

Food-Specific Serum IgG4 and IgE Titers to Common Food Antigens in Irritable Bowel Syndrome

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INTRODUCTION: Food hypersensitivity is a common perception among irritable bowel syndrome (IBS) patients. Data from dietary elimination and food challenge studies support an etiopathological role of diet in IBS, but there are no well-established tests to identify food hypersensitivity.

AIM: To compare IgG4 and IgE titers to common food antigens in IBS and controls.

METHOD: One hundred and eight IBS [52 diarrhea-predominant (D-IBS); 32 constipation-predominant (C-IBS); 24 alternating (Alt-IBS)], and 43 controls were included in the study. IgG4 and IgE titers and skin prick testing (SPT) to 16 common foods including milk, eggs, cheese, wheat, rice, potatoes, chicken, beef, pork, lamb, fish, shrimps, soya bean, yeast, tomatoes, and peanuts were measured.

RESULTS: IBS had significantly higher IgG4 titers ($\mu\text{g/L}$) to wheat (395 IQR \pm 1,011 vs 0 IQR \pm 285, $p < 0.001$), beef (1,079 IQR \pm 930 vs 617 IQR \pm 435, $p < 0.001$), pork (481 IQR \pm 379 vs 258 IQR \pm 496, $p < 0.001$), and lamb (241 IQR \pm 460 vs 167 IQR \pm 232, $p = 0.009$) compared to controls. These differences were maintained across all three subgroups. The antibody titers to potatoes, rice, fish, chicken, yeast, tomato, and shrimps were not significantly different. No significant difference in IgE titers was observed between IBS and controls. SPT was positive for only a single antigen in 5 of 56 patients tested with the same panel of foods. No correlation was seen between the pattern of elevated IgG4 antibody titers and patients' symptoms.

CONCLUSION: Serum IgG4 antibodies to common foods like wheat, beef, pork, and lamb are elevated in IBS patients. In keeping with the observation in other atopic conditions, this finding suggests the possibility of a similar pathophysiological role for IgG4 antibodies in IBS.

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BACKGROUND

Food hypersensitivity is a common perception in up to a fifth of the general population (1). Symptoms such as headaches, skin allergy, asthma, angioedema, behavioral changes, and intestinal symptoms are frequently attributed to adverse food reactions (2, 3). On account of the difficulty in diagnosing food hypersensitivity, its true prevalence is unknown but has been estimated to be approximately 5% in the general population (4, 5). The perception of food hypersensitivity is much higher in irritable bowel syndrome (IBS), with 20–65% of patients attributing their symptoms to food hypersensitivity (6, 7). Several studies have shown symptomatic improvement in response to exclusion diets in IBS patients, with wheat, dairy products, beef, corn, and coffee being the most commonly incriminated foods (6).

Elevated levels of food-specific serum IgE and IgG4 antibodies have been associated with food-hypersensitivity-induced atopic conditions such as asthma, hay fever, and

atopic dermatitis, although the significance of elevated IgG4 antibodies has been questioned (8–13). Exclusion diets have been shown to improve symptoms in these conditions.

In 1982 Jones *et al.* demonstrated, for the first time, symptomatic response to an elimination diet in IBS patients (14). Since then, several studies have reproduced these findings, demonstrating a benefit from exclusion diets in up to two-thirds of IBS patients, with diarrhea-predominant subgroup showing the highest response rate (6, 15–17). Identification of the offending foods by dietary elimination and challenge can be cumbersome and poor patient compliance limits the usefulness of this approach in clinical practice. There is limited understanding of the pathophysiology of food hypersensitivity and this is paralleled by a paucity of available diagnostic options. Use of skin prick tests to identify the offending foods has shown equivocal results (18, 19). It has been suggested that food-specific serum IgE and IgG4 antibodies may have a pathophysiological role in IBS patients in a manner similar to other atopic conditions.

Serum IgG4 antibodies to common food antigens is being offered as a screening test for food hypersensitivity in IBS patients by some laboratories. At present there is no convincing scientific evidence to support this practice. Some of our patients who have used exclusion diet based on elevated IgG4 titers have reported improvement in their symptoms. This study attempts to explore the significance of food-specific IgG4 antibody titers in IBS patients.

The aims of this study were two-fold. Firstly, to compare the food-specific IgG4 and IgE antibody titers and the response to skin prick testing (SPT) to common food antigens in IBS patients and healthy controls and secondly, to correlate these to patients' symptoms.

