Clinical and Experimental Allergy, **37**, 823–830

ORIGINAL PAPER

Alterations of food antigen-specific serum immunoglobulins G and E antibodies in patients with irritable bowel syndrome and functional dyspepsia

X. L. Zuo*, Y. Q. Li*, W. J. Li*, Y. T. Guo*, X. F. Lu*, J. M. Li* and P. V. Desmond†

**Department of Gastroenterology, Qilu Hospital, Shandong University, Jinan, China and †Department of Gastroenterology, St Vincent's Hospital, Fitzroy Vic., Australia*

Clinical and Experimental Allergy

Summary

Background Post-prandial worsening of symptoms as well as adverse reactions to one or more foods are common in the patients with functional gastrointestinal diseases, such as irritable bowel syndrome (IBS) and functional dyspepsia (FD). However, the role played by true food allergy in the pathogenesis of these diseases is still controversial and there are no well-established tests to identify food allergy in this condition.

Objective To investigate serum food antigen-specific IgG, IgE antibody and total IgE antibody titres in controls and patients with IBS and FD, and to correlate symptoms with the food antigen-specific IgG titres in IBS and FD patients.

Methods Thirty-seven IBS patients, 28 FD patients and 20 healthy controls participated in this study. Serum IgG and IgE antibody titres to 14 common foods including beef, chicken, codfish, corn, crab, eggs, mushroom, milk, pork, rice, shrimp, soybean, tomatoes and wheat were analysed by ELISA. Serum total IgE titres were also measured. Last, symptomatology was assessed in the study.

Results IBS patients had significantly higher titres of IgG antibody to crab ($P = 0.000$), egg ($P = 0.000$), shrimp ($P = 0.000$), soybean ($P = 0.017$) and wheat ($P = 0.004$) than controls. FD patients had significantly higher titres of IgG antibody to egg ($P = 0.000$) and soybean ($P = 0.017$) than controls. The percentage of individuals with detectable positive food antigen-specific IgE antibodies of the three groups did not show any significant differences ($P = 0.971$). There were no significant differences between IBS patients, FD patients and controls in the serum total IgE antibody titres ($P = 0.978$). Lastly, no significant correlation was seen between symptom severity and serum food antigen-specific IgG antibody titres both in IBS and FD patients.

Conclusion Serum IgG antibody titres to some common foods increased in IBS and FD patients compared to controls. But there is no significant correlation between symptom severity and elevated serum food antigen-specific IgG antibodies in these patients.

Correspondence:
Prof. YanQing Li, Department of Gastroenterology, Qilu Hospital of Shandong University, Jinan 250012, China.
E-mail: mx8902@163.com

Keywords food allergy, functional dyspepsia, IgE, IgG, irritable bowel syndrome
Submitted 25 November 2006; revised 6 March 2007; accepted 16 March 2007

Introduction

Food allergy is a complex area of medicine. Up to 20% of the population have adverse reactions to food and claim to be of food allergy or food intolerance [1]. The percentage of food allergy is much higher in some functional diseases of the gastrointestinal (GI) tract such as irritable bowel syndrome (IBS), with 20–65% of patients attributing their symptoms to food allergy [2–4]. However, the role played by true food allergy in the pathogenesis of IBS and other functional diseases of the GI tract are still controversial and there are no well-established tests to identify food allergy in this condition [5–7].

Previously, food allergy was believed to be associated with an IgE-mediated immune response to a particular allergen in the diet. Therefore, the standardized skin prick

testing and RAST testing were frequently used to diagnose food allergy [8, 9]. However, there is no evidence that shows IgE does indeed play an important role in hypersensitive reactions to food in IBS patients [10–14]. The gold standard in this condition is the double blind placebo-controlled food challenge test under careful supervision in a hospital. But this test is cumbersome, time consuming and of poor patient compliance, which limits its use in clinical practise. Fortunately, accumulating data in recent years have indicated that IgG-mediated immune response, which characteristically gives a more delayed response following exposure to a particular antigen, is of great importance in food allergy [15–17] and the measurement of serum IgG titres opens up a new avenue for diagnosing food allergy in patients suffering adverse reactions to foods.

Post-prandial worsening of symptoms as well as adverse reactions to one or more foods are common [18, 19] and dietary elimination can lead to symptomatic improvements in patients with IBS [3, 20–22]. Recently, Zar et al. [23] have shown elevated IgG titres in IBS patients while Atkinson et al. [24] have demonstrated that food elimination based on IgG antibodies may be effective in reducing IBS symptoms. Other studies have shown food allergy is also present in functional dyspepsia (FD), which has overlapping symptoms with IBS [25, 26]. However, fewer studies focus on the role of food allergy in the pathogenesis of FD and the level of serum food antigen-specific IgG antibodies in FD has not been investigated.

