

Ant ulcer drugs promote oral sensitization and hypersensitivity to hazelnut allergens in BALB/c mice and humans^{1–3}

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ABSTRACT

Background: Hazelnut allergy can be a consequence of sensitization to cross-reactive pollen, especially from the Fagales family. However, severe allergic reactions after ingestion of hazelnuts without associated pollen allergy have been reported. In these cases, oral sensitization by hazelnut ingestion is plausible.

Objective: We have reported that antiulcer drugs promote oral sensitization to digestion-labile food allergens. Because hazelnut proteins were sensitive to gastric digestion in our in vitro assay, we aimed to analyze the effect of antiulcer treatment on oral sensitization to hazelnut proteins.

Design: BALB/c mice were fed hazelnut extract with or without antiulcer drugs. In parallel, gastroenterologic patients ($n = 153$) were screened during antiulcer treatment for specific immunoglobulin (Ig) E to hazelnut and inhalative allergens in vitro and in vivo.

Results: Mice fed hazelnut extract in combination with antiulcer drugs formed anaphylactogenic IgG1 toward hazelnut and developed type I skin reactivity to hazelnut extract. In the human study population, 5 of 153 (3.3%) patients developed hazelnut-specific IgE, 4 of 5 developed specific skin reactivity, 3 of 5 had a positive result to oral provocation, and 2 of 5 manifested a food allergy to hazelnut after a 3-mo course of antiulcer treatment. Immunoblot testing with recombinant allergens showed that hazelnut, but not Fagales pollen, was the genuine elicitor in mice and humans.

Conclusion: Our experimental and epidemiologic data suggest that the intake of antiulcer drugs may lead to the induction of immediate-type food hypersensitivity toward hazelnut. *Am J Clin Nutr* 2005;81:154–60.

KEY WORDS Food allergy, hazelnuts, antiulcer drugs, epidemiology, oral sensitization, digestion

INTRODUCTION

The prevalence of food allergy is steadily increasing and now affects 6–8% of children younger than 3 y and $\approx 2\%$ of the adult population in the industrialized world (1–3). Of the different edibles, hazelnut has been found in several studies to be the most frequent cause of IgE-mediated food allergy, with a prevalence between 21% and 53% in patients with food allergies in Europe (4–7).

Although patients who reported adverse reactions to hazelnuts mainly showed oral symptoms, gastrointestinal and skin reactions were also reported (6, 8). Most important, severe systemic reactions—including shortness of breath, asthma, and hypotension—affect nearly 5% of nut-allergic patients (6, 9). Symptoms

have been observed after the consumption of only 6–7 mg hazelnut protein, amounts comparable with those hidden in dietary products (10, 11). Because tree nut allergy is responsible for fatal or near-fatal outcomes, it represents a significant health concern (12).

Two possible mechanisms for the induction of food allergy have been postulated. On the one hand, sensitization can occur via inhalation of airborne allergens. The subsequent symptoms to a food compound are then elicited by cross-reactive immunoglobulin (Ig) E antibodies. On the other hand, food allergens can directly sensitize the organism via the oral route, but it is proposed that a prerequisite for allergenicity of food is its ability to reach the intestinal mucosa in an intact form, which implies survival of the gastric digestion (13).

Hazelnut allergy can be a consequence of primary sensitization with cross-reactive birch pollen allergens. For instance, the major birch pollen allergen Bet v 1 is homologous to Cor a 1 in hazelnut (14). However, severe allergic reactions after ingestion of hazelnuts without any association to tree pollen allergy have been observed (10, 15–17). The role of hazelnut as a potent and independent elicitor of food allergy was further confirmed by double-blind, placebo-controlled food challenges in a multicenter study (8). In these cases, oral sensitization by hazelnut ingestion is plausible. However, it is puzzling that most of the major allergens in hazelnut extracts are not resistant to gastric and pancreatic digestion (18, 19) and, therefore, do not show the features of classic food allergens (20).

For these reasons, we analyzed the effect of antiulcer drugs on oral sensitization to hazelnut proteins. Recently, we reported that antiulcer drugs promote oral sensitization to digestion-labile fish allergens because they prevent peptic degradation of proteins (21). In the present study, we fed our BALB/c mouse food allergy model (21) hazelnut extract in combination with antiulcer medication and examined the resulting immune response in vitro as

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well as *in vivo*. Furthermore, we collected epidemiologic data from gastroenterologic patients who developed hazelnut-specific IgE during antiulcer treatment and identified the genuine allergen responsible for sensitization.