MATERIALS AND METHODS

Patients

A total of 122 patients with a suspected diagnosis of IBS who attended the gastroenterology outpatient clinics were screened for this study. The diagnosis was based on Rome II criteria (20). The patients were further classified as diarrhea-predominant (D-IBS), constipation-predominant (C-IBS), and alternators (Alt-IBS) based on a detailed history and examination as well as response to a questionnaire based on Rome II criteria. Routine investigations including full blood count, serum urea and electrolytes, liver function tests, thyroid function tests, calcium, and ESR/CRP were carried out to screen for concomitant diseases, which would lead to exclusion from the study. A colonoscopy or flexible sigmoidoscopy with barium enema was carried out in all patients. Patients were excluded from the study if any of the following conditions were present: (i) inflammatory bowel disease, celiac disease, known lactose intolerance, or other significant gastrointestinal disorder; (ii) "advanced" cardiac, respiratory, renal, or hepatic disease; (iii) concurrent malignancy; (iv) major psychiatric disorders including history of drug or alcohol abuse; (v) previous abdominal surgery except uncomplicated appendectomy; (vi) use of any medication, which is known to perturb gastrointestinal sensorimotor function; and (vii) pregnancy. Local ethics committee approval was obtained for the protocol and full written informed consent was obtained from the subjects.

Of the 122 patients screened, 14 patients were excluded either because they did not fulfill the inclusion criteria ($n = 7$) or had an alternative diagnosis made (4 functional pain disorder, 3 inflammatory bowel disease). Therefore, a total of 108 IBS patients (17 males, 91 females) were included in the study, comprising 52 D-IBS patients (mean age: 41 yr, SD ± 12.8), 32 C-IBS patients (mean age: 42.7 yr, SD ± 12.4), and 23 alternators (mean age: 35.3 yr, SD ± 9.4).

Healthy Controls

The control group, recruited from the members of the hospital staff, comprised of 43 asymptomatic healthy subjects (14 males, 29 females; mean age: 38.2 yr, SD ± 9.46). They were

specifically asked questions about bowel symptoms and were included in the study only if they had no symptoms suggestive of IBS. The same exclusion criteria were used for screening the controls as for the patients.

Questionnaire

All patients and controls completed a symptom assessment questionnaire based on Rome II criteria (20). It recorded information on the site and frequency of abdominal pain, relief with defecation, stool frequency, and stool form. In addition, data on stool urgency, sensation of incomplete evacuation, bloating, straining, and passage of mucus were collected. Subjects were asked to record the frequency of these symptoms (5 = everyday, 4 = most days, 3 = once per week, 2 = at least once per month, 1 = less than once per month, and 0 = none). Patients were also asked to indicate if their symptoms were related to any particular type of food and if they avoided any food because it disagreed with them.

A second questionnaire was administered to patients to evaluate the severity of their symptoms for the 10-day period prior to the interview, using a previously validated questionnaire (21). In this questionnaire, a visual analogue scale (0–100) was used to quantify the severity of pain and bloating, satisfaction with bowel habit, and effect of IBS on life in general. Frequency of abdominal pain was recorded as the number of days the patient experienced pain out of the previous 10 days (expressed as a percentage). A cumulative total score was then calculated. As pain is the central symptom in IBS, it is scored for both frequency and severity thereby giving it a greater weight in the total score.

In addition, the subjects were also required to complete a Hospital Anxiety and Depression questionnaire (H.A.D. scale) (22). This quantifies the level of anxiety and depression experienced by subjects by recording response to 14 questions (scored from 0–3), with the higher scores indicating a greater level of anxiety or depression. It gives a maximum score of 21 for anxiety and depression each.

Serum Antibody Measurement (IgG4 and IgE)

Serum IgG4 antibody titers to 16 common foods including milk, eggs, wheat, cheddar cheese, rice, yeast, potato, peanut, cod fish, chicken, lamb, beef, pork, tomatoes, and soya bean were measured. The antibody titers were expressed as $\mu\text{g/L}$ and the measured range was between 1.5 $\mu\text{g/L}$ and 30,000 $\mu\text{g/L}$. Blood samples were allowed to stand for 20–30 min before being centrifuged at 3,000 cycles per min for 15 min. The serum was separated and frozen at -20°C for subsequent analysis. Samples were processed in a central laboratory (Allergy Diagnostic Laboratory, Oxfordshire, UK) using a commercially available fluoro-enzyme-immunoassay (Pharmacia CAP System Specific IgG4 FEIA, Uppsala, Sweden). This is an *in vitro* test system for the quantitative measurement of antigen-specific IgG4 in human serum. The laboratory was blinded to the diagnosis by coding the samples.

