as a personal history of at least one of the following atopic symptoms: eczema, asthma or rhinoconjunctivitis. This is consistent with current diagnostic criteria for allergic diseases.²⁴

MATERIALS AND METHODS

Study population

Patients consecutively referred by primary physicians or specialist doctors to Haukeland University Hospital (Bergen, Norway), because of gastrointestinal complaints self-attributed to food hypersensitivity, were eligible for inclusion in the study. Confirmed inflammatory bowel disease, pregnancy, lactation and a history of severe anaphylactic reactions were exclusion criteria. After having given consent, patients were examined first in the allergy clinic, thereafter in the gastroenterology clinic. The study was approved by the Western Norway Regional Committee for Medical Research Ethics.

Allergological assessment

The allergological examination included a structured and detailed anamnesis about atopic symptoms, family history of atopy, and suspected food items. Skin prick

tests (SPT) using standard panels for food and inhalant allergens were performed (ALK Abello, Hørsholm, Denmark). All patients were tested for seven common food items (six commercial allergens – egg white, cow's milk, codfish, wheat, peanut, soy – and a native wheat flour suspension in saline) and eight inhalants (birch, timothy, mugwort, *Dermatophagoides pteronyssinus*, *Cladosporium herbarum*, cat, dog, horse). Additional tests for other allergens were performed when indicated according to the patients' history. The SPTs were performed in duplicate by placing a drop of each extract on the volar side of the forearm and perforating the skin with a standard lanzett (1 mm tip) through the drop. Histamine (10 mg/mL) and saline were used as positive and negative controls respectively. Skin wheal diameters were measured after 15 min. A positive SPT response was defined as net wheal mean diameter equal to or greater than 3 mm. The serum levels of total IgE and specific IgE antibodies, as well as of the inflammation markers tryptase and ECP, were measured at the Laboratory of Clinical Biochemistry, Haukeland University Hospital using the ImmunoCAP system (Phadia, Uppsala, Sweden) according to the manufacturer's instructions. Serum total IgE and specific IgE antibodies towards the major food and inhalant allergens denoted above for SPT were determined in all patients. Additional specificities were analysed if indicated by patient's history.

When allergy was suspected according to history and SPT/IgE results, or when non-IgE-mediated hypersensitivity to a specific staple food item was suspected according to the patient's history, successive trials with elimination diet, open provocation test and DBPCFC were performed by a dietician. The trials began with (open) elimination of the most strongly suspected food item from the diet for 2–3 weeks. When the elimination diet led to symptom relief, an open provocation test was performed, and when the open provocation tests were positive, a DBPCFC with the same allergen was performed. Procedures for the provocation tests are described in detail elsewhere.⁸ Only staple food items, like flour (mainly wheat), milk and egg white were used as test allergens in these trials.

Gastroenterological assessment

General examinations. The gastroenterological examinations were performed 2–4 weeks after the allergological assessment and included upper endoscopy with

biopsies taken from the gastric mucosa to diagnose *Helicobacter pylori* (*H. pylori*) infection (with rapid urease test). Three duodenal biopsy specimens were also taken for the exclusion of coeliac disease and for analyses of eosinophils, mast cells and IgE-positive cells, as described below. Serological (antigliadin and antiendomysium antibodies) and genetic (HLA-DQ) tests were used as additional tools to exclude coeliac disease. Inflammatory bowel disease was excluded by measuring calprotectin in gut lavage fluid, as previously described,²⁵ and by measuring intestinal permeability (see below). Microscopy and cultures of stool samples were performed when indicated, to rule out parasitic infections. A screening questionnaire based on the Rome II criteria was applied for the diagnosis of IBS.²⁶ The presence and severity of six gastrointestinal symptoms (nausea, bloating, abdominal pain, diarrhoea, constipation and anorexia) were assessed by the Irritable Bowel Syndrome Symptom Questionnaire (IBS-SQ).²⁷ The symptoms were rated on a scale from 0 (no symptoms) to 10 (severe symptoms).

Cellular stainings in duodenal biopsies. The duodenal biopsies were immediately formalin-fixed and paraffin-embedded. Serial sections were cut at 4 µm. One section was stained with haematoxylin-eosin and used for evaluation of inflammation and for the quantitative assessment of eosinophils. The procedures for immunostaining of IgE and mast cells were briefly as follows: sections were incubated overnight at 55 °C, and thereafter deparaffinated and rehydrated. Antigen retrieval was performed by microwaving the sections in TrisEDTA buffer (tryptase-staining) or citrate buffer (IgE-staining). Peroxidase activity was blocked by hydrogen peroxide. Primary antibodies were, for staining of mast cells, a monoclonal antibody against mast cell tryptase (clone AA1, DAKO, Glostrup, Denmark) at a dilution of 1:800, and for staining of IgE, anti-IgE (epsilon-Heavy Chain, clone Ab-1, Thermo Fischer Scientific, Fremont, USA) at a dilution of 1:500 was used. The reaction was finally developed using the EnVision detection kit (DAKO), with diaminobenzidine (DAB) as colour reagent.