The aim of this study is to compare serum food antigen-specific IgG and IgE antibodies in controls and patients with IBS and FD, and to correlate symptoms with the food antigen-specific IgG and IgE titres in IBS and FD patients.

Materials and methods

Patients

Thirty-seven IBS patients (12 men and 25 women; mean age 36 years) and 28 FD patients (nine men and 19 women; mean age 35 years) participated in this study. All patients were recruited from the Department of Gastroenterology of Qilu Hospital, Shandong University and were diagnosed by using the Rome II criteria [27] for IBS (pain or abdominal discomfort accompanied by two or three symptoms, such as relief with defecation and/or with alterations in the frequency of evacuations or in the shape of the feces for at least 12 weeks, which need not be consecutive, in the preceding 12 months; in the absence of organic GI diseases) and for FD (persistent or recurrent symptoms, such as pain or discomfort in the upper abdomen at least 12 weeks earlier, not necessarily serial, during the preceding 12 months). Organic GI disorders were excluded by routine laboratory tests and endoscopies with biopsies.

Controls

The control group consisted of 20 healthy subjects (six men and 14 women, mean age 36.5 years) recruited from the community. All control subjects were free of GI symptoms and had no evidence of acute or chronic illnesses.

None of the patients or healthy individuals was on any medication at the time of the study. The patient groups and the control group did not differ significantly with respect to mean age and sex ratio.

Informed, preferably written, consent was obtained from each subject. The study has been performed according to the Declaration of Helsinki and the procedures have been approved by Qilu hospital ethics committee.

Measurement of serum food antigen-specific immunoglobulins G and E antibodies. Serum samples were collected from all subjects and stored at $-20\,^{\circ}C$ for subsequent analysis. Serum IgG and IgE antibody titres to 14 common foods including beef, chicken, codfish, corn, crab, eggs, mushroom, milk, pork, rice, shrimp, soybean, tomatoes and wheat were analysed by ELISA.

Serum food antigen-specific immunoglobulin G enzyme-linked immunosorbent assay. Serum food antigen-specific IgG ELISA was performed with Allerquant Food Allergy Screening ELISA Kit (Biomerica, Inc. Newport Beach, CA, USA).

According to the instruction of the ELISA Kit, 50, 100, 200 and 400 U/mL of Food IgG Calibrator were prepared and added to the microplate together with blanks and positive controls. This is the calibration curve to be used in the assay. Serum samples were diluted to 1/100 and 25 μL of each serum sample were taken and added to 2.5 mL of serum dilute regents. Then 100 μL of the diluted patient serum were placed into the microwells coated with 14 food antigens for 60 min at room temperature. And then, 100 μL of food IgG–HRP conjugate were added to each well after washing and the plates were incubated for 30 min at room temperature. Another 100 μL of working substrate mix (TMB and H_2O_2) were applied to each well and the plates were covered and incubated for 10 min at room temperature. The reaction was stopped using 50 mL per well of 1 N sulphuric acid. At last, the plates were read using a Dynex ELISA plate reader (Dynex Technologies, Inc., Chantilly, VA, USA) at 450 nm. The IgG concentration to each food antigen was expressed as units per millitre.

Plates with microwells were washed three times with washing buffer between steps, and were incubated at room temperature for each stage of the assay.

Serum food antigen-specific immunoglobulin E and total immunoglobulin E enzyme-linked immunosorbent assay. Serum food antigen-specific IgE and total IgE ELISA were performed with the IVT Allergy Profile kit (In vitro

Technologies Inc. Arlington, TX, USA). It is a qualitative test designed to identify IgE levels in the test samples. A positive result is identified visually by a gradual yellow to purple colour change around a reactive segment within the capillary. This kit is also semiquantitative in that the rate of conversion from yellow to purple and the intensity of the purple colour is proportional to the level of IgE antibody in the patient sample. All components were allowed to come to the room temperature before use. The test procedures are as follows.

Firstly, each serum sample was added to a properly labelled multiple immunoassay device and allowed to react for 100 min at room temperature. Then was the sample expelled from the device and the device washed with 1 mL wash solution. Secondly, the conjugate reagent was added to the device and allowed to react for 100 min. Then, was the conjugate reagent expelled from the device and the device washed with wash solution. Thirdly, the substrate indicator was added to the device and the characteristic yellow to purple colour change was within the observed next 30 min period.