SUBJECTS AND METHODS

Preparation of hazelnut extract

Commercially available raw hazelnuts were used to prepare an extract, as described previously (21). The protein concentration of the extract was determined according to the method of Bradford (22) with the use of a Bio-Rad Protein Assay (Bio-Rad, Munich, Germany).

Simulated gastric fluid (SGF) consisted of 3.2 mg pepsin/mL (Sigma, Steinheim, Germany) in 0.03 mol NaCl/L (adjusted to pH 1.2 with 1 mol HCl/L). Hazelnut extract (50 μ L of 3.3 mg/mL) was added to 200 μ L SGF at 37 °C and incubated for 0, 0.5, 1, 5, 15, and 30 min. Hazelnut extract in double-distilled water, in SGF at pH 1.2 without pepsin or in SGF at pH 5.0 with pepsin (imitating antacid conditions), was incubated for 2 h. The reaction was quenched with 75 μ L of 1 mol NaOH/L and then the tubes were placed on ice. Aliquots of the samples (20 μ L) were separated under reducing conditions on a 12% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) gel, and Coomassie brilliant blue staining was performed.

Immunizations of BALB/c mice

Four- to 6-wk-old female BALB/c mice ($n = 5$ per group) were obtained from the Institute for Laboratory Animal Science and Genetics (University of Vienna) and were treated according to European Community rules of animal care (23) (permission number GZ 66.009/211-Pr/4/2003 of the Austrian Ministry of Science).

Mice in group A received hazelnut extract orally [2 mg in 100 μ L phosphate-buffered saline (PBS)]. For further mouse groups, the amounts of antiulcer drugs were correlated to the dosage recommended for use in humans and calculated according to the body weights of the mice. Mice in group B were fed sucralfate (2 mg; Ulcogant, Merck, Vienna) and 100 μ L hazelnut extract. Mice in group C were treated with omeprazole (11.6 μ g; Losec, AstraZeneca, Vienna) intramuscularly and then fed hazelnut extract 1 h later. Mice in group D remained completely naive. Other groups of mice were treated with antiulcer drugs but received no hazelnut extract: group E received sucralfate (2 mg suspended in 100 μ L PBS orally), and group F received omeprazole (11.6 μ g intramuscularly). Immunizations were performed on days 1, 44, 92, and 112. Blood samples were taken before and on days 43, 73, 97, 132, and 186 after the initial immunization. Skin tests were conducted on day 217.

Detection of hazelnut-specific antibodies in enzyme-linked immunosorbent assays

Microtiter plates (Maxisorp, Nunc, Roskilde, Denmark) were coated with hazelnut extract (5 μ g per well in 100 μ L 50 mmol/L NaHCO₃, pH 9.6). Samples were diluted 1:100 for IgG1 and IgG2a or diluted 1:10 for IgE. Enzyme-linked immunosorbent assay (ELISA) was performed as described previously (24).

Isolation and characterization of murine IgG antibodies

Pooled sera of mice immunized with sucralfate (group B) were used for purification of IgG on a protein A sepharose column

(Prosep-A, Montagé Life Science Kits; Millipore, Bedford, MA). IgG1 and IgG2a were eluted separately according to the manufacturer's instructions and were concentrated and stored in glycerol at –20 °C. This concentrate as well as the mouse serum pool before the purification of antibodies was separated on an 8% SDS-PAGE gel under nonreducing conditions. For control purposes, standard mouse IgE (2.5 μ g/lane; PharMingen, San Diego) or standard mouse IgG1 (1.5 μ g/lane; mouse standard panel, Southern Biotechnology, Birmingham, AL) were added, and the gel stained by Coomassie brilliant blue. To determine the absolute amount of hazelnut-specific IgG1, IgG2a, and IgE, the concentrated eluent was examined in an ELISA (as described above).

Skin tests of immunized mice

On day 217, Evans blue (100 μ L of 5 mg/mL) was injected into the tail vein of mice. Subsequently, 30 μ L of hazelnut extract (50 μ g/mL), hazel pollen allergen Cor a 1 (2.5 μ g/mL; Biomay, Vienna), grass pollen allergen Phl p 5 (2.5 μ g/mL; Biomay), mast cell degranulation compound 48/80 (20 μ g/mL; Sigma), or PBS were administered intradermally into the shaved abdominal skin. After 20 min, mice were killed and skinned. The diameter of the color reaction was measured on the inside of the abdominal skin. The color intensity was determined by using a hand-held reflection densitometer (Vipdens, Brixen, Italy). Skin reactivity indexes were calculated as described previously (25, 26) (diameter of skin reactivity $\times$ densitometrical signal intensity).