The assessment of cells was performed using an Olympus microscope BX51 (Olympus, Germany) with a computer-linked camera (DP71, Olympus, Germany). The cells were assessed and counted at 400× magnification. Computer-assisted image analysis was used (analySIS, Olympus Soft Imaging Solutions GmbH,

Germany) to measure the size of each counted area. All cell countings were performed by K.L., who was blinded to patients' identity and clinical data. Countings were performed in the lamina propria of all intact longitudinally sectioned areas of three different biopsies from each patient. Mean scores were then calculated on the basis of all counted areas per patient. All results are given as number of cells/mm².

Intestinal permeability. A nasoduodenal feeding tube (Freka Feeding Tube, Fresenius Kabi, GmbH, Germany) was positioned with its tip in the distal part of the duodenum, by aid of endoscopy. Two litres of isotonic polyethylene glycol solution (PEG, MW 3350, Laxabon, Tika, Sweden) was then administered through the tube over a 40 min period, using a peristaltic pump (Watson Marlow 505S/RL, Falmouth, UK). Approximately 50 µCi of ⁵¹CrEDTA (Amersham International, Amersham, UK) was added to the solution. Urine was then collected for 5 h. The total urine volume was measured and a sample was analysed for radioactivity using a gamma counter (model 1282 Compugamma counter; LKB-Wallac, Turku, Finland). The percentage of administered dose of ⁵¹CrEDTA excreted in urine was calculated as previously described.²⁵ This method for measuring intestinal permeability was originally developed for the purpose of a combined permeability and ultrasound (hydrosonography) study,²⁸ where a large amount of fluid was necessary for optimal visualization of the bowel. Permeability results based on this procedure have shown good correlation with other markers of intestinal inflammation (i.e. calprotectin).²⁵

Statistics

Statistical analyses were performed using the GRAPHPAD PRISM 5 statistical software package (GraphPad Software Inc, San Diego, CA, USA). Data that were not normally distributed are reported as medians with interquartile ranges (IQR), and Mann-Whitney *U*-tests were used for comparison of medians. Mean age of the two groups was compared by an unpaired *t*-test. Correlations between different parameters within the same group were evaluated by Spearman's correlation coefficient or, after log-transformation, with Pearson's correlation coefficient. Chi-square statistics (Fisher's exact test) was used for comparisons of proportions. All tests were two-tailed and the level of significance was set to *P* < 0.05.

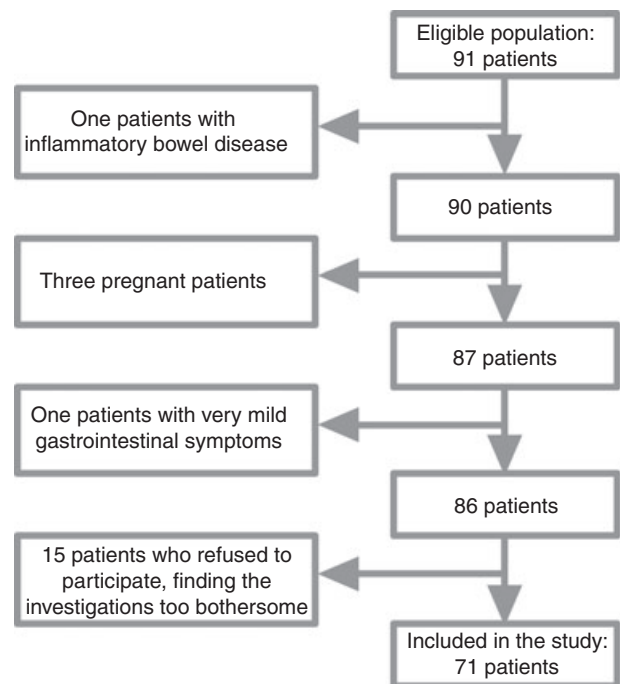


Figure 1. Overview of patients.

RESULTS

Participants

Patients were recruited between September 2005 and May 2007. A total of 91 patients were eligible for inclusion, and the final number of patients who were included in the study was 71 (Figure 1). The patients who were included in the study did not differ significantly from the not-included with respect to age and gender.

Atopic disease

Forty-three of 71 patients (61%) fulfilled our criteria of having atopic disease. The mean age of these patients (32.2 year, range 17–65) was significantly lower than in patients without atopic disease (44.7 year, range 18–70, *P* = 0.0002). The distribution of gender did not differ significantly between groups. There were more women than men in the atopic group (*n* = 30, 70%) as well as among patients without atopic disease (*n* = 22, 79%).

A family history of atopic disease was reported by 29 (67%) of the atopic patients and by 10 (36%)

patients without atopic disease. The most prevalent atopic symptom among the patients was rhinoconjunctivitis, which was reported by 41 (95%) of the atopic patients. A majority had seasonal (49%) or a combination of seasonal and perennial rhinitis (33%). A history of asthma was reported by 12 (28%) of the atopic patients, while 11 (26%) had a history of atopic eczema. All patients had experienced at least one atopic symptom within the last year. In the non-atopic group, two patients had a personal history of atopic eczema and rhinoconjunctivitis in their childhood, but had no symptoms or indications of specific IgE production at the time of the study.