Highly allergic reactions to a particular allergen caused a rapid purple colouration around that specific segment within 5 min. Weaker allergen took progressively longer to effect the colour change. Negative reactions showed equivalent colour to the inert spacers. On the total IgE segment, low total IgE levels ($\leqslant$30 IU/mL) were evident by a yellow to grey colouration, distinguishable from normal IgE levels (60 $\pm$ 30 IU/mL), which yield moderately purple colour throughout, and elevated IgE levels (>90 IU/mL), which yield intense purple colouration after 30 min incubation with the substrate indicator.

Symptom questionnaire. Symptomatology was assessed in the study. All the patients completed a symptom assessment questionnaire based on Rome II criteria to evaluate the severity of symptoms for the 1 week period before the interview. Questions targeting the presence and severity of abdominal pain/discomfort, bloating, bowel urgency/diarrhoea, constipation, early satiety, nausea and belching were asked, for example 'Have you experienced abdominal pain/discomfort during this week?' If the subjects reported 'yes', they were then asked to grade the severity of that symptom using the scale 1 = mild, 2 = moderate, 3 = intense and 4 = severe. If the subjects reported 'no', the score was recorded as 0. The total score of each patient is the sum of each symptom score.

Statistical analysis. Linear models were fitted to the log transformation of the variables, such as the IgG and total IgE titres. A one-way ANOVA was carried out to test for differences in levels of antibody titres between the groups. Tamhane's T2 test was used to test for pairwise differences between IBS, FD and control groups. The χ^2 tests were used to test for the differences of the percentage of individuals with positive IgE antibodies between groups. Comparison between groups for interval data (age, weight) was carried out with *t*-test. The correlation between the individual symptom scores and the IgG titres was analysed by Pearson's correlation test. Significance was accepted at 5% level ($P < 0.05$). The statistics package SPSS v 13.0 running on Windows XP Professional was used for the analyses.

Results

Serum food antigen-specific immunoglobulin G antibody titres

The serum IgG antibody titres of each of the IBS, FD and control groups to each food antigen are shown in this study together with the results of the ANOVA (Table 1). There were significant differences between the three groups in their IgG responses to crab, egg, shrimp, soybean and wheat. IBS patients had significantly higher titres of IgG antibody to crab, egg, shrimp, soybean and wheat than controls and higher titres of IgG antibody to crab, egg, shrimp and wheat than FD patients. FD patients had significantly higher IgG antibody titres to egg and soybean than controls and lower IgG antibody titres than IBS patients to crab, egg, shrimp and wheat (Fig. 1). There were no significant differences between the three groups in their IgG responses to beef, chicken, codfish, corn, mushroom, milk, pork, rice and tomatoes.

Table 1. Serum IgG antibody titres to food antigens showing the means in IBS, FD and control groups, the standard error of the mean (SEM) and *P*-value from the ANOVA

	Mean $\pm$ SEM (U/mL)			
	IBS ($n = 37$)	FD ($n = 28$)	Control ($n = 20$)	*P*-value
Beef	32.86 ± 0.46^a	32.43 ± 0.44^a	32.10 ± 0.60^a	0.556
Chicken	28.65 ± 0.51^a	27.68 ± 0.58^a	27.20 ± 0.55^a	0.171
Codfish	32.86 ± 0.49^a	32.89 ± 0.52^a	32.55 ± 0.59^a	0.902
Corn	31.87 ± 0.43^a	31.82 ± 0.46^a	31.29 ± 0.60^a	0.617
Crab	50.27 ± 0.89^a	37.53 ± 0.95^b	37.90 ± 0.78^b	0.000
Eggs	119.3 ± 11.8^a	69.71 ± 4.63^b	51.93 ± 3.74^c	0.000
Mushroom	27.78 ± 0.51^a	26.93 ± 0.63^a	27.25 ± 0.49^a	0.515
Milk	33.14 ± 0.51^a	32.36 ± 0.64^a	32.41 ± 0.98^a	0.615
Pork	31.54 ± 0.45^a	31.82 ± 0.53^a	30.80 ± 0.59^a	0.437
Rice	28.68 ± 0.52^a	29.54 ± 0.75^a	28.41 ± 0.83^a	0.493
Shrimp	65.05 ± 3.70^a	45.39 ± 1.62^b	43.25 ± 1.73^b	0.000
Soybean	55.83 ± 3.35^a	53.29 ± 1.89^a	43.60 ± 1.95^b	0.017
Tomatoes	34.65 ± 0.78^a	33.79 ± 0.98^a	34.05 ± 1.23^a	0.782
Wheat	60.59 ± 3.4^a	49.39 ± 2.05^b	48.10 ± 2.01^b	0.004

Means with different superscripts across rows were significantly different ($P < 0.05$).

IBS, irritable bowel syndrome; FD, functional dyspepsia.