Passive cutaneous anaphylaxis

Naive mice were shaved on the abdomen, and then 30 μ L hazelnut-specific IgG1 (purified from pooled sera from group B mice, *see above*) (5 μ g/mL), standard IgG1 (5 μ g/mL), 48/80 histamine release compound (20 μ g/mL; Sigma), and 0.9% NaCl as well as glycerol as negative controls were injected intradermally. After 1 h, 100 μ L of hazelnut extract (2.5 mg/mL in 0.9% NaCl) with 5 mg/mL Evans blue was injected intravenously, and after another 30 min the mice were killed and skinned. Skin reactivity indexes were calculated as described above.

Patients

Sera of 153 adult patients (mean age: 65.9 y; 66 men and 87 women) from a gastroenterology clinic in Szolnok, Hungary, were collected. None of the patients reported adverse reactions to inhalant or food allergens; however, IgE immunoblots were performed to screen their allergic state. Because of dyspepsia or chronic gastritis, patients were treated orally for 3 mo with an H₂-receptor blocker—ranitidine ($n = 48$; Zantac: 2 $\times$ 150 mg/d; Pfizer Inc, New York), famotidin ($n = 83$; Quamatel: 40 $\times$ 2 mg/d; Medimpex, London), or ranitidine bismuth citrate ($n = 5$; Pylorid: 2 $\times$ 400 mg/d; GlaxoSmithKline, Uxbridge, United Kingdom)—or with the proton pump inhibitor omeprazole ($n = 17$; Losec: 1 $\times$ 20 mg/d; AstraZeneca, Vienna). Patients who received no treatment ($n = 50$; mean age: 59.9 y) were used as the control group. The study was conducted in accordance with the 2002 guidelines of the Ministry of Health in Hungary for clinical studies, analogous to the Helsinki criteria for clinical studies.

Serum IgE and skin tests in patients

Sera were tested for allergen-specific IgE against hazelnut and inhalative allergens from tree pollen (eg, birch, hazel, and alder),

grass pollen, weed pollen, molds, house dust mites, insect venoms, and animal dander. Immunoblot testing (AllergyScreen; MEDIWISS Analytic, Moers, Germany) was performed before and 3 and 8 mo after the onset of antiulcer treatment. This system allows the detection of IgE by color reactions through formation of precipitates on nitrocellulose test strips. The coloration is directly proportional to the specific antibody content of the serum sample. Evaluation is carried out after complete drying with a CCD camera (Rapid Reader), which assigns the results to the test classes 0–6 of absolute IgE amounts. Results were considered positive at a class of ≥ 2 (≥ 0.75 kU/L). Additionally, total IgE was determined before and at the 3-mo time point (ALLERgen Total IgE; Adaltis, Milano, Italy). Patients with hazelnut-specific IgE in their sera were further subjected to skin tests with hazelnut extract (ALK-Abelló, Horsholm, Denmark) at the 8-mo time point.

Oral provocation of patients

Single-blind, placebo-controlled oral provocation with hazelnut (Test Dose; Lofarma, Milano, Italy) was performed 11–13 mo after the onset of antacid therapy in the 5 patients with hazelnut-specific IgE. Patients were tested with hazelnut (doses of 0.15, 0.6, 3.0, 15.0, and 60.0 mg/caps) and with talcum (210 mg/caps) as a negative control. The different doses were given at an interval of 60 min. The tests were stopped at the first appearance of symptoms.