Twenty-one (58%) of the patients with seasonal rhinoconjunctivitis reported oral allergy syndrome (OAS); i.e. itching and a feeling of swelling in mouth and throat, after intake of typical pollen-related foods such as fruits, nuts, and raw vegetables. However, when it came to patients' suspected causes of gastrointestinal symptoms, patients with atopic disease most frequently suspected cow's milk (72%), wheat (65%) and hen's egg (42%). Also in the non-atopic group, cow's milk (79%) and wheat (61%) stood out as the most commonly suspected causes of gastrointestinal complaints.

IgE sensitization and markers of inflammation

The median level of serum total IgE in patients with atopic disease (median 161.0, IQR 61.0–613.0) was significantly higher than in non-atopic patients (median 27.0, IQR 12.0–70.3, $P = <0.0001$). Twenty-four patients with atopic disease (56%) had an elevated level of total IgE (>120 kU/L). The median serum concentration of ECP was also significantly higher in patients with atopic disease (median 13.9, IQR 8.9–22.0) than in non-atopic patients (median 9.1, IQR 4.9–14.8, $P = 0.037$). There was no significant difference in median serum tryptase concentration between the groups (atopic patients' median 3.6, IQR 2.4–5.1 and non-atopic patients' median 4.3, IQR 3.1–5.7).

All 43 patients with atopic disease were sensitized to at least one inhalant allergen, as verified by SPT and/or serum specific IgE antibodies (Table 1). Twenty-nine of the atopic patients (67% of the atopic group, 41% of the whole patient sample) were also sensitized to at least one food allergen (Table 2). In the non-atopic group, two patients had elevated specific IgE antibodies to pollen (>0.35 kUa/L), one of them also with an elevated level of serum total IgE (>120 kU/L), but they had never experienced atopic

Table 1. Prevalences of IgE sensitization to inhalant allergens in patients with self-reported food hypersensitivity and atopic disease

Allergen	Number of patients	% of patients ($n = 43$)
Birch	32	74
Timothy grass	31	72
Dog	27	63
<i>Dermatophagoides pteron.</i>	23	54
Cat	20	47
Grey alder	15	35
Hazel	15	35
Mugwort	13	30
Horse	10	23
<i>Cladosporium herbarum</i>	5	12
Other moulds	3	7

Table 2. Prevalences of IgE sensitization to food allergens in patients with self-reported food hypersensitivity and atopic disease

Allergen	Number of patients	% of patients ($n = 43$)
Wheat	20	47
Peanut	14	33
Rye	12	28
Hazelnut	11	26
Barley	10	23
Soy bean	9	21
Oat	7	16
Shrimp	5	12
Crab	5	12
Carrot	4	9
Egg white	4	9
Cow's milk	3	7

symptoms of any kind. Two patients had elevated serum total IgE without a history of atopic symptoms, and with no positive test results indicating specific IgE production or any other obvious explanations for their high total IgE levels.

Provocation tests

In the atopic group, trials with elimination diet and provocation tests with wheat as the test allergen was indicated and performed in 13 of the 20 patients who

had positive specific IgE and/or SPT to wheat. The elimination diet was positive in 12 patients. They underwent open provocation, which came out positive in six cases. None of these six patients came out with a positive DBPCFC. Five other patients underwent DBPCFC with milk, with negative results. In another three patients, food allergy toward shrimp and crab was highly suspected based on history and corresponding positive tests, but these patients refrained from provocation tests because of previous strong reactions. Positive test results for nut allergens corresponded with a history of OAS (and rhinoconjunctivitis) in all cases, but because nuts rarely were suspected as a cause of gastrointestinal complaints, provocation tests with nuts were not performed in this study.

Seven of the non-atopic patients went through trials with elimination diet and provocation tests. All seven had symptom relief after the elimination diet, and five of them had a positive open provocation test. One came out with a positive DBPCFC, in which food hypersensitivity to cow's milk (non-allergic food hypersensitivity or non-IgE-mediated food allergy) was confirmed.

Gastroenterological assessments

A total of 66 patients (93%) fulfilled the Rome II criteria of having IBS, 39 in the atopic group (91%) and 27 (97%) in the non-atopic patient group. A diarrhoea-predominant symptom pattern was more prevalent in atopic (65%) than in non-atopic (50%) patients, but the difference was not statistically significant. The frequency of constipation-predominant IBS was 30% in the atopic group and 35% in the non-atopic group, while 5% and 15% of the respective groups were unable to decide which of the two bowel patterns that predominated.

There were no significant differences between atopic and non-atopic patients with respect to the spectrum and severity of gastrointestinal symptoms (IBS-SQ; Table 3). Five patients with atopic disease and five non-atopic patients were *H. pylori* positive. The duodenal mucosa was macroscopically normal in all patients. Histological evaluation showed mild unspecific inflammatory changes of the duodenal mucosa in five patients with atopic disease, and in two non-atopic patients. Among atopic patients who were sensitized to pollen allergens ($n = 41$), 21 (51%) had the biopsy sampling performed off-season (between October and February).