In this technique, antigen of interest covalently coupled to a cellulose cap reacts with the specific IgG4 in the subject's

serum. After washing away the nonspecific IgG4 antibodies, enzyme-labeled monoclonal antibodies (mouse origin) against IgG4 are added to form a complex. After incubation, unbound enzyme anti-IgG4 is washed away and the bound complex is then incubated with a developing agent (fluorescence substrate). The conjugate enzyme reacts with substrate forming a fluorescent product. After stopping the reaction, the fluorescence of the eluate is measured in fluorometer (FluoroCount 96, Uppsala, Sweden). The higher the fluorescence value the more specific IgG4 is present in the specimen. To classify test results, fluorescence units (FU) for patient samples are compared directly with FU for calibrators run in parallel. The mean fluorescence in FU for each calibrator is plotted against the concentration on lin-log graph paper to construct a calibration curve. The calibration curve is then used to determine the concentration of the patient samples from the calibration curve and is multiplied with dilution factor to give a range of 1.5 $\mu\text{g/L}$ and 30,000 $\mu\text{g/L}$.

Food-specific serum IgE antibody titers to the same panel of 16 foods were measured using a similar fluoro-enzyme-immunoassay technique in a blinded fashion (Allergy Diagnostic Laboratory). The minimum concentration of the IgE antibody measurable with this technique was 0.35 kU/L.

Skin Prick Testing

In a subgroup of 56 patients and 5 controls, SPT to the same panel of food antigens was performed to look for immediate hypersensitivity reaction (type I) at the Allergy and Immunology Clinic, St. Helier Hospital, Carshalton, UK. The food antigens were procured from ALK Abello, Horsholm, Denmark and Hollister-Stier, Toronto, Canada. Food antigens were stabilized in 50% glycerin and prepared from carefully identified foods according to the manufacturers' own thoroughly evaluated extraction techniques.

A qualified immunology nurse specialist carried out the skin testing. All patients undergoing skin testing had to avoid antihistamines and other drugs known to interfere with skin testing for the previous 7 days. A positive histamine and negative diluents control was used in each case. Wheal size was recorded (in mm) as the mean of two diameters perpendicular to each other. The results of the skin testing were read after 15 min and only accepted if the histamine positive control returned a wheal diameter 3 mm greater than the negative

control. A positive response to a food antigen was recorded if it returned a wheal diameter 3 mm greater than the negative control. Where two test foods adjacent to each other returned a positive result, a fresh lancet was used to retest both foods to ensure that a false positive result had not been produced by allergen carry over on the lancet.

Statistical Analysis

The food-specific serum IgG4 and IgE antibody titers and SPT results were compared between the healthy controls and IBS patients. In addition, the differences between various IBS subgroups and healthy controls were also assessed. The data were analyzed using nonparametric tests (Mann-Whitney U-test). A p -value of <0.01 was used as the threshold for significance to avoid a type I error.

In addition, correlation between individual symptom scores and the antibody titers was also calculated using the Pearson's test and univariate logistic regression analysis was carried out.

RESULTS

Symptom Profile

No significant difference was observed in the severity of pain and bloating, satisfaction with bowel habits, and effect of IBS on life in general between the different IBS subgroups (Table 1). IBS patients had significantly higher anxiety scores (9.6 IQR \pm 4.5 vs 4.4 IQR \pm 4.0, $p < 0.001$) and depression scores (5.4 IQR \pm 3.9 vs 1.6 IQR \pm 2.6, $p < 0.001$) as compared to healthy controls (Table 2). No significant differences were seen in the anxiety and depression scores between different IBS subgroups. Thirty-eight percent of patients indicated that their symptoms were caused by certain foods and 61% stated that they avoided certain foods.