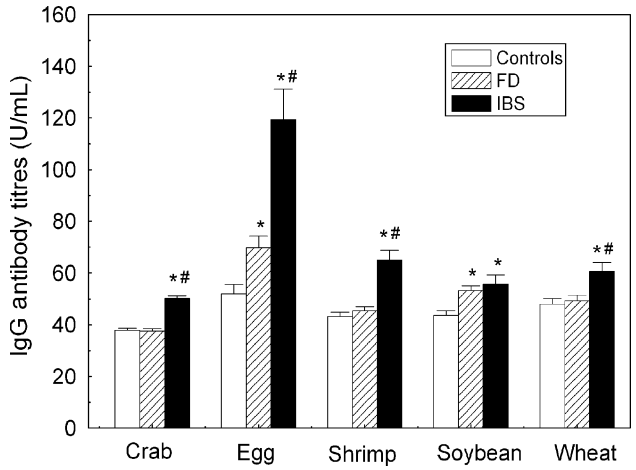

Fig. 1. Comparisons of serum IgG antibodies to crab, egg, shrimp, soybean and wheat between irritable bowel syndrome (IBS), functional dyspepsia (FD) and control groups. IBS patients have significantly higher IgG antibodies to crab, egg, shrimp, soybean and wheat than controls. FD patients have significantly higher IgG antibodies to egg and soybean than controls. IBS patients have significantly higher IgG antibodies to crab, egg, shrimp and wheat than FD patients. The data were expressed with the geometric means and the standard errors of the means. $^{*}P < 0.05$ compared with controls; $^{\#}P < 0.05$ compared with FD patients.

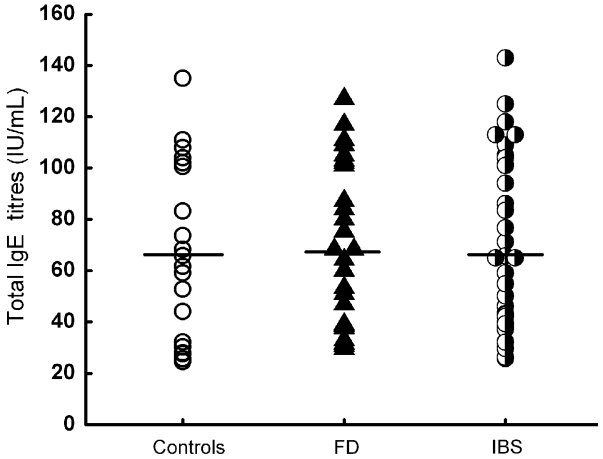

Fig. 2. Comparision of total IgE antibody titres to food antigens between irritable bowel syndrome (IBS), functional dyspepsia (FD) and control groups. The data were expressed with the individual values in the three groups. Horizontal bars represent the means for every group. There were no significant differences between IBS patients, FD patients and controls in the serum total IgE antibody titres ($P = 0.978$).

Serum food antigen-specific immunoglobulin E antibody titres

In terms of serum food antigen-specific IgE antibodies, 10 of 37 IBS patients, eight of 28 FD patients and six of 20 controls had detectable positive IgE ELISA results to beef, codfish, eggs, mushroom, soybean and wheat (Table 2). The percentage of individuals with detectable positive IgE antibodies of the three groups did not show any significant differences (27.03% for IBS, 28.57% for FD and 30% for controls, $\chi^2 = 0.059$, $P = 0.971$). In IBS patients, only two had positive IgE antibodies against codfish, three against eggs, one against mushroom and four against wheat. Of 28 FD patients, one had positive IgE antibodies against beef, one against codfish, two against eggs, one against soybean and three against wheat. In addition, one control had positive IgE antibodies against codfish, two against eggs, one against mushroom and two against wheat. The number of patients and controls with positive IgE antibodies against each individual food antigen was too small to apply a statistical test.

Table 2. Summary of the individuals with positive IgE ELISA results in IBS, FD and control groups

	IBS ($n = 37$)	FD ($n = 28$)	Control ($n = 20$)
Beef	0	1	0
Chicken	0	0	0
Codfish	2	1	1
Corn	0	0	0
Crab	0	0	0
Eggs	3	2	2
Mushroom	1	0	1
Milk	0	0	0
Pork	0	0	0
Rice	0	0	0
Shrimp	0	0	0
Soybean	0	1	0
Tomatoes	0	0	0
Wheat	4	3	2

IBS, irritable bowel syndrome; FD, functional dyspepsia.