Western blotting and dot blots

Hazelnut extract (2 mg/mL) was separated under reducing conditions in a 12% SDS-PAGE and electroblotted onto a nitrocellulose membrane (Schleicher&Schuell, Dassel, Germany). One blot strip was blocked with PBS and 0.5% Tween, and staining was performed with commercially available black ink in PBS and 0.05% Tween (1:1000) to control the blotted protein (27). Remaining blot strips were blocked with buffer (50 mmol/L sodium phosphate buffer, pH 7.5; 0.5% Tween 20, 0.5% bovine serum albumin); incubated with hazelnut-specific mouse IgG concentrate (1:100), mouse preimmune serum (1:100), or human sera (1:10) overnight at 4 °C; washed, and incubated with ^{125} I-labeled sheep-anti mouse Ig (1:1000) or with ^{125} I-labeled anti-human IgE (1:20) overnight. Additionally, mouse IgG was also tested in birch pollen or mugwort pollen extract (prepared as hazelnut extract). The reaction was visualized by exposing blots to BIOMAX-MS films (Kodak, Rochester, NY). For dot blots, 1 μ g/dot of recombinant Bet v 1, Cor a 1, or Art v 1 (Biomay) or 2 μ g/dot of hazelnut extract was applied onto nitrocellulose, blocked, and incubated as above with sera from the 5 hazelnut-allergic patients in the antiulcer group or from pools of randomly selected birch pollen- or mugwort-allergic patients.

Statistical analysis

IgG1 values are summarized over time for each mouse in each group as the area under the curve and compared between 2 groups by applying a Mann-Whitney *U* test to these area under the curve values. The 2 comparisons of the sucralfate and the omeprazole group, respectively, to the group fed only with hazelnut were corrected for multiplicity by using the Bonferroni method. *P* values ≤ 0.05 were considered to be statistically significant. All computations were performed with the use of SPSS software version 11.5 for WINDOWS (SPSS Inc, Chicago).

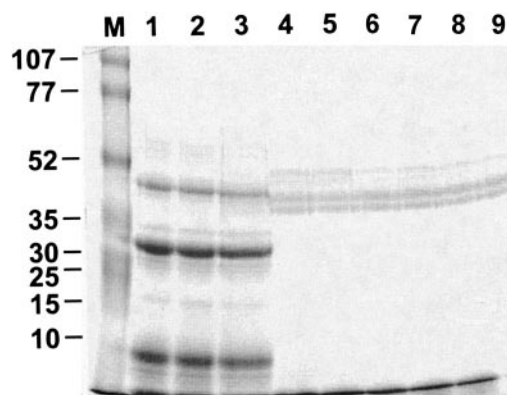


FIGURE 1. Hazelnut proteins remain undigested by pepsin at elevated pH. Hazelnut extract was incubated with pepsin in simulated gastric fluid (SGF) at pH 1.2 for different time intervals. Aliquots were separated on a 12% sodium dodecyl sulfate–polyacrylamide gel electrophoresis gel, and Coomassie brilliant blue staining was performed. Protein molecular weight marker (lane M), hazelnut extract in double-distilled water (lane 1), hazelnut extract in SGF at pH 1.2 without pepsin (lane 2), hazelnut extract in SGF at pH 5.0 with pepsin (lane 3), and hazelnut extract incubated at pH 1.2 with pepsin for 30 s (lane 4), 1 min (lane 5), 5 min (lane 6), 15 min (lane 7), and 30 min (lane 8). Lane 9 shows the pepsin solution without hazelnut.

RESULTS

Digestion assay

Hazelnut extract was incubated in SGF with pepsin at pH 1.2 for different time intervals (**Figure 1**). All proteins in our extract were digested within 30 s (lanes 4–8). Incubating the extract in double-distilled water (lane 1), in SGF at pH 1.2 without pepsin (lane 2), or in SGF with pepsin at pH 5.0 imitating hypoacidic conditions (lane 3) did not affect the hazelnut proteins, even after 2 h of incubation.

Humoral immune response of immunized mice

Mice were fed with hazelnut extract alone or in combination with the antiulcer drugs sucralfate (an aluminum sucrose hydrogen sulfate) or omeprazole (a proton pump inhibitor). Feedings during treatment with sucralfate or omeprazole induced hazelnut-specific IgG1 in 4 of 5 mice and in 3 of 5 mice, respectively, whereas feedings with hazelnut extract alone did not induce allergen-specific antibodies in any of the mice (**Figure 2**). A comparison of the induced IgG1 levels (summarized over time as the area under the curve) between the sucralfate group and the group fed hazelnut only is significant (Bonferroni corrected *P* = 0.032), whereas the data for the omeprazole group showed no significant difference (*P* = 0.754).