Table 3. Severity of gastrointestinal symptoms in atopic and non-atopic patients, according to the Irritable Bowel Syndrome Symptom Questionnaire (IBS-SQ)

	Atopic patients ($n = 43$)		Non-atopic patients ($n = 28$)		<i>P</i>
	Mean	s.d.	Mean	s.d.	
Nausea	4.0	2.8	3.2	3.2	0.194
Bloating	6.1	2.5	6.7	2.4	0.282
Pain	5.1	2.4	5.7	2.9	0.260
Diarrhoea	5.7	2.6	4.4	3.3	0.103
Constipation	2.5	2.6	2.4	2.6	0.873
Anorexia	1.9	2.5	1.3	2.3	0.395

Quantitative assessment of duodenal mucosal cells

The median number of IgE-positive cells pr/mm^2 duodenal mucosa was significantly higher in atopic (median 73.2, IQR 32.7–104.9) patients than in non-atopic (median 28.2, IQR 4.9–71.8, $P = 0.003$) patients (Figure 2a). A majority of cells were peripherally stained and appeared as ring-formed structures (Figure 2b), which is indicative of membrane-bound IgE. This finding is consistent with a previous study based on double-staining, which concluded that IgE-positive cells in human intestinal mucosa are mainly mast cells 'armed' with IgE.²⁹ Among atopic patients who were sensitized to pollen allergens, there were no significant differences between in-season and off-season sampled biopsies with respect to counts of eosinophils, tryptase- and IgE-positive cells (data not shown). Hence, when comparing the non-atopic patients with only the off-season sampled biopsies from atopic patients, the numbers of IgE-positive cells still remained significantly higher in the atopic group (median 82.7, IQR 32.4–99.4, $P = 0.018$). There were no significant differences in eosinophil numbers between atopic (median 121.3, IQR 94.0–179.1) and non-atopic patients (median 116.5, IQR 89.4–190.5, $P = 0.962$). Neither was there a significant difference in tryptase-positive mast cell counts between patients with (median 175.7, IQR 151.9–210.1) and without (median 173.8, IQR 133.3–214.8) atopic disease. Patients who were sensitized to food allergens and patients who were sensitized to inhalant allergens only did not differ significantly with respect to duodenal cell numbers.

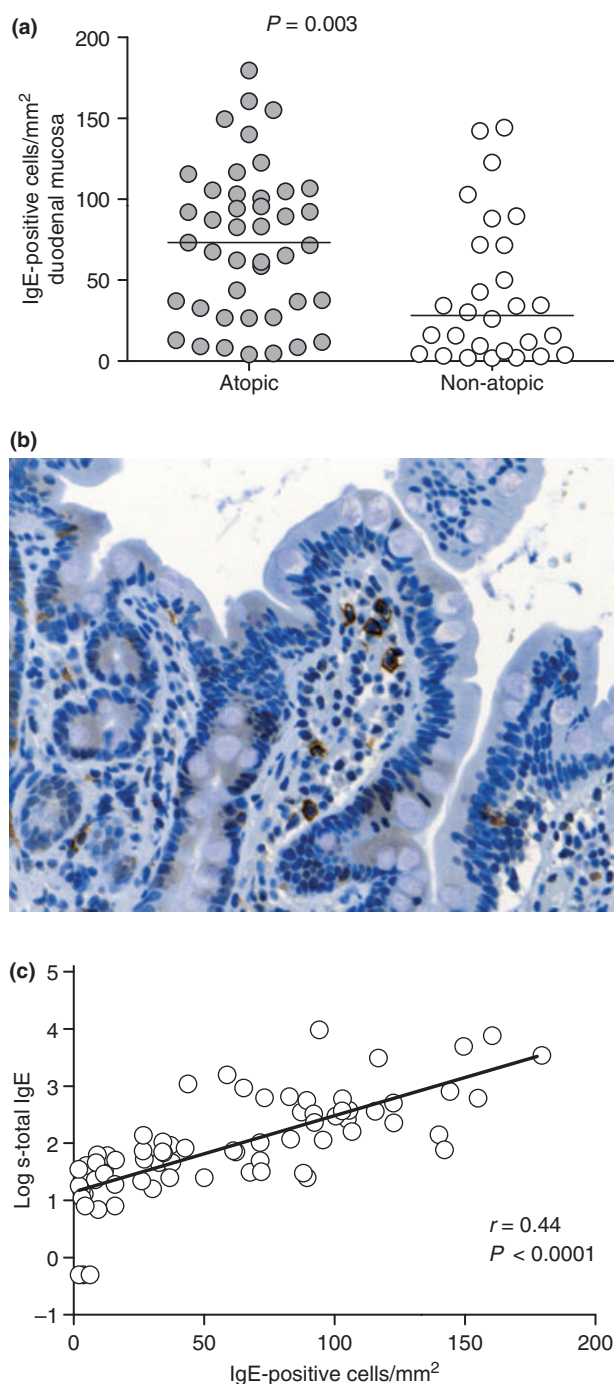


Figure 2. IgE-positive cells in the duodenal mucosa. (a) IgE-positive cells in patients with self-reported food hypersensitivity, with ($n = 43$) and without ($n = 28$) atopic disease. (b) IgE-‘armed’ cells in the duodenal mucosa of patients with self-reported food hypersensitivity (400 $\times$ magnification). (c) Correlation between log-transformed levels of serum total IgE and duodenal IgE-positive cells in patients with self-reported food hypersensitivity ($n = 71$).