Serum total immunoglobulin E antibody titres

The serum total IgE antibody titres were also measured in this study. There were no significant differences between IBS patients, FD patients and controls (means $\pm$ SEM 66.87 ± 7.65 for controls, 68.69 ± 5.9 for FD patients and 67.30 ± 5.49 for IBS patients, $P = 0.978$, Fig. 2).

Correlation with symptoms

The symptom severity of each FD and IBS patient was scored according to the symptom questionnaire. The relation between the symptoms and elevated IgG responses to some food antigens was observed and no significant correlation was seen between symptom severity and serum food antigen-specific IgG antibody titres both in IBS and FD patients (Fig. 3).

Discussion

This study demonstrated a significant increase in IgG antibody titres to several common foods in patients with functional diseases of the GI tract compared with healthy

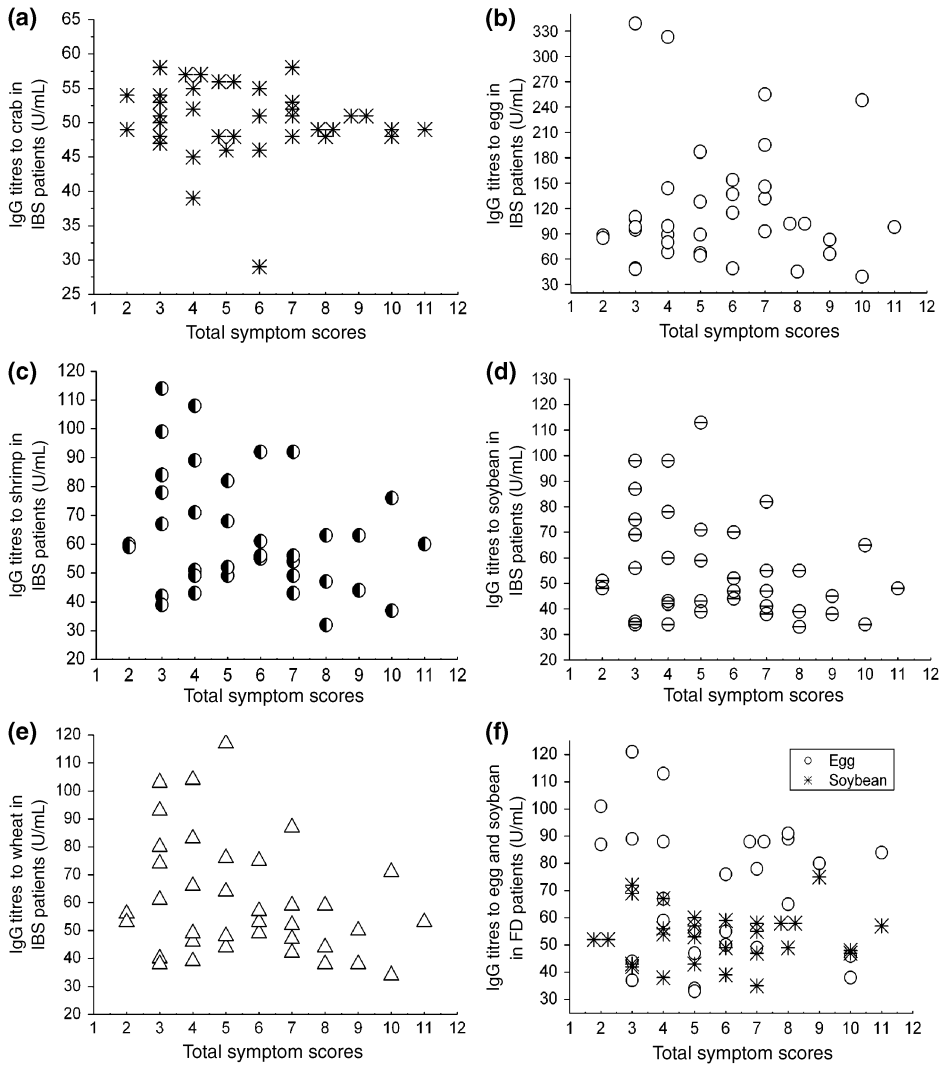

Fig. 3. The correlation between abdominal symptom scores and IgG titres to food antigen in irritable bowel syndrome (IBS) patients and functional dyspepsia (FD) patients. (a–e) The individual plots of values for food antigen-specific IgG titres and symptom scores in 37 IBS patients. No significant correlations are seen between symptom scores and IgG titres to crab (a, $P = 0.426$), eggs (b, $P = 0.657$), shrimp (c, $P = 0.800$), soybean (d, $P = 0.854$) and wheat (e, $P = 0.794$). (f) The individual plots of values for food antigen-specific IgG titres and symptom scores in 28 FD patients. No significant correlations were seen between symptom scores and IgG titres to eggs ($P = 0.460$) and soybean ($P = 0.986$).

subjects. In IBS patients, elevated food antigen-specific IgG antibodies to crab, egg, shrimp, soybean and wheat were observed while elevated food antigen-specific IgG antibodies to egg and soybean were observed in FD patients. It should be mentioned that, consistent with the previous study [23], the antibodies against wheat were significantly elevated in IBS patients. However, we also got some raised food antigen-specific antibodies different from the previous study [23], such as elevated antibody titres to crab and shrimp instead of beef. This might be due to the difference between a Chinese diet and a western diet. More specifically, most of the subjects in this study are from a coastal area (where this study was performed) in China and they are used to consume less beef and more seafood.