Serum IgG4

IBS patients had significantly higher IgG4 antibody titers to wheat ($p \leq 0.001$), beef ($p < 0.001$), pork ($p < 0.001$), lamb ($p = 0.009$), and soya bean ($p = 0.012$) as compared to healthy controls (Table 3). In addition, borderline significance was seen for egg yolk ($p = 0.048$) and egg white ($p = 0.065$). No significant difference was seen for milk, cheese, yeast, potato, rice, chicken, fish, shrimps, peanuts, and tomatoes. At a cut off value of 250 $\mu\text{g/L}$, IBS patients had

Table 1. Symptom Severity Score in IBS Subgroups

	D-IBS Mean (\pm SEM)	C-IBS Mean (\pm SEM)	Alt-IBS Mean (\pm SEM)
Pain severity	47.37 (\pm 4.2)	54.92 (\pm 5.7)	40.56 (\pm 7.6)
Pain frequency	45.58 (\pm 5.1)	58.85 (\pm 6.6)	45.0 (\pm 9.3)
Bloating severity	46.42 (\pm 4.3)	57.58 (\pm 5.2)	39.44 (\pm 8.7)
Satisfaction with bowel habits	74.95 (\pm 3.5)	77.81 (\pm 4.2)	67.44 (\pm 6.1)
Effect of IBS on life in general	67.09 (\pm 3.6)	64.77 (\pm 3.7)	83.94 (\pm 23.8)
Total score	281.84 (\pm 15.3)	313.96 (\pm 19.5)	231.63 (\pm 33.6)

Table 2. Hospital Anxiety Scores in Controls and IBS Subgroups

	Controls	D-IBS	C-IBS	Alt-IBS
Anxiety score*	4.40 (± 0.8)	9.46 (± 0.7)	10.21 (± 0.7)	9.16 (± 0.9)
Depression score*	1.6 (± 0.4)	5.50 (± 0.6)	5.64 (± 0.7)	4.63 (± 0.8)

*Values are given as mean (\pm SEM).

elevated IgG4 antibodies to an average of 8 foods (range: 1–12) whereas the controls had elevated antibodies to 5 foods (range: 1–12). All the IBS subgroups showed reactivity to a similar range of foods. Only a small fraction (<20%) of patients and controls had detectable antibodies to yeast, potato, rice, chicken, fish, and shrimps. The number of patients and controls with elevated IgG4 titers at cut off levels of 250 μ g/L as well as 1,000 μ g/L are shown in Table 4.

The subgroup analysis showed that this pattern was maintained in D-IBS, C-IBS, and Alt-IBS as compared to controls for wheat, beef, pork, and lamb. D-IBS group had significantly higher titers against wheat (398 IQR \pm 1,204, $p = 0.001$), beef (991 IQR \pm 931, $p < 0.001$), pork (480 IQR \pm 655, $p < 0.001$), and soya bean (0 IQR \pm 315, $p = 0.002$) (Figs. 1 and 2). The differences for peanuts (0 IQR \pm 301, $p = 0.031$), lamb (236 IQR \pm 371, $p = 0.066$), egg yolk (464 IQR \pm 2,002, $p = 0.08$), and rice (0 IQR \pm 79, $p = 0.028$) were of borderline statistical significance (Figs. 2 and 3). In the C-IBS group, IgG4 titers were significantly higher for wheat (398 IQR \pm 1,204, $p = 0.008$), lamb (254 IQR \pm 363, $p = 0.008$), beef (1,109 IQR \pm 947, $p < 0.001$), and pork (482 IQR \pm 345, $p < 0.001$) whereas borderline significance was observed for egg white (888 IQR \pm 4,418, $p = 0.09$) and milk (16,970 IQR \pm 9,657, $p = 0.06$) (Figs. 1 and 3). Similarly, in the Alt-IBS group, IgG4 titers were significantly higher for wheat (402 IQR \pm 829, $p = 0.001$), beef (1,341 IQR \pm 1,086, $p < 0.001$), and pork (482 IQR \pm 317, $p = 0.001$) whereas the differences for lamb (247 IQR \pm 478, $p = 0.06$) and soya bean (0 IQR \pm 258, $p = 0.06$) were of borderline significance (Figs. 1 and 2). The antibody titers to potatoes, fish, chicken, yeast, tomato, and shrimps were not significantly different between IBS patients and the control group. There was no significant difference in the IgG4