Relationships between serum markers and duodenal cells

The numbers of IgE-positive cells in duodenal mucosa were significantly correlated to log-transformed levels of serum total IgE (Figure 2c). There was no significant correlation between serum ECP and duodenal eosinophil counts ($r = 0.29$, $P = 0.066$), or between serum tryptase and duodenal tryptase-positive mast cell counts ($r = -0.13$, $P = 0.560$).

Relationships between gastrointestinal symptoms and findings

There were no significant correlations between the severity of gastrointestinal symptoms (IBS-SQ) and numbers of duodenal eosinophils, IgE-positive cells, tryptase-positive mast cells or intestinal permeability (data not shown).

Intestinal permeability

Three patients did not complete the permeability assessment (they expelled the nasoduodenal tube). Another seven samples were excluded from the analysis because of visible contamination of the urine samples with faecal material. As illustrated in Figure 3, the median level of urinary excretion of $^{51}\text{CrEDTA}$ was significantly higher in atopic patients (median 0.10, IQR 0.08–0.13) than in non-atopic patients (median 0.08, IQR 0.05–0.10). There was no significant difference in intestinal permeability between atopic patients

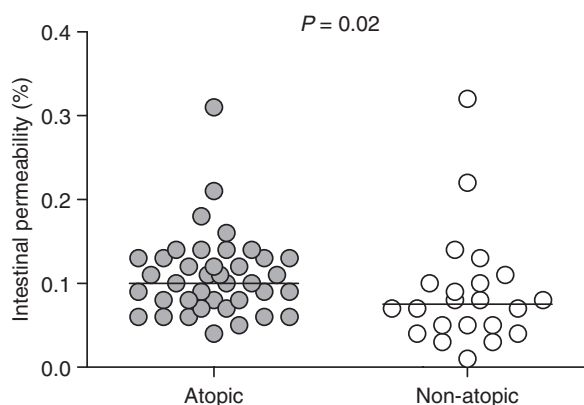


Figure 3. Intestinal permeability in patients with self-reported food hypersensitivity, with ($n = 39$) and without ($n = 22$) atopic disease.

who were sensitized to food allergens and patients who were sensitized to inhalant allergens only. Among patients who were sensitized to pollen allergens, there was no significant difference in intestinal permeability between those who had the measurement done in-season and those who were examined off-season (data not shown).

DISCUSSION

Patients with gastrointestinal complaints self-attributed to food hypersensitivity had a high prevalence of IBS (93%) and a high prevalence of atopic disease (61%). Elevated numbers of IgE-positive cells in the duodenal mucosa and increased intestinal permeability were notable findings in the atopic patients compared with non-atopic patients. The IgE-sensitization was most often directed towards inhalant allergens and to pollen-associated food allergens. However, DBPCFCs were negative; hence, the clinical significance of the sensitization is uncertain.

The prevalence rate of atopic diseases in these patients is considerably higher than in the general population, in particular the prevalence of allergic rhinitis (58% vs. 17%–29%).³⁰ A similarly high prevalence of atopic disease was found in another study of patients with IBS and perceived food intolerance ($n = 29$), where 60% had atopic disease as defined by three or more positive SPTs to inhalant allergens.³¹ Whether such findings reflect an association between atopic disease and gastrointestinal symptoms *per se* remains uncertain, as gastroenterology patients who attribute their complaints to food hypersensitivity is a selected group.^{32, 33} However, a high prevalence of allergic symptoms in unselected gastroenterology patients¹¹ and, *vice versa*, a high prevalence of IBS-like symptoms in allergic patients,^{10, 11} both suggest that atopic disease and gastrointestinal complaints may be associated, independently of whether the patients suspect food hypersensitivity as the main cause of their symptoms or not.

Our finding of elevated counts of IgE-positive cells in the duodenal mucosa of atopic patients with self-reported food hypersensitivity is consistent with some previous studies. In adult patients with suspected food hypersensitivity mainly manifested as diarrhoea, Bengtsson *et al.* found that the prevalence of duodenal IgE-positive cells was significantly higher among patients with a positive SPT to a suspected food allergen than among SPT-negative patients.³⁴ Notably, all

patients with a food-positive SPT also had positive SPTs to inhalant allergens and the prevalence of rhinitis was significantly higher in patients with positive SPTs than in those with negative SPTs. In patients with atopic eczema, numbers of duodenal IgE-positive cells are found to be increased in both children¹⁵ and adults,¹⁶ and in patients with confirmed food allergy, IgE-positive cells are found to be increased in the duodenal and jejunal mucosa.^{35, 36}

The increase in duodenal IgE-positive cells in atopic patients in the present study was not accompanied by an increase in the numbers of eosinophils or tryptase-positive mast cells. This is in contrast with some previous studies.^{13, 14, 37} On the other hand, our results are consistent with those of Kalimo *et al.*, who found the numbers of duodenal mast cells to be similar in patients with atopic eczema and controls, despite a significant increase in IgE-positive cells in the atopic patients.¹⁶