Food allergy can involve different organs and systems such as the digestive tract, the skin, the respiratory tract and the cardiovascular system. While dermatologic, respiratory and systemic manifestations of food allergy are well recognized, the reactions manifested primarily in the digestive tract can be difficult to recognize, diagnose and treat. This is due to the protean ways food can cause GI symptoms, the relatively poorly understood pathophysiologic mechanisms and the limited diagnostic methods available to objectively identify afflicted individuals. These deficiencies are, in part, a consequence of the

difficulty accessing the GI tract to establish mechanisms of disease and develop methods to diagnose and treat food allergy [28, 29]. Often, patients of this nature are classified as being psychosomatic or being functional without defining the real problem. It has been recognized for some time now that several 'functional diseases' might be associated with food allergy [30–34].

The mechanisms that underlie these increases in IgG antibody responses to some common foods remain speculative. The role of IgG and IgA antibodies in the coeliac disease is well studied [35]. Coeliac disease occurs due to a delayed immune reaction to gluten in wheat. This causes intestinal membrane damage, IgG/IgA change and the resultant diarrhoea, abdominal bloating and anaemia. Raised IgG antibodies to food antigens have also been reported in patients with asthma caused by milk allergy and patients with atopic dermatitis and/or bronchial asthma caused by soybean allergy [17, 36]. Exclusions of the offending foods from diet have shown to improve symptoms in these diseases. Raised food-specific IgG antibodies may play a similar pathophysiological role in IBS and FD patients. The allergic food antigens transported by way of M cells into the lamina propria activate T helper cells and B cells, increase the production of IgG and cytokines. Then the increased IgG antibodies and cytokines lead to the inflammation response of the gut, which is now believed to play an important role in the pathogenesis of IBS through inducing alterations in GI peristalsis, abdominal discomfort and bowel dysfunction.

In addition to the immune inflammation reaction explanation, another possible mechanism that may account for the elevated IgG antibodies is the alteration in the permeability of gut mucosa. Theoretically, any increases in the gut mucosal permeability in IBS and FD patients might increase the uptake of undigested protein and increase antigenic load presented to the mucosal immune system. This may lead to the increased IgG antibody titres even with a normal physiological response of the gut immune system. If the above hypothesis is correct, then a generalized increase in the IgG antibodies to all 14 food antigens should have been observed. However, in this study we found that the food-specific IgG antibodies increased to only some rather than to all food antigens. The possible explanation is that the patients might have selective gut permeability to food antigens and the increase of food-specific IgG titres is a specific reaction rather than a non-specific response to increased gut mucosal permeability.

In terms of serum food antigen-specific IgE antibody and total IgE antibody titres, no significant difference was found in IBS, FD patients and controls. Furthermore, there were fewer individuals who had positive food antigen-specific IgE responses compared with food antigen-specific IgG responses in the studied population. This came as no surprise, as several other studies also demonstrate that serum IgE measurements do not correlate well with the mucosal allergic response in the intestine [28, 37]. This suggests that an IgE-mediated hypersensitivity response to food is unlikely to play an important role in most of the IBS and FD patients.

Both IBS and FD patients reported that GI symptoms often occur after certain food intake. There was also a great overlap in the post-prandial dyspeptic symptoms in the two groups of patients, such as gas problems, pain, nausea and upper abdominal discomfort. This came as no surprise, as a high prevalence of overlap between FD and IBS has been universally reported and some shared common pathophysiological disturbances might exist in these GI functional diseases, such as delayed gastric emptying, visceral hypersensitivity including food hypersensitivity [38–40]. But, interestingly, we were unable to correlate the level of food-specific IgG antibodies with the severity of symptoms both in IBS and FD patients in this study. The underlying mechanisms of the pathogenesis have not yet been fully defined. Maybe some patients with food sensitivities have non-allergic food reactions and the elevated IgG antibodies to food may be secondary to 'inflammation' and therefore be more of an epi-phenomenon. In these patients, there are no real food allergies or immunity responses while experiencing symptoms. Psychological factors have also been suggested to be of great importance for the reported food intolerance in these patients [41, 42]. In addition, IBS and FD symptoms may also be related to abnormal intestinal bacteria, caffeine, alcohol, low dietary fibre, overgrowth of intestinal yeasts and excessive dietary sugars.