Purification and characterization of hazelnut-specific IgG antibodies

Hazelnut-specific antibodies induced through medication with sucralfate were purified from sera, concentrated, and analyzed in an SDS-PAGE gel. The concentrate mainly contained IgG antibodies, compared with murine IgE and IgG standards (data not shown). The absolute amounts of subclasses present in the eluted fraction were determined by ELISA. The final concentration of IgG1 in the eluate was 5.1 μ g/mL, and the content of IgG2a was 1.7 μ g/mL. The serum pool before antibody extraction contained 2.5 μ g/mL IgG1 and 2.3 μ g/mL IgG2a.

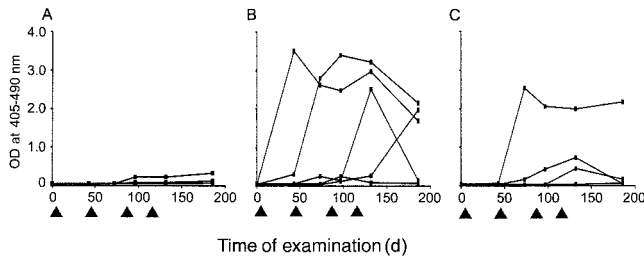


FIGURE 2. Hazelnut feedings during antiulcer treatment induce specific immunoglobulin (Ig) G1 in mice. Mice ($n = 5$) were fed hazelnut extract with or without antiulcer drugs, and serum antibody concentrations were measured in enzyme-linked immunosorbent assay. The lines connect hazelnut-specific IgG1 concentrations in sera taken at different time points from individual mice. Mice that were fed hazelnut extract concomitant with sucralfate (B) had significantly greater amounts of IgG1 than the mice fed hazelnut extract only (A) ($P = 0.032$). Feedings with hazelnut extract after intramuscular application of omeprazole (C) showed only a trend toward greater amounts of IgG1 ($P = 0.754$). Serum samples were collected before immunization (day 0) and on days 43, 73, 97, 132, and 186. Arrow heads indicate the time points of feedings. OD, optical density.

Skin tests of mice

The *in vivo* relevance of the induced IgG1 antibodies was analyzed by type I skin tests in immunized animals. Mice were tested intradermally with hazelnut extract, recombinant Cor a 1 (major hazel pollen allergen), Phl p 5 (major grass pollen allergen), compound 48/80, and PBS. None of the mice immunized with hazelnut extract only (group A) showed a positive skin reaction to hazelnut extract. In contrast, 3 of 5 mice that received hazelnut extract in combination with sucralfate (group B) showed type I skin reactivity to hazelnut. In the group premedicated with omeprazole (group C), only 1 mouse had a positive skin reaction, although 3 of 5 mice had formed hazelnut-specific IgG1 antibodies (Figure 3). No reactivity was achieved with Cor a 1 from hazel pollen or Phl p 5 from grass pollen (Figure 3).

Passive cutaneous anaphylaxis

To examine the biological activity of purified IgG1 induced by feeding hazelnut during sucralfate treatment (group B), these antibodies were used for passive cutaneous anaphylaxis experiments in naive mice ($n = 10$). Animals were tested intradermally with purified IgG1 or with purchased standard IgG1 with unknown specificity. All mice displayed positive skin reactivity with purified hazelnut-specific IgG1 after intravenous administration of hazelnut extract (Figure 4). In contrast, no reaction was observed when testing with irrelevant IgG1 (data not shown).

Specific IgE of patients treated with antiulcer drugs

A total of 153 gastroenterologic patients with no history of allergy were treated with antiulcer drugs for 3 mo. Sera were examined for hazelnut-specific IgE in immunoblot before and 3 and 8 mo after commencement of antiulcer medication. None of the patients showed hazelnut-specific IgE before the treatment, but 5 patients (3.3%) had developed hazelnut-specific IgE (class of ≥ 2 , >0.75 kU/L) 3 mo after the onset of treatment. These 5 patients showed no cross-reactive sensitizations against early blooming trees, such as birch, alder, or hazel from the Fagales family. None of the patients in the untreated control group ($n = 50$) developed specific IgE during the observation period. Because of the smaller control group, no statistical significance could be calculated.