Table 3. Food-Specific IgG4 Antibody Titers—Controls Versus IBS

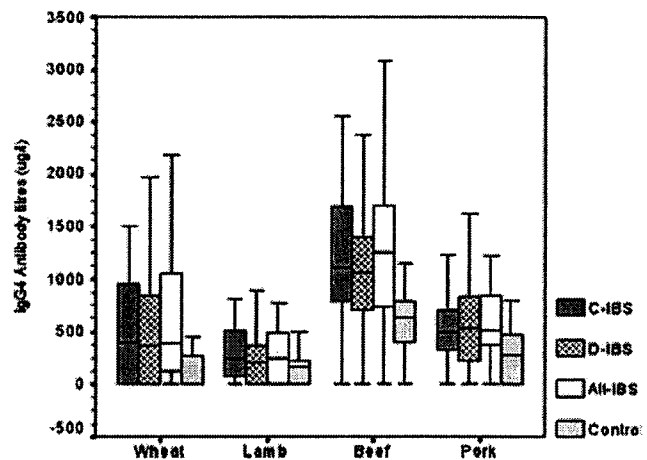
	Controls μ g/L (\pm IQR)	IBS μ g/L (\pm IQR)	p -Values
Egg white	508 ($\pm 1,308$)	768 ($\pm 3,119$)	0.065
Egg yolk	218 (± 759)	564 ($\pm 2,598$)	0.048
Milk	13,070 ($\pm 12,924$)	15,204 ($\pm 15,456$)	NS
Cheese	673 ($\pm 1,690$)	673 ($\pm 1,690$)	NS
Wheat	0 (± 285)	395 ($\pm 1,011$)	<0.001
Lamb	167 (± 232)	241 (± 460)	0.009
Beef	617 (± 435)	1,079 (± 930)	<0.001
Pork	258 (± 496)	481 (± 379)	<0.001
Soya bean	0 (± 0)	0 (± 213)	0.012

Table 4. Number of Subjects in IBS and Control Groups with Elevated IgG4 Antibody Titers at Cut-Off Value of ≥ 250 μ g/L and 1,000 μ g/L for Different Foods Tested

Food Antigen Tested	IgG4 Titer ≥ 250 μ g/L, n (%)		IgG4 titer $\geq 1,000$ μ g/L, n (%)	
	IBS	Control	IBS	Control
Egg white	83 (76.9)	26 (60.5)	43 (39.8)	16 (37.2)
Egg yolk	65 (60.2)	21 (48.8)	42 (38.9)	8 (18.6)
Cheese	87 (80.6)	33 (76.7)	62 (57.4)	18 (41.9)
Wheat	65 (60.2)	12 (27.9)	28 (25.9)	4 (9.3)
Beef	96 (88.9)	34 (79.1)	59 (54.6)	6 (14)
Pork	85 (78.7)	22 (51.2)	15 (13.9)	0
Lamb	48 (44.4)	7 (16.3)	6 (5.6)	0
Fish	4 (3.7)	1 (2.3)	0	0
Chicken	12 (11.1)	7 (16.3)	0	0
Shrimps	3 (2.8)	1 (2.3)	0	0
Rice	11 (10.2)	0	0	0
Potatoes	10 (9.3)	6 (14)	3 (2.8)	0
Peanuts	27 (25)	7 (16.3)	9 (8.3)	5 (11.6)
Tomatoes	(28.7)	9 (20.9)	(7.4)	1
Soya	27 (25)	4 (9.3)	4 (3.7)	0
Yeast	6 (5.6)	4 (9.3)	0	0

antibody titers between the three IBS subgroups for the foods tested except for peanuts for which titers were significantly higher in D-IBS versus C-IBS ($p = 0.004$) (Fig. 2).

There was a large variability in the IgG4 antibody response to different food groups. The antibody titers induced by dairy products (cheese, egg yolk, and egg white) were generally higher than wheat, red meats, tomato, and peanuts. Among the red meats, beef titers were higher than pork and lamb. In contrast, chicken, fish, shrimps, yeast, rice, and potatoes were associated with lower antibody titers (Table 4).

**Figure 1.** Comparison of IgG4 antibodies to wheat, lamb, beef, and pork between IBS subgroups and controls. C-IBS versus control (wheat, $p = 0.008$; lamb, $p = 0.008$; beef, $p < 0.001$; pork, $p < 0.001$); D-IBS versus control (wheat, $p = 0.001$; lamb, $p = 0.066$; beef, $p < 0.001$; pork, $p < 0.001$). Alt-IBS versus control (wheat, $p = 0.001$; lamb, $p = 0.06$; beef, $p < 0.001$; pork, $p = 0.001$).