In conclusion, increased antigen-specific IgG antibody titres for some foods were found in IBS and FD patients compared with controls but there is no evidence that these findings contribute to the pathogenesis of these functional GI diseases. Future studies along these lines are expected to lead to a better understanding about the role of elevated food antigen-specific IgG antibodies in these functional GI diseases.

Acknowledgements

The authors appreciate the considerable assistance from the Gastroenterology kinetic laboratory and the Central Laboratory of Immunity in Qilu Hospital, Shandong University. This study was founded by a research grant (NSFC, 30570831) from National Natural Science Foundation of China and a grant (Y2005C02) from the Department of Science and Technology of Shandong Province of China.

References

1 Young E, Stoneham MD, Petruckevitch A, Barton J, Rona R. A population study of food intolerance. *Lancet* 1994; **343**:1127–30.

2 Bischoff SC, Crowe SE. Gastrointestinal food allergy: new insights into pathophysiology and clinical perspectives. *Gastroenterology* 2005; **128**:1089–113.

3 Nanda R, James R, Smith H, Dudley CR, Jewell DP. Food intolerance and the irritable bowel syndrome. *Gut* 1989; **30**:1099–104.

4 Dainese R, Galliani EA, De Lazzari F *et al*. Discrepancies between reported food intolerance and sensitization test findings in irritable bowel syndrome patients. *Am J Gastroenterol* 1999; **94**:1892–7.

5 Niec AM, Frankum B, Talley NJ. Are adverse food reactions linked to irritable bowel syndrome? *Am J Gastroenterol* 1998; **93**:2184–90.

6 Jones VA, McLaughlan P, Shorthouse M, Workman E, Hunter JO. Food intolerance: a major factor in the pathogenesis of irritable bowel syndrome. *Lancet* 1982; **2**:1115–7.

7 McKee AM, Prior A, Whorwell PJ. Exclusion diets in irritable bowel syndrome: are they worthwhile? *J Clin Gastroenterol* 1987; **9**:526–8.

8 Roudebush P. Diagnosis and management of adverse food reactions. In: Bonagura JD, ed. *Kirk's current veterinary therapy XII*. Philadelphia: W.B. Saunders Co, 1995; 59–64.

9 Spergel JM, Brown-Whitehorn T. The use of patch testing in the diagnosis of food allergy. *Curr Allergy Asthma Rep* 2005; **5**:86–90.

10 Zwetchkenbaum J, Burakoff R. The irritable bowel syndrome and food hypersensitivity. *Ann Allerg* 1988; **61**:47–49.

11 Zar S, Kumar D, Benson M.J. Review article: food hypersensitivity and irritable bowel syndrome. *Aliment Pharm Ther* 2001; **15**:439–43.

12 Petitpierre M, Gumowski P, Girard JP. Irritable bowel syndrome and hypersensitivity to food. *Ann Allergy* 1985; **54**:538–40.

13 Barau E, Dupont C. Modifications of intestinal permeability during food provocation procedures in pediatric irritable bowel syndrome. *J Pediatr Gastroenterol Nutr* 1990; **11**:72–77.

14 Roussos A, Koursarakos P, Patsopoulos D, Gerogianni I, Philippou N. Increased prevalence of irritable bowel syndrome in patients with bronchial asthma. *Respir Med* 2003; **97**:75–79.

15 Crowe SE, Perdue MH. Gastrointestinal food hypersensitivity: basic mechanisms of pathophysiology. *Gastroenterology* 1992; **103**:1075–95.

16 Host A, Husby S, Gjesing B, Larsen JN, Lowenstein H. Prospective estimation of IgG, IgG subclass and IgE antibodies to dietary proteins in infants with cow's milk allergy. Levels of antibodies to whole milk protein, BLG and ovalbumin in relation to repeated milk challenge and clinical course of cow's milk allergy. *Allergy* 1992; **47**:218–29.

17 Awazuhara H, Kawai H, Maruchi N. Major allergens in soybean and clinical significance of IgG4 antibodies investigated by IgE and IgG4 immunoblotting with sera from soybean-sensitive patients. *Clin Exp Allergy* 1997; **27**:325–32.

18 Ragnarsson G, Bodemar G. Pain is temporally related to eating but not to defaecation in the irritable bowel syndrome (IBS). Patients' description of diarrhea, constipation and symptom variation during a prospective 6-week study. *Eur J Gastroenterol Hepatol* 1998; **10**:415–21.

