

Case Report

IgE antibodies against bovine serum albumin in a case of eosinophilic gastroenteritis

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Eosinophilic gastroenteritis is a disease characterized histologically by an eosinophilic infiltration of the gut. The cause of this disease remains unclear, although both food allergy and food intolerance have been implicated in its pathogenesis. We report the case of a 22-year-old man in whom gastrointestinal symptoms first appeared in childhood, with involvement of mucosa and muscularis layers of stomach and bowel. He presented high IgE blood levels, and his prick test was positive to bovine, pig, and lamb sera. Immunoblots from calf, pig, and lamb sera, incubated with the patient's serum and revealed by autoradiography, demonstrated the presence of a 65-kDa protein band that was recognized by IgE antibodies but not by IgG. This band corresponded to bovine serum albumin, while IgE did not show reactivity with human albumin. These data suggest a possible role for IgE-mediated hypersensitivity mechanisms in the pathogenesis of eosinophilic gastroenteritis.

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Eosinophilic gastroenteritis is a disease characterized histologically by an eosinophilic infiltration of serosa, mucosa, or muscularis layers of the gut. Patients complain of gastrointestinal symptoms (abdominal pain, vomiting, and diarrhea) which may be associated with weight loss, and growth delay in children. The cause of this disease remains obscure, and the pathogenic mechanisms are poorly understood, although both food allergy and food intolerance have been implicated (1, 16). In this regard, a familial and personal history of atopy is frequently observed (2, 16), and serum levels of IgE are often elevated in patients with mucosal involvement (18). Moreover, specific IgE and IgG antibodies against certain foods, such as milk, eggs, wheat, peanuts, pork, and beef, have been detected (2, 3), but in most cases target antigens have not been identified.

The present study describes a case of eosinophilic gastroenteritis in a patient who developed beef, pork, and lamb meat hypersensitivity. The objective was to characterize meat antigens and to study the isotype of the antibodies specific for these antigens.

Material and methods

Patient

The patient was a 22-year-old man referred from the gastroenterology department of our hospital to undergo an immunoallergologic study in our department. From childhood, he had suffered multiple episodes of eosinophilic gastroenteritis (diagnosed by clinical and pathologic criteria), which required treatment with systemic corticosteroids.

At the onset of the study in our department, the patient complained of gastrointestinal symptoms (soft stools, abdominal pain). No evidence of parasitic or extraintestinal disease was observed. Gastroduodenal endoscopy showed duodenal stenosis with an inflammatory component. Biopsy showed moderate eosinophilic infiltration involving mucosa and muscularis layers. Laboratory studies showed a hemoglobin concentration of 9.5 mg/dl. The total leukocyte count was 5600/mm³. The differential count showed lymphocytes 49%, neutrophils 32%, eosinophils 12% (672/mm³), and basophils 1%.

Total serum proteins were somewhat low (55.4 g/l; normal range 60–80). Stool examination for blood was negative.

The patient's past medical history included bronchial asthma that had been completely cured at the age of 12. Since 1988, he had had iron deficiency anemia that was treated with oral iron without satisfactory response.

The results of the immunoallergologic study indicated the instauration of a meat exclusion diet. A control study performed 2 months later showed a partial improvement of clinical symptoms as well as a decrease in total IgE levels to 747 IU/ml and a normalization of eosinophil count (240/mm³).

Prick test

The prick test was performed with inhalant allergens and various foods (fish, meat, fruit, vegetables, and milk proteins) LETI, SA, Barcelona, Spain). One percent histamine solution and 0.9% saline serum were used as positive and negative controls, respectively. The results were evaluated at 20 min, comparing the diameter of the wheal induced by antigen with the histamine control. A reaction was considered to be positive when the antigen diameters were either equal (+ + +) to or greater (+ + + +) than those caused by histamine (5).

Quantitation of immunoglobulins

The concentration of immunoglobulins (IgA, IgG, and IgM) was determined by immunoephelometry (Behring, Munich, Germany). Total and specific IgE was determined by commercially available immunoradiometric assay (Pharmacia IgE RIACT, Uppsala, Sweden) and fluoroallergosorbent test (FAST, 3M Diagnostic Systems, Santa Clara, CA, USA), respectively, according to the manufacturers' instructions.