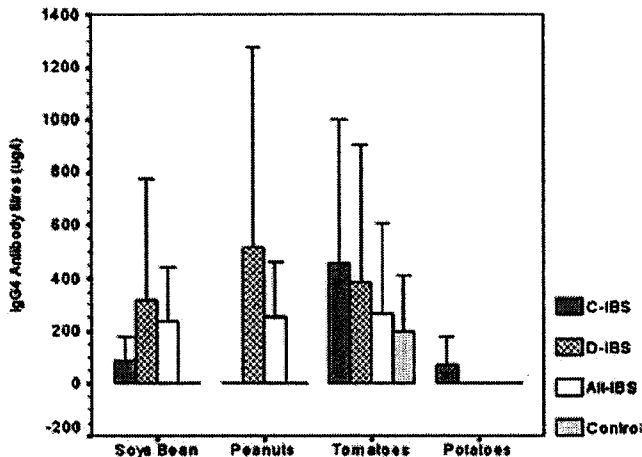


Figure 2. Comparison of IgG4 antibodies to soya bean, peanuts, tomatoes, and potatoes between IBS subgroup and controls. D-IBS versus control (soya bean; $p = 0.002$, peanuts; $p = 0.03$); D-IBS versus C-IBS (peanuts; $p = 0.004$).

Serum IgE

Food-specific serum IgE antibody titers were detectable in only 9 of 108 IBS patients. Four IBS patients had detectable antibody titers to wheat, rice, potatoes, peanuts, and soya bean. One patient had elevated IgE antibody titers to all the food articles tested suggesting a nonspecific increase in IgE antibodies. Of the remaining 4 patients, 3 had detectable IgE antibody response to shrimp and 1 to yeast only. Of the 43 controls, 8 had detectable food-specific IgE antibody titers. Five of these had detectable antibody titers against peanuts and tomatoes. Some of these controls also had antibodies against wheat, rice, potatoes, and soya bean, a pattern similar to IBS patients. In addition, 2 controls had antibodies against shrimps and 1 against milk. The number of patients and controls with elevated IgE antibody titers was too small to apply a statistical test.

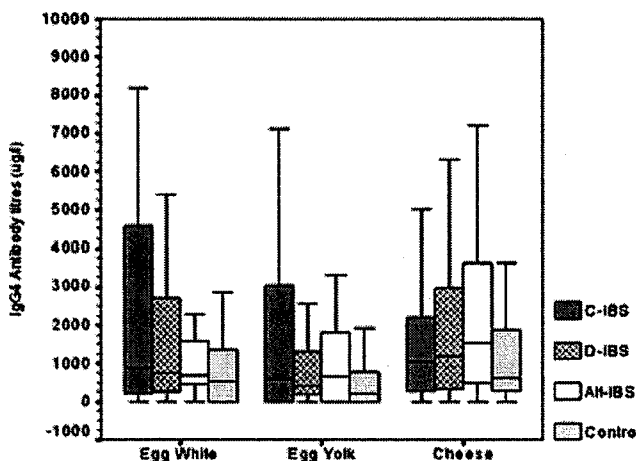


Figure 3. Comparison of IgG4 antibodies to egg white, egg yolk, and cheddar cheese between IBS subgroups and controls. No significant difference observed.

SPT

Fifty-six IBS patients and 5 controls had SPT with the same panel of food antigens. The low uptake was due to the fact that the test was based at a separate center requiring a separate visit. In addition, due to the low yield the testing was abandoned in the later part of the study. Out of the 56 IBS patients, 5 patients had positive skin test to shrimp antigen. In addition, 2 patients had significant cutaneous reaction to three antigens each (chicken, peanuts, and soya bean in one and cod, beef, and tomato in the other). However, both these patients also had mild cutaneous reactivity to other food antigens and negative control and a marked reaction to positive control, suggesting a nonspecific increased cutaneous reactivity. Of the 5 controls tested, one reacted to shrimp antigen and another to tomato antigen. SPT results showed no concordance with either IgE or IgG4 antibody response for the panel of food antigens tested.

Correlation with Symptoms

No significant correlation was seen between symptom severity and frequency indices and serum IgG4 antibody titers. Logistic regression analysis did not reveal any joint effect of combination of symptoms such as pain, bloating, stool frequency, straining, urgency, and sensation of incomplete evacuation on the IgG4 antibody titers.