19 Dainese R, Galliani EA, De Lazzari F, Di Leo V, Naccarato R. Discrepancies between reported food intolerance and sensitization test findings in irritable bowel syndrome patients. *Am J Gastroenterol* 1999; **94**:1892–7.

20 Whorwell P, Lea R. Dietary treatment of the irritable bowel syndrome. *Curr Treat Options Gastroenterol* 2004; **7**:307–16.

21 Bentley SJ, Pearson DJ, Rix KJ. Food hypersensitivity in irritable bowel syndrome. *Lancet* 1983; **2**:295–7.

22 Farah DA, Calder I, Benson L, MacKenzie JF. Specific food intolerance: its place as a cause of gastrointestinal symptoms. *Gut* 1985; **26**:164–8.

23 Zar S, Benson MJ, Kumar D. Food-specific serum IgG4 and IgE titers to common food antigens in irritable bowel syndrome. *Am J Gastroenterol* 2005; **100**:1550–7.

24 Atkinson W, Sheldon TA, Shaath N, Whorwell PJ. Food elimination based on IgG antibodies in irritable bowel syndrome: a randomised controlled trial. *Gut* 2004; **53**: 1459–64.

25 Soares RL, Figueiredo HN, Maneschy CP, Rocha VR, Santos JM. Correlation between symptoms of the irritable bowel syndrome and the response to the food extract skin prick test. *Braz J Med Biol Res* 2004; **37**:659–62.

26 Park MI, Camilleri M. Is there a role of food allergy in irritable bowel syndrome and functional dyspepsia? A systematic review. *Neurogastroenterol Motil* 2006; **18**:595–607.

27 Thompson WG, Longstreth GF, Drossman DA *et al*. Functional bowel disorders and functional abdominal pain. *Gut* 1999; **45** (Suppl. 2):II43–7.

28 Bischoff SC, Mayer JH, Manns MP. Allergy and the gut. *Int Arch Allergy Immunol* 2000; **121**:270–83.

29 Crowe SE. Gastrointestinal food allergies: do they exist? *Curr Gastroenterol Rep* 2001; **3**:351–7.

30 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.

31 Niec AM, Frankum B, Talley NJ. Are adverse food reactions linked to irritable bowel syndrome? *Am J Gastroenterol* 1998; **93**:2184–90.

32 Iacono G, Cavataio F, Montalto G *et al*. Intolerance of cow's milk and chronic constipation in children. *N Engl J Med* 1998; **339**:1100–4.

33 Read NW. Food and hypersensitivity in functional dyspepsia. *Gut* 2002; **51** (Suppl. 1):i50–3.

34 Spanier JA, Howden CW, Jones MP. A systematic review of alternative therapies in the irritable bowel syndrome. *Arch Intern Med* 2003; **163**:265–74.

35 O'Farrelly C, Kelly J, Hekkens W *et al*. Alpha gliadin antibody levels: a serological test for coeliac disease. *Br Med J (Clin Res Ed)* 1983; **286**:2007–10.

36 Shakib F, Brown HM, Phelps A, Redhead R. Study of IgG subclass antibodies in patients with milk intolerance. *Clin Allergy* 1986; **16**:451–8.

37 Schwab D, Raithel M, Klein P *et al*. Immunoglobulin E and eosinophilic cationic protein in segmental lavage fluid of the small and large bowel identify patients with food allergy. *Am J Gastroenterol* 2001; **96**:508–14.

38 Gwee KA, Chua AS. Functional dyspepsia and irritable bowel syndrome, are they different entities and does it matter? *World J Gastroenterol* 2006; **12**:2708–12.

39 Stanghellini V, Tosetti C, Barbara G *et al*. Dyspeptic symptoms and gastric emptying in the irritable bowel syndrome. *Am J Gastroenterol* 2002; **97**:2738–43.

40 Haag S, Talley NJ, Holtmann G. Symptom patterns in functional dyspepsia and irritable bowel syndrome: relationship to disturbances in gastric emptying and response to a nutrient challenge in consulters and non-consulters. *Gut* 2004; **53**:1445–51.

41 Houghton LA, Whorwell PJ. Towards a better understanding of abdominal bloating and distension in functional gastrointestinal disorders. *Neurogastroenterol Motil* 2005; **17**:500–11.

42 Addolorato G, Marsigli L, Capristo E, Caputo F, Dall'Aglio C, Baudanza P. Anxiety and depression: a common feature of health care seeking patients with irritable bowel syndrome and food allergy. *Hepatogastroenterology* 1998; **45**:1559–64.