Detection of precipitating antibodies

Precipitating antibodies against beef, lamb, and pig meat and purified BSA were analyzed by conventional double (Ouchterlony) immunodiffusion technique. The agarose (Sigma, St Louis, MO, USA) gel (1% w/v) was prepared with phosphate-buffered saline (PBS). An amount of 50 µl of serum from the patient was placed in the central well. Purified bovine serum albumin (BSA) (Sigma, St Louis, MO, USA) and commercially available sera from calf, lamb, and pig (LETI, Barcelona, Spain) were placed in the peripheral wells in a seven-well pattern (8).

Gel electrophoresis and Western blotting

For detection of specific antigen, 10% polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS–PAGE) was performed with the discontinuous buffer system of Laemmli (9). The wells were loaded with commercially available sera from calf, lamb, and pig (LETI, Barcelona, Spain). Appropriate molecular mass markers (Bio-Rad, CA, USA) were included in each run. Polyacrylamide gel was stained with 0.5% Coomassie brilliant blue. After the runs, protein bands of the gel were transferred to nitrocellulose strips at 15 V overnight by the method of Towbin et al. (17). After transfer, nitrocellulose membranes were soaked under agitation for 1 h in 10% methanol and PBS, pH 7.4, followed by immersion in 0.1% Tween 20 (Fluka Chemie AG, Buchs, Switzerland) in PBS overnight at 4°C to block unbound membrane sites.

Developing of nitrocellulose strips by autoradiography

Nitrocellulose strips were incubated overnight with patient serum or control serum (from nonatopic subject) diluted 1:4 with 0.1% Tween 20 in PBS; after washing, the strips were incubated with ¹²⁵I-labeled anti-IgE (0.5 µg, 222 kBq, Phadebas RAST, Pharmacia Diagnostics AB, Uppsala, Sweden) in a volume of 5 ml for 4–6 h. After washing with 0.05% Tween 20 in PBS, the nitrocellulose strips were air-dried at room temperature and loaded into a Kodak-Omatic cassette (KP. 69065-c) together with radiographic film (AGFA CURIX RP2). The cassette was then stored at –80°C for periods of 24–48 h (7).

Developing of nitrocellulose strips by the immunoenzymatic method

Other nitrocellulose strips were incubated with the same sera for 2 h. After washing with 0.05% Tween 20 in PBS, the strips were incubated with 5 ml 0.001% horseradish peroxidase (HRP)-conjugated rabbit antihuman IgG (DAKOPATTS, Glostrup, Denmark) in PBS for 2 h at room temperature. After two washes, these strips were developed under agitation with 0.02 M acetate buffer containing 20 mg aminoethylcarbazole (Sigma, St Louis, MO, USA), 12 ml dimethylsulfoxide (Sigma, St Louis, MO, USA), and 0.150 ml 30% hydrogen peroxide until full color development (usually 5–10 min).

In a second step, SDS–PAGE and Western blotting were performed with both purified bovine serum albumin (BSA) (Sigma, St Louis, MO, USA) and human serum. The nitrocellulose strips were then incubated with patient and control sera by the methods already described.

Results

Prick test

The prick test showed strong positivity to bovine serum (+ + +), and a weaker positive reaction to pig and lamb sera (+ +) was also observed.

Quantitation of immunoglobulins

Serum levels of immunoglobulins were within the normal range (IgA 1.09 g/l, IgG 11.7 g/l, and IgM 1.25 g/l; normal ranges: IgA 0.5–4.3 g/l, IgG 9.2–17.8 g/l, and IgM 0.82–2.82 g/l). Total IgE was 2040 IU/ml (reference limit less than 200 IU/ml), and specific IgE against both beef and pig meat was 1.5 IU/ml (class 2).

Detection of precipitating antibodies

Patient serum did not precipitate either BSA or any protein from calf, lamb, and pig sera.

Immunoprints

Autoradiography of immunoblots from calf, pig, and lamb sera incubated with the patient serum showed a 65-kDa protein band that was recognized by IgE antibodies from patient serum (Fig. 1). This reactivity was not observed when strips were incubated with HRP-conjugated anti-IgG. The molecular mass of the band suggested BSA as the specific target of IgE, and this hypothesis was verified by incubating the patient serum with blotted, purified BSA, as shown in Fig. 2. Moreover, patient serum showed IgE immunoreactivity against an unidentified minor protein band of 45 kDa present only in blotted pig serum.