DISCUSSION

This study has demonstrated a consistent increase in IgG4 antibody titers across the three IBS subgroups compared to controls for wheat, beef, pork, lamb, and soya bean out of 16 foods tested. Wheat, beef, and dairy products have been incriminated in the pathogenesis of IBS patients in previous studies based on the response to exclusion diets (6). These findings suggest that elevated food-specific IgG4 antibody titers to common foods may have a pathophysiological role in IBS patients. The differences in IgG4 titers to dairy products between IBS patients and controls, however, were neither significant nor consistent in this study, although differences of borderline significance were noted for individual subgroups. These results were against expectation as dairy products are frequently incriminated in IBS patients. The antibody titers for milk were extremely high for both controls and IBS patients. The reagents used for this analysis have extremely high affinity for milk antibodies, which might have resulted in spuriously high-milk IgG4 antibody titers for both patients and controls and may not represent the true *in situ* antibody titers. This in turn could have potentially masked a true difference in milk antibodies between patients and controls.

The pathophysiological processes that underlie this increase in IgG4 antibody response to common articles of foods, remain speculative. Raised IgG4 antibodies to food antigens have been reported in several atopic conditions. The role of IgG and IgA anti gliadin antibodies in the celiac disease is well known (23). In contrast to healthy infants, in whom

IgG antibody titers diminish with time, atopic children continue to produce IgG antibodies to milk and egg proteins, suggesting an underlying disturbance of immune regulation (24, 25). Raised titers of IgG4 antibodies have been reported in patients with eczema and/or asthma caused by milk intolerance (12). Similarly, Awazuhara *et al.* demonstrated strongly reactive IgG4 antibodies in patients with atopic dermatitis and/or bronchial asthma, caused by soya bean hypersensitivity (26). Exclusion of the offending foods from diet has shown to improve symptoms in these conditions. Elevated food-specific IgG4 antibodies may play a similar pathophysiological role in IBS patients.

While food-specific IgG4 antibody production has been proposed to play a pathogenetic role in food hypersensitivity, some studies have suggested that IgG4 production may be a physiological response of the gut immune system to dietary antigens challenge (27, 28). Transient alteration in the permeability of gut mucosa from any insult can theoretically increase antigenic load presented to the mucosal immune system. An increased IgG4 antibody response therefore may be a secondary phenomenon and represent a normal physiological rather than a pathological response of the gut immune system. If this hypothesis is correct and increased mucosal permeability was the primary event in IBS patients, then a generalized increase in IgG4 titers to all the food antigens tested should have been observed. However, the observed increase in antibody titers was selective, which argues against the above supposition. One possible explanation is that IBS patients may have differential gut permeability to food antigens. Some of the triggers recognized to play a role in IBS such as gastroenteritis, antibiotics, altered microbial flora, and stress, have the potential of increasing the gut permeability. Once an increased antigenic exposure has induced a pathological mucosal immune response in "preprogrammed" individuals, it can potentially self-perpetuate the increased mucosal permeability through a variety of immune mechanisms. Irrespective of the initiating event, the differential increase in IgG4 titers to food antigens suggests that it is a specific reaction rather than a nonspecific response to increased mucosal permeability.

A large variability was observed in the IgG4 antibody response to different food groups. High titers were observed for dairy products, intermediate titers for beef, lamb, pork, wheat, tomato, and peanuts whereas low titers were observed for chicken, fish, shrimps, yeast, rice, and potatoes. This may be attributable to a difference in the inherent antigenicity of various foods, the effect of modification of the protein structure by the digestive processes, differential permeability of the gut mucosa to different foods thereby influencing the antigen load presented to the immune cells, and/or a consequence of differential modification of the gut immune response by different food groups.

It is also important to note that a considerable overlap was observed in the antibody titers for individual food articles between IBS and controls. A number of possible mecha-

nisms may account for this. First, a subconscious avoidance of the triggering foods by IBS patients may have potentially attenuated the antibody response. Second, the intermittent nature of symptoms in IBS patients is a well-recognized phenomenon, which may reflect fluctuations in the underlying immunopathological processes. This, in turn, may be mirrored by fluctuations in the antibody response over time. Third, there may be hidden or "subclinical" IBS individuals in the control group. Finally, transient alterations in gut permeability in healthy individuals in response to various triggers may induce transient IgG4 response to food antigens, which unlike IBS patients does not persist long term.

The three IBS subgroups showed similar pattern of IgG4 antibody response and no individual "signature" for the food articles tested was observed for any subgroup. With the exception of peanuts there were no significant differences between any of the IBS bowel habit subgroups with respect to the prevalence of IgG antibodies. The significance of raised IgG4 antibody titers for peanuts in D-IBS compared to C-IBS remains unknown. Overall, the data suggest that the IBS population shares a common immunopathological response to dietary food antigens. However, it should be stressed that only 16 common food articles were tested in this study. It is a possibility that employing a broader food panel may have demonstrated a different response for each IBS subgroup.