With respect to intestinal permeability in atopic patients, previous studies have provided conflicting results. Our finding of increased urinary excretion of ⁵¹CrEDTA in atopic patients with gastrointestinal complaints is consistent with the results of Dunlop *et al.*, who showed that adult IBS patients with hay fever, asthma or eczema had increased proximal small intestinal permeability as compared with IBS patients without atopic symptoms.²¹ Another study based on ⁵¹CrEDTA showed increased intestinal permeability in adult patients with asthma when compared with healthy controls,³⁸ but there was no significant difference between patients with allergic asthma and patients with non-allergic asthma. In a study of adult patients with atopic eczema, however, the intestinal absorption of ⁵¹CrEDTA did not differ from that in healthy controls.³⁹ Some studies based on the absorption of inert sugar molecules found increased intestinal permeability in patients with atopic disease,^{17–19} while others have not found any significant differences between atopic patients and controls.⁴⁰ By increasing the mucosal uptake of allergens, gut barrier dysfunction may be an important pathogenetic factor for the development of atopic diseases. On the other hand, increased permeability may also be a secondary phenomenon and a result of (allergic) inflammation or other damage of the intestinal mucosal barrier. The cross-sectional design of the present study does not provide information with respect to the direction of causality in patients with self-reported food hypersensitivity. Our two patient groups were also largely overlapping with respect to intestinal permeability values,

leaving the clinical significance of this finding uncertain, and the question whether and how an atopic subtype of IBS is associated with altered intestinal permeability needs to be further elucidated.

Consistent with some previous studies,^{29, 34} the IgE-positive cells observed in the present study appeared to be mainly mast cells. Activation of intestinal mast cells may play a role in the development of IBS-like symptoms.⁴¹ We did not assess the activation of mast cells locally in the gastrointestinal tract; hence, it is uncertain whether the 'armed' mast cells we observed are more easily activated and contribute more to the development of symptoms than 'unarmed' mast cells. An alternative possibility is that they are merely 'passively' sensitized by systemic circulating non-specific IgE antibodies, and that their function is no different from other mast cells as long as they are not triggered by specific antigens. However, recent studies in animals have shown that membrane-bound IgE may exert a role on mast cell biology and activation, also in the absence of specific antigens.⁴²

Although we did not find any correlation between intestinal IgE-positive cells and the severity of gastrointestinal symptoms in the present study, some previous studies have found an association. In a study of patients with seasonal allergic rhinitis, the numbers of IgE-positive cells and eosinophils in the duodenal mucosa were found to be increased during the pollen season as compared with off-season, and some of the patients also reported seasonal worsening of gastrointestinal symptoms.⁴³ In another study, patients with gastrointestinal complaints and food hypersensitivity confirmed by a positive DBPCFC (mainly serum IgE-negative) showed significantly higher numbers of IgE-positive cells, eosinophils and T cells during symptomatic than during asymptomatic periods.⁴⁴

If the presence of elevated numbers of IgE-coated intestinal mast cells plays a role in symptom generation, it may have implications for treatment as well. Mast cell stabilizers, such as disodium cromoglycate, are used in the treatment of atopic diseases and have also been tried in the treatment of IBS, possibly with particular success in those with food-positive SPT.^{45–47} None of our patients was sensitized to food allergens only; they were also sensitized to inhalant allergens. Moreover, the numbers of IgE-positive (mast) cells and the intestinal permeability did not differ between

patients who were sensitized to food and those who were sensitized to inhalant allergens only. The latter finding is consistent with the previous suggestion that individuals with atopic diseases may have a non-specific alteration in the mucosal defence, whether or not they are sensitized to food allergens.¹⁷ Whether the effect of a mast cell stabilizer on IBS symptoms would extend to patients sensitized to non-food allergens could be an interesting topic for future studies.

In summary, the present study showed that patients with abdominal complaints self-attributed to food hypersensitivity had a high prevalence of IBS. Patients' own perceptions of gastrointestinal food hypersensitivity corresponded poorly with IgE sensitization, and food allergy was not confirmed by DBPCFC. Nevertheless, the prevalence of other atopic diseases was high and these atopic patients had increased density of duodenal IgE-bearing cells compared with non-atopic patients. Hence, we found indications of 'atopic bowel' in a substantial subgroup of patients with self-reported food hypersensitivity. Recently, 'atopic IBS' was suggested as a new subgroup of IBS, with the intestinal mucosal mast cell as a possible pivotal pathophysiological factor.^{11, 48} The atopic IBS patients in the present study did not differ from non-atopic patients in terms of gastrointestinal symptoms. However, our results indicate that 'atopic bowel' may be a relevant topic for future research on the pathogenesis and pathophysiology of food-related gastrointestinal complaints.

ACKNOWLEDGEMENTS

Declaration of personal interests: Kristine Lillestøl, Gülen Arslan Lied, Jørgen Valeur and Ragna Lind have research grants from the University of Bergen and the Western Norway Regional Health Authority. We thank Brynhild Haugen (Department of Pathology, Haukeland University Hospital) and Aud-Sissel Eriksen Hjart-holm and Gro Olderøy (Department of Medicine, Haukeland University Hospital) for skillful and diligent assistance with the preparation of biopsy samples and permeability measurements respectively. We also acknowledge the other members of the MAI (Food Allergy and Intolerance) investigation team at Haukeland University Hospital, for important contributions in the collection of data, and for many enriching discussions. *Declaration of funding interests:* None.