No immunoreactivity was observed when immunoblots of calf, pig, and lamb sera or purified BSA were incubated with control serum. As a test of whether BSA-specific antibodies from the patient cross-reacted with human albumin, blotted human serum was incubated with serum from either a patient or a control subject and revealed with ^{125} I-labeled anti-IgE. Autoradiography failed to show a positive reaction (data not shown).

Discussion

Eosinophilic gastroenteritis is an uncommon disorder that occurs most frequently in adulthood. The pathogenesis of the lesion remains unknown, but in patients with predominant involvement of the mucous layer, high serum IgE levels are often present (18), and in some cases food allergy has been reported (2, 3). It is known that challenge with certain foods induces an increase in the serum IgE levels

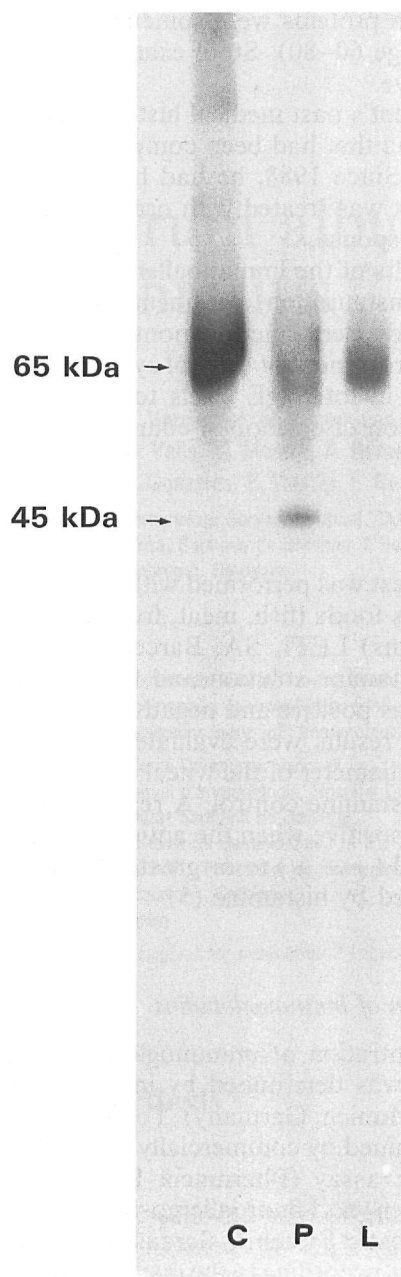


Fig. 1. Immunoblots from calf (c), lamb (l), and pig (p) sera. Sera were subjected to SDS-PAGE and transferred to nitrocellulose strips. Diluted sera (1:4) from patient and healthy subject were incubated with strips, and presence of BSA-specific antibodies was revealed by further incubation with ^{125} I-labeled anti-IgE antibody. Autoradiography showed 65-kDa protein band in all three immunoblots, and another protein band of 45 kDa only in blotted pig serum. No reactive bands were observed when strips were incubated with serum of nonatopic subject (not shown).

and typical gastroenteritic symptoms in some patients (2, 13), although in other patients immunologic studies have revealed no abnormalities (3). IgG, IgA, IgM, and IgE antibodies against BSA have been demonstrated in some cases (11), as well as cross-reacting, precipitating antibodies against BSA, and against lamb, beef, and pig meat (11, 15).