No significant difference was observed in the cutaneous reactivity or specific IgE antibody response in IBS subgroups and controls for the food antigens tested. The number of patients with either elevated IgE antibodies or with cutaneous reactivity was too small to have pathophysiological significance in the vast majority of IBS patients. In fact, the control population had a higher proportion of individuals with detectable IgE titers as compared to the patient population studied. In addition, the patients who had detectable IgE antibodies, did not consistently have a corresponding increase in IgG4 antibody titers, suggesting that the mechanism underlying the two are independent of each other.

Interestingly, the study did not show any significant correlation between serum IgG4 antibody titers and symptom severity and frequency indices using Pearson's test or logistic regression analysis. It is possibly due to the fact that IBS subgroups do not reflect distinct pathophysiologies. On the other hand, the severity and frequency of symptoms as experienced by the IBS sufferer are heavily influenced by various social and cultural factors and hence symptoms may fail to discriminate between different underlying pathophysiologies.

The lack of skin reactivity and IgE response is not unexpected. In previous exclusion diet studies in IBS patients, which used SPT and/or IgE as evidence of food hypersensitivity, positive correlation with dietary challenge was demonstrated in only one (15, 29, 30). It has been proposed that food hypersensitivity may be a heterogeneous condition and either IgG4 or IgE (and in some cases both) may be the predominant antibody response. Awazuhara *et al.* demonstrated increased

IgE and IgG4 antibodies of variable reactivity and specificity in patients with atopic dermatitis and/or bronchial asthma caused by soya bean hypersensitivity (26). Similarly, el Rafei *et al.* showed that measurement of both food-specific IgG4 and IgE levels correlated with a positive history of food hypersensitivity in 90% of patients tested compared to 60% for either IgG4 or IgE alone (31). However, the small number of patients with detectable IgE antibodies in this study suggests that an IgE-mediated hypersensitivity reaction is unlikely to play a role in the majority of IBS patients. The significance of raised IgE antibodies observed in a small subgroup of the study population remains unknown.

In a recent study by Atkinson *et al.*, a 3-month elimination diet based on IgG antibodies to common foods demonstrated a 10% greater reduction in symptom score compared to a sham diet in IBS patients (32). A positive IgG response for a given food was defined as a level of three times the background signal obtained by the sample against a no food allergen-coated control using enzyme-linked immunosorbent assay. On average patients were sensitive to approximately 6–7 foods. Unlike the present study, quantitative assessment of IgG response was not carried out and comparison was not made with the control population. Nonetheless, improvement in symptoms on elimination diet suggests that IgG-based hypersensitivity reactions have a causative role in the pathogenesis of IBS. A fresh approach to understanding the pathophysiology of IBS incorporating the role of IgG-based food hypersensitivity through interaction between genetic susceptibility, impaired gut barrier function, luminal antigen milieu (dietary and microbial), and immune dysregulation is emerging (33).

In summary, this study demonstrates that IBS patients have elevated food-specific IgG4 antibodies to common foods like wheat, beef, pork, lamb, and soya bean. This observation is consistent for D-IBS, C-IBS, and Alt-IBS subgroups. In keeping with the observation in other atopic conditions, these findings suggest the possibility of a similar pathophysiological role for IgG4 antibodies in IBS patients. The response to exclusion diet based on elevated food-specific IgG4 in future studies may be useful in establishing the significance of these findings. On the contrary, SPT and serum IgE antibody titers to common food antigens did not yield any significant difference between IBS patients and controls.

WHAT IS ACCEPTED AND WHAT THIS RESEARCH ADDS

- (i) The prevalence of food hypersensitivity in general population is estimated at 5%.
- (ii) Food hypersensitivity has been incriminated in various atopic conditions.
- (iii) Elevated food-specific serum IgE and IgG4 antibodies have been associated with food-hypersensitivity-induced atopic conditions such as atopic dermatitis, hay fever, and asthma.
- (iv) Up to 65% of IBS patients attribute their symptoms to adverse food reactions.
- (v) Food-specific serum IgG4 antibodies to common foods such as wheat, beef, pork, lamb, and soya bean are elevated in IBS patients.
- (vi) Food-specific IgG4 antibody-induced hypersensitivity may play a pathogenetic role in a subgroup of IBS patients.

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