REFERENCES

- 1 Rona RJ, Keil T, Summers C, *et al.* The prevalence of food allergy: a meta-analysis. *J Allergy Clin Immunol* 2007; 120: 638–46.
- 2 Woods RK, Stoney RM, Raven J, Walters EH, Abramson M, Thien FC. Reported adverse food reactions overestimate true food allergy in the community. *Eur J Clin Nutr* 2002; 56: 31–6.
- 3 Asero R, Ballmer-Weber BK, Beyer K, *et al.* IgE-mediated food allergy diagnosis: current status and new perspectives. *Mol Nutr Food Res* 2007; 51: 135–47.
- 4 Jansen JJ, Kardinaal AF, Huijbers G, Vlieg-Boerstra BJ, Martens BP, Ockhuizen T. Prevalence of food allergy and intolerance in the adult Dutch population. *J Allergy Clin Immunol* 1994; 93: 446–56.
- 5 Young E, Stoneham MD, Petrukevitch A, Barton J, Rona R. A population study of food intolerance. *Lancet* 1994; 343: 1127–30.
- 6 Schafer T, Bohler E, Ruhdorfer S, *et al.* Epidemiology of food allergy/food intolerance in adults: associations with other manifestations of atopy. *Allergy* 2001; 56: 1172–9.
- 7 Woods RK, Thien F, Raven J, Walters EH, Abramson M. Prevalence of food allergies in young adults and their relationship to asthma, nasal allergies, and eczema. *Ann Allergy Asthma Immunol* 2002; 88: 183–9.
- 8 Arslan G, Kahrs GE, Lind R, Froyland L, Florvaag E, Berstad A. Patients with subjective food hypersensitivity: the value of analyzing intestinal permeability and inflammation markers in gut lavage fluid. *Digestion* 2004; 70: 26–35.
- 9 Zar S, Kumar D, Benson MJ. Food hypersensitivity and irritable bowel syndrome. *Aliment Pharmacol Ther* 2001; 15: 439–49.
- 10 Powell N, Huntley B, Beech T, Knight W, Knight H, Corrigan CJ. Increased prevalence of gastrointestinal symptoms in patients with allergic disease. *Postgrad Med J* 2007; 83: 182–6.
- 11 Tobin MC, Moparty B, Farhadi A, DeMeo MT, Bansal PJ, Keshavarzian A. Atopic irritable bowel syndrome: a novel subgroup of irritable bowel syndrome with allergic manifestations. *Ann Allergy Asthma Immunol* 2008; 100: 49–53.
- 12 Kokkonen J, Simila S, Herva R. Gastrointestinal findings in atopic children. *Eur J Pediatr* 1980; 134: 249–54.
- 13 Pires GV, Souza HS, Elia CC, *et al.* Small bowel of patients with asthma and allergic rhinitis: absence of inflammation despite the presence of major cellular components of allergic inflammation. *Allergy Asthma Proc* 2004; 25: 253–9.
- 14 Wallaert B, Desreumaux P, Copin MC, *et al.* Immunoreactivity for interleukin 3 and 5 and granulocyte/macrophage colony-stimulating factor of intestinal mucosa in bronchial asthma. *J Exp Med* 1995; 182: 1897–904.
- 15 Caffarelli C, Cavagni G, Romanini E, Caruana P, De Angelis G. Duodenal IgE-positive cells and elimination diet responsiveness in children with atopic dermatitis. *Ann Allergy Asthma Immunol* 2001; 86: 665–70.
- 16 Kalimo K, Lammintausta K, Klemi P, Leino R, Panula P, Kalimo H. Mast cells and IgE in intestinal mucosa in adult atopic dermatitis patients. *Br J Dermatol* 1988; 119: 579–85.
- 17 Jackson PG, Lessof MH, Baker RW, Ferrett J, MacDonald DM. Intestinal permeability in patients with eczema and food allergy. *Lancet* 1981; 1: 1285–6.
- 18 Majamaa H, Isolauri E. Evaluation of the gut mucosal barrier: evidence for increased antigen transfer in children with atopic eczema. *J Allergy Clin Immunol* 1996; 97: 985–90.
- 19 Pike MG, Heddle RJ, Boulton P, Turner MW, Atherton DJ. Increased intestinal permeability in atopic eczema. *J Invest Dermatol* 1986; 86: 101–4.
- 20 Ukabam SO, Mann RJ, Cooper BT. Small intestinal permeability to sugars in patients with atopic eczema. *Br J Dermatol* 1984; 110: 649–52.
- 21 Dunlop SP, Hebden J, Campbell E, *et al.* Abnormal intestinal permeability in subgroups of diarrhea-predominant irritable bowel syndromes. *Am J Gastroenterol* 2006; 101: 1288–94.
- 22 Venge P. Monitoring the allergic inflammation. *Allergy* 2004; 59: 26–32.
- 23 Payne V, Kam PC. Mast cell tryptase: a review of its physiology and clinical significance. *Anaesthesia* 2004; 59: 695–703.
- 24 Johansson SG, Bieber T, Dahl R, *et al.* Revised nomenclature for allergy for global use: Report of the Nomenclature Review Committee of the World Allergy Organization, October 2003. *J Allergy Clin Immunol* 2004; 113: 832–6.
- 25 Berstad A, Arslan G, Folvik G. Relationship between intestinal permeability and calprotectin concentration in gut lavage fluid. *Scand J Gastroenterol* 2000; 35: 64–9.
- 26 Thompson WG, Longstreth GF, Drossman DA, Heaton KW, Irvine EJ, Muller-Lissner SA. Functional bowel disorders and functional abdominal pain. *Gut* 1999; 45(Suppl 2): II43–7.
- 27 Kane SV, Sandborn WJ, Rufo PA, *et al.* Fecal lactoferrin is a sensitive and specific marker in identifying intestinal inflammation. *Am J Gastroenterol* 2003; 98: 1309–14.
- 28 Folvik G, Bjerke-Larsen T, Odegaard S, Hausken T, Gilja OH, Berstad A. Hydrosonography of the small intestine: comparison with radiologic barium study. *Scand J Gastroenterol* 1999; 34: 1247–52.
- 29 Rognum TO, Brandtzaeg P. IgE-positive cells in human intestinal mucosa are mainly mast cells. *Int Arch Allergy Appl Immunol* 1989; 89: 256–60.
- 30 Bauchau V, Durham SR. Prevalence and rate of diagnosis of allergic rhinitis in Europe. *Eur Respir J* 2004; 24: 758–64.
- 31 Smith MA, Youngs GR, Finn R. Food intolerance, atopy, and irritable bowel syndrome. *Lancet* 1985; 2: 1064.
- 32 Monsbakken KW, Vandvik PO, Farup PG. Perceived food intolerance in subjects with irritable bowel syndrome - etiology, prevalence and consequences. *Eur J Clin Nutr* 2006; 60: 667–72.
- 33 Simren M, Mansson A, Langkilde AM, *et al.* Food-related gastrointestinal symptoms in the irritable bowel syndrome. *Digestion* 2001; 63: 108–15.
- 34 Bengtsson U, Rognum TP, Brandtzaeg P, *et al.* IgE-positive duodenal mast cells in patients with food-related diarrhea. *Int Arch Allergy Appl Immunol* 1991; 95: 86–91.
- 35 Caffarelli C, Romanini E, Caruana P, Street ME, De Angelis G. Clinical food hypersensitivity: the relevance of duodenal immunoglobulin E-positive cells. *Pediatr Res* 1998; 44: 485–90.
- 36 Rosekrans PC, Meijer CJ, Cornelisse CJ, van der Wal AM, Lindeman J. Use of morphometry and immunohistochemistry of small intestinal biopsy specimens in the diagnosis of food allergy. *J Clin Pathol* 1980; 33: 125–30.