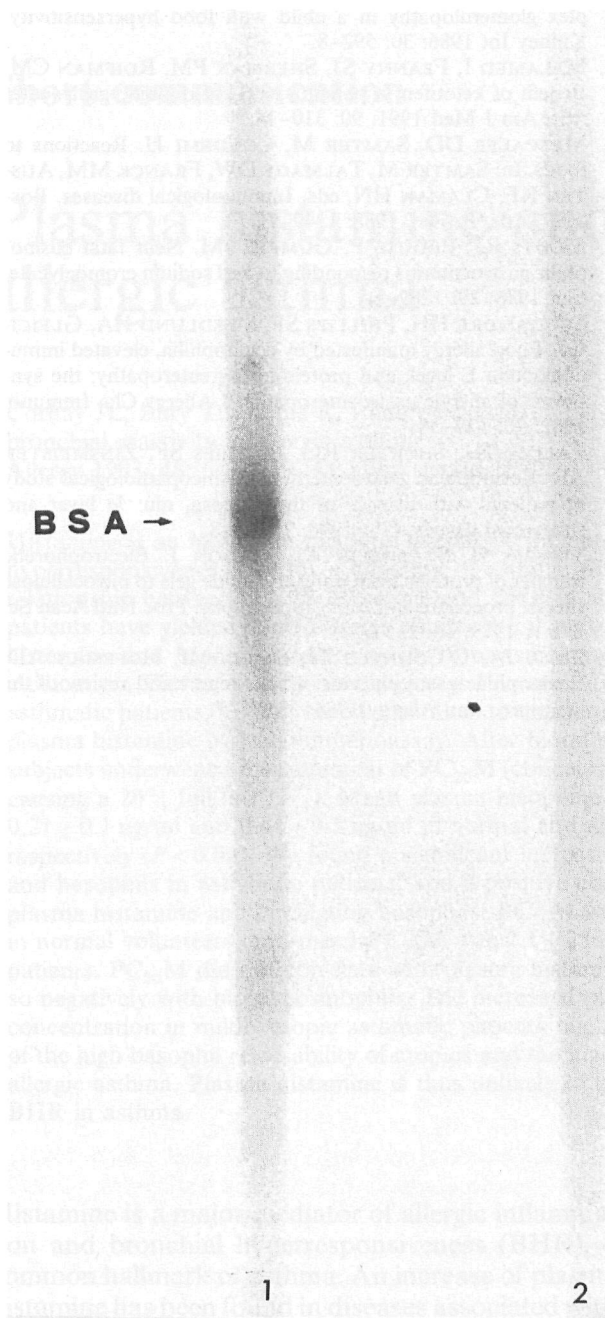


Fig. 2. Immunoblots from purified bovine serum albumin (BSA). Purified BSA was subjected to SDS-PAGE and transferred to nitrocellulose strips. Diluted sera (1:4) from patient and healthy control were incubated with strips, and presence of BSA-specific antibodies was revealed by further incubation with ^{125}I -labeled anti-IgE antibody. Autoradiography showed immunoreactivity when immunoblots were incubated with patient serum (1). No reactive band was observed when strips were incubated with serum of nonatopic subject (2).

Our patient, as in other cases (11, 15), presented high IgE levels and immediate hypersensitivity to lamb, pig, and bovine meat. We demonstrated that IgE antibodies of the patient were reactive to albumin, which is present in bovine, lamb, and pig sera,

but they did not cross-react with human albumin. IgE antibodies against a 45-kDa band present in the pig sera were also detected, but this band has not yet been identified. In contrast to another study (11), the patient did not have detectable IgG antibodies against any protein of these sera. It is possible that high levels of IgE arose in relation to bronchial asthma suffered during childhood (the pathogenesis of that asthma was not studied), but this possibility is slight since the patient was asymptomatic for many years, skin tests to inhalant allergens were negative, and meat intake did not trigger bronchial asthma.

The implication of meat in the pathogenesis of the disease is supported by the fact that the patient showed partial improvement of clinical symptoms and a decrease of IgE concentration and of eosinophil count 2 months after starting a meat-free diet. In our case, the chronic course of the disease implicated anatomic and functional alterations of the gut that probably accounted for the incomplete success of the diet. Other studies have also reported a transitory improvement in the symptoms after eliminating the inciting food (3, 4, 18) or after using mast cell stabilizers such as ketotifen (12) and oral sodium cromoglycate (6, 14). However, other cases did not respond to sodium cromoglycate treatment (10, 13).

Our results suggest that an IgE-mediated mechanism can play a role in the pathogenesis of some eosinophilic gastroenteritis. However, mechanisms other than immediate hypersensitivity could be implicated, since some patients with predominantly muscularis disease lack specific food sensitivity and have normal IgE levels, while trial elimination diets do not improve the symptoms (3, 16).

The present results further indicate that in patients with eosinophilic gastroenteritis it is important to determine the presence of food hypersensitivity as well as to characterize the antigens involved in the disease because of their cross-reactivity with other food antigens, since an incomplete elimination diet could account for the absence of clinical response.

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