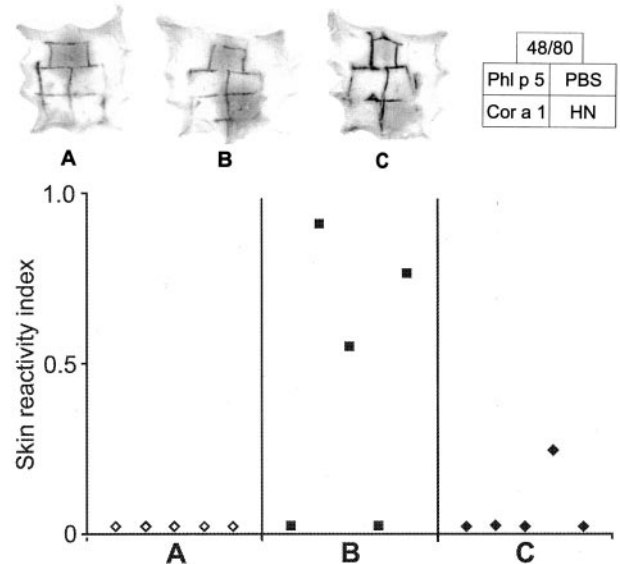


FIGURE 3. Results of skin tests in mice fed hazelnut extract with or without antiulcer drugs. Immunized mice ($n = 5$) were tested intradermally on the abdominal skin with hazelnut extract (HN), Cor a 1, compound 48/80 as positive control, and Phl p 5 and phosphate-buffered saline (PBS) as negative controls. Group A was fed hazelnut extract only, group B was given sucralfate orally combined with hazelnut extract, and group C was treated with omeprazole before feedings with hazelnut extract. Representative skin tests of individual mice (top) and skin reactivity indexes (diameter of skin reactivity $\times$ densitometrical signal intensity) of all mice (bottom) are shown.

Skin testing and oral provocations

In the 5 patients who developed hazelnut-specific IgE, skin-prick tests were performed after 8 mo, and 4 of 5 patients had a positive reaction to hazelnut. Two of these patients reported clinical symptoms after eating hazelnut (oral allergy syndrome) or after consuming chocolate containing hazelnut (acute urticaria) and 3 of them reacted in oral provocation tests with hazelnut (Table 1).

Specificity of murine and human anti-hazelnut antibodies

The binding patterns of purified murine IgG1 of the sucralfate-treated mice and of IgE from human patients, who had developed

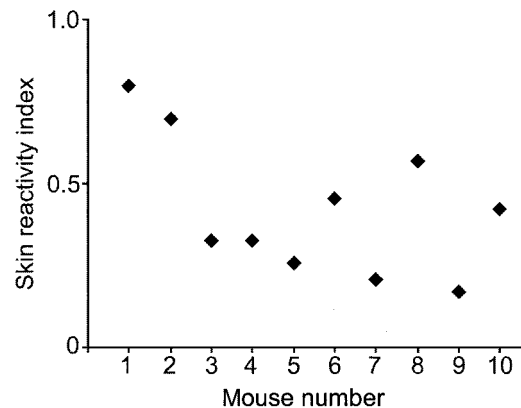


FIGURE 4. Induction of passive cutaneous anaphylaxis by purified hazelnut-specific immunoglobulin (Ig) G1. Naive mice ($n = 10$) were tested intradermally on the shaved abdominal skin with purified hazelnut-specific IgG1. Skin reactivity indexes were calculated after application of hazelnut extract (diameter of the skin reactivity $\times$ densitometrical signal intensity).

TABLE 1Characteristics of patients treated with antiulcer drugs for 3 mo¹

No. and sex	Age	HN-specific IgE (class) ²			Total IgE (kU/L)		SPT, 8 mo ³	Clinic, 8 mo ⁴	PROV, 12 mo ⁵	Medication ⁶
		0 mo	3 mo	8 mo	0 mo	3 mo				
	y								mg	
1 M	59	0	2	0	102	119	3/7	—	—	A
2 M	40	0	2	0	128	541	6/12	—	3	B
3 M	55	0	2	0	210	1000	—	—	—	C
4 F	67	0	3	3	68	314	6/16	AU	0.6	C
5 F	68	0	5	4	3	3	5/14	OAS	0.6	A