- 37 Bagnato GF, Di Cesare E, Caruso RA, *et al.* Gastric mucosal mast cells in atopic subjects. *Allergy* 1995; **50**: 322–7.
- 38 Benard A, Desreumeaux P, Huglo D, Hoorbeke A, Tonnel AB, Wallaert B. Increased intestinal permeability in bronchial asthma. *J Allergy Clin Immunol* 1996; **97**: 1173–8.
- 39 Bjarnason I, Goolamali SK, Levi AJ, Peters TJ. Intestinal permeability in patients with atopic eczema. *Br J Dermatol* 1985; **112**: 291–7.
- 40 Barba A, Schena D, Andreass MC, *et al.* Intestinal permeability in patients with atopic eczema. *Br J Dermatol* 1989; **120**: 71–5.
- 41 Santos J, Guilarte M, Alonso C, Malagelada JR. Pathogenesis of irritable bowel syndrome: the mast cell connection. *Scand J Gastroenterol* 2005; **40**: 129–40.
- 42 Kawakami T, Kitaura J. Mast cell survival and activation by IgE in the absence of antigen: a consideration of the biologic mechanisms and relevance. *J Immunol* 2005; **175**: 4167–73.
- 43 Magnusson J, Lin XP, Dahlman-Hoglund A, *et al.* Seasonal intestinal inflammation in patients with birch pollen allergy. *J Allergy Clin Immunol* 2003; **112**: 45–50.
- 44 Lin XP, Magnusson J, Ahlstedt S, *et al.* Local allergic reaction in food-hypersensitive adults despite a lack of systemic food-specific IgE. *J Allergy Clin Immunol* 2002; **109**: 879–87.
- 45 Stefanini GF, Bazzocchi G, Prati E, Lanfranchi GA, Gasbarrini G. Efficacy of oral disodium cromoglycate in patients with irritable bowel syndrome and positive skin prick tests to foods. *Lancet* 1986; **1**: 207–8.
- 46 Stefanini GF, Prati E, Albini MC, *et al.* Oral disodium cromoglycate treatment on irritable bowel syndrome: an open study on 101 subjects with diarrheic type. *Am J Gastroenterol* 1992; **87**: 55–7.
- 47 Stefanini GF, Saggioro A, Alvisi V, *et al.* Oral cromolyn sodium in comparison with elimination diet in the irritable bowel syndrome, diarrheic type. Multicenter study of 428 patients. *Scand J Gastroenterol* 1995; **30**: 535–41.
- 48 Tobin MC, Keshavazian A, Farhardi A. Atopic irritable bowel syndrome: same old hat or a new entity? *Expert Rev Gastroenterol Hepatol* 2008; **2**: 457–9.