¹ HN, hazelnut; IgE, immunoglobulin E; PROV, oral provocation with hazelnut extract; SPT, skin-prick test with hazelnut.² Classes of specific IgE: 0 = <0.35 kU/L, 1 = 0.35–0.75 kU/L, 2 = 0.75–3.5 kU/L, 3 = 3.5–17.5 kU/L, 4 = 17.5–50 kU/L, 5 = 50–100 kU/L, and 6 = >100 kU/L.³ Reaction is given as mm induration/mm erythema.⁴ Patient manifested acute urticaria (AU) after eating chocolate containing hazelnut; patient showed oral allergy syndrome (OAS) (local urticaria in mouth) after eating hazelnut.⁵ Symptoms appeared within minutes after swallowing the capsule with hazelnut extract in patient 2 (pruritus) and in patients 4 and 5 (urticaria).⁶ A, omeprazole; B, ranitidine bismuth citrate; C, ranitidine.

hazelnut reactivity during antiulcer treatment, was compared in a hazelnut immunoblot. The antibodies induced through antiulcer medication in mice (panel A, lane 1) predominantly bound to proteins in the molecular mass range of 33 to 65 kDa in hazelnut extract as shown in **Figure 5**. These proteins are also relevant for IgE binding in the human patients (panel B, lanes 4–8). IgE binding to the higher-molecular-weight hazelnut compounds was also observed after testing in a pool of patients allergic to birch pollen (panel B, lane 10) but not after testing in a mugwort-specific serum pool (panel B, lane 9). To reveal the genuine sensitizer, we performed immunodots using recombinant allergens (panel C). None of the antiulcer medication–treated patients, who developed hazelnut-specific IgE, reacted with the Fagales pollen allergens Bet v 1 or Cor a 1 (panel C, lanes 4–8), which were strongly recognized by the serum pool of birch pollen–sensitized patients (panel C, lane 10). One of the 5 hazelnut patients reacted with recombinant Art v 1 from mugwort pollen (panel C, lane 7), which was strongly and exclusively recognized by a serum pool of mugwort pollen–sensitized patients (panel C, lane 9).

DISCUSSION

Of the increasing rates of food allergy, the most obvious rise has occurred in peanut and tree nut allergies (28–30). An important difference between these foods is their stability to gastric digestion: some allergens in peanuts have been shown to be resistant to gastric digestion (13, 19). In agreement with the findings of other reports (18, 19), we showed in the present study that hazelnut allergens are degraded within seconds when incubated with SGF. Hazelnut allergy is mainly ascribed to cross-reactivity with birch pollen (16). Nevertheless, several studies have reported severe anaphylactic reactions without an association with pollen allergy (15–17) or even monosensitization to hazelnut (10).

In a recent study we showed that treatment of mice with antiulcer drugs leads to the induction of T helper 2 cell responses against usually harmless dietary proteins (21). By different

mechanisms (acid neutralization, proton pump inhibition, or H₂-receptor blocking), these medications elevate the gastric pH and prevent peptic degradation of proteins.

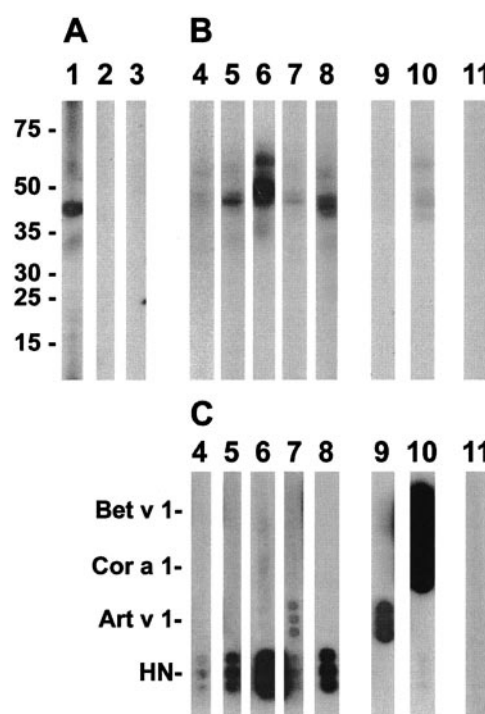


FIGURE 5. Specificity of purified mouse immunoglobulin (Ig) G1 and of human IgE from hazelnut-allergic patients treated with antiulcer drugs. A: Hazelnut-specific purified mouse IgG1 was tested on hazelnut extract (lane 1), birch pollen extract (lane 2), and mugwort extract (lane 3). Bound IgG1 was detected by radiolabeled anti-mouse Ig antibody. B: Hazelnut extract blots were incubated with sera from patients ($n = 5$) who were treated with antiulcer drugs and developed hazelnut-specific IgE (lanes 4–8), with a pool of sera from mugwort pollen–allergic patients (lane 9), with a pool of sera from birch pollen–allergic patients (lane 10), or with buffer (lane 11). C: Dot blots with triplicates of the recombinant allergens Bet v 1, Cor a 1, and Art v 1 or of hazelnut extract (HN) were tested with sera or buffer as in section B. In sections B and C, IgE was detected with radiolabeled anti-human IgE antibody.

Our *in vitro* digestion experiments showed that, at pH 5.0, peptic digestion of all hazelnut proteins in an extract was hindered. Our working hypothesis was that in this setting intact proteins could reach the mucosa of the intestine, where they are capable of priming sensitization and inducing T helper 2 cell responses. As a proof of concept, we used our BALB/c mouse model and fed hazelnut extract either without or in combination with antiulcer drugs. Sucralfate, which is known to bind and neutralize hydrochloric acid (31), was chosen as an example for an aluminum compound. It might support IgE induction similarly to aluminum hydroxide, which is used routinely as an experimental and clinical adjuvant in vaccine formulations, and induces T helper 2 cell-type responses. The proton pump inhibitor omeprazole was selected as a substance without any adjuvant properties. Similarly to H₂-receptor blockers, omeprazole reduces the net acid output and hinders pepsin activation. Therefore, we considered that both H₂-receptor blockers and proton pump inhibitors should have the same clinical and experimental effects. Most of the mice in both groups treated with antiulcer drugs concomitantly with hazelnut feedings developed hazelnut-specific IgG1 antibodies. The IgG1 levels were more pronounced in the sucralfate-treated group, which may have been due to 1) the timely application in combination with hazelnut extract in the experimental situation, and 2) the possible adjuvant effect of the aluminum compound. The anaphylactogenic capacity of the IgG1 antibodies induced by feedings with sucralfate was shown in passive cutaneous anaphylaxis. It is already known that, at least in the animal model, specific IgG in complex with its antigen can bind directly to Fc γ RIII on mast cells and thereby achieve triggering capacity (32, 33). Although IgE was below the detectable level, type I skin tests were positive in 3 of 5 mice fed hazelnut extract in combination with sucralfate and in 1 of 5 of the omeprazole-treated mice. In these skin tests, Cor a 1 was also included, but the reaction was negative in all mice, probably because the commercially available recombinant Cor a 1 used in our experiments came from hazel pollen and is only 63% homologous to Cor a 1 from hazelnut (34).

To analyze the effect of our observations in a human group, we screened 153 gastroenterologic patients, who were treated for gastric complaints such as dyspepsia or gastritis but were not instructed to follow a special diet. Before antiulcer medication, no hazelnut-specific IgE was present in any of the sera, but 5 patients developed hazelnut-specific IgE during the 3-mo treatment with ranitidine or omeprazole; an untreated control group ($n = 50$) did not. Thus, the occurrence of hazelnut sensitization in these patients (3.3%) was higher than the prevalence of all tree nut allergies combined in a general population (0.2–0.7% of the US population, 0.38% of the UK population) (29, 35). The levels of specific IgE decreased until the 8-mo investigation, which pointed toward a low memory response. Nevertheless, skin-prick tests at this time point were positive in 4 of the 5 patients. In addition, oral provocation with hazelnut gave positive results in 3 of the 5 patients even at the 12-mo time point after onset of therapy. These reactions may be due to the longer survival time of IgE fixed to mast cells via the high affinity receptor Fc ϵ RI compared with IgE in serum (36). Two of these 5 patients reported acute urticaria or oral allergy syndrome after consumption of hazelnuts or chocolate containing hazelnuts. Importantly, none of the 5 patients had or developed *de novo* IgE specific for cross-reactive airborne allergens from early blooming trees (eg, birch pollen) during our observation period. This suggests that

sensitization to hazelnuts in the 5 patients was not due to primary sensitization with cross-reactive pollen. To definitively answer this question, we performed immunodots using recombinant Bet v 1 and Cor a 1 as marker allergens for Fagales pollen sensitization (14, 37) and Art v 1 for mugwort pollen allergy (38). The results indicate that the patients were not pollen sensitized but that hazelnut was the genuine elicitor of IgE formation. Taken together, our data suggest that antiulcer treatment, or other conditions leading to insufficient protein degradation in the stomach, may lead to sensitization with hazelnut proteins without the involvement of an inhalative allergy. 

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