

Antacid medication inhibits digestion of dietary proteins and causes food allergy: A fish allergy model in Balb/c mice

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Background: Digestible proteins were supposed to be irrelevant for oral sensitization and induction of food allergy. Approximately 10% of the adult population uses antacids for the treatment of dyspeptic disorders, drugs that hinder peptic digestion. In these patients, proteins that are normally degradable might act as food allergens.

Objective: We aimed to study the influence of antacid intake on the allergenicity of dietary proteins, taking sturgeon caviar and parvalbumin, the major fish allergen, as examples.

Methods: Caviar proteins and recombinant parvalbumin from carp, rCyp c 1, were applied for intragastric feedings with or without the antacids sucralfate, ranitidine or omeprazole, using a Balb/c mouse model.

Results: Both caviar proteins and parvalbumin were rapidly degraded in an in vitro digestion assay at pH 2.0, but not at pH 5.0, imitating the effect of antacids. The groups fed with caviar in combination with ranitidine hydrochloride intramuscularly or sucralfate orally had significant levels of caviar-specific IgE antibodies ($P < .01$), T-cell reactivity, and elevated counts of gastrointestinal eosinophils and mast cells. Food allergy in these groups was further evidenced by oral provocation tests and positive immediate-type skin reactivity. In contrast, feedings with caviar alone led to antigen-specific T-cell tolerance. None of the groups showed immune reactivity against the daily mouse diet. As a proof of the principle, feeding mice with parvalbumin in combination with ranitidine or omeprazole intramuscularly induced allergen-specific IgE antibodies ($P < .05$).

Conclusions: When antacid medication impairs the gastric digestion, IgE synthesis toward novel dietary proteins is promoted, leading to food allergy. (*J Allergy Clin Immunol* 2003;112:616-23.)

Key words: Food allergy, caviar, parvalbumin, IgE, nutrition, antacids, digestion, animal model

Abbreviations used

ig: Intragastric
im: Intramuscular
ip: Intraperitoneal

IgE-mediated food allergy represents an increasing health risk in industrialized countries and affects both children and adults.^{1,2} Proteins resistant to gastrointestinal digestion are especially prone to elicit IgE-mediated immediate-type hypersensitivity reactions, among which anaphylactic shock is the most severe.³ In contrast, easily digestible proteins, for example dietary homologues of pollen allergens, elicit local reactions such as itching or swelling of the oral mucosa (the so-called oral allergy syndrome).^{4,5} For these reasons, digestion assays using pepsin at low pH or trypsin at alkaline conditions have become a standard procedure to examine the allergenic properties of food proteins.^{3,6} Recent reports have, however, shown that some food allergens might also be quickly degraded in these assays.⁷ For the peanut allergens Ara h 1 and Ara h 2, obviously the trimeric protein structure plays an important role for their stability and is probably responsible for the persistence of allergenic Ara h 1 and 2 fragments in digestion assays.⁸ It has been demonstrated that only digestion for 24 to 48 hours destroys their IgE epitopes.⁹ These data are, however, in disagreement with a recent report indicating that Ara h 1 and 2 are digestible within 0.5 to 5 minutes and therefore should not be capable of sensitization.¹⁰

The question whether insufficient digestion, as it might occur under antacid treatment of dyspeptic patients, might support the induction of food allergy has never been asked. It has been reported that dietary epitopes can induce B-cell responses when transformed into a digestion-resistant state, or when digestion is hindered.^{3,6} At present, there are no epidemiologic studies available on the correlation of antacid treatment and sensitization to dietary antigens. Food allergy might affect the adult population, often without atopic background. They are professionally active and stressed and, therefore, prone to have dyspepsia. According to recent studies, 10% of the middle-aged population uses antacids for dyspeptic disorders.¹¹ Adult food allergy is typically characterized by sensitization to a single or few compounds of the diet, with a long list of the most exotic pos-

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sible elicitors like fruits, nuts, spices, or seafood.¹²⁻¹⁶ We have recently reported a case of an adult nonatopic patient with selective food allergy to Russian Beluga caviar.¹⁷ It is remarkable that this patient's first time consumption of caviar coincided with the intake of sucralfate (aluminium saccharose hydrogen sulfate) for the treatment of a gastric ulcer. On the second consumption of caviar, the patient responded with severe anaphylaxis.

Beluga proteins and the major fish allergen parvalbumin are degraded within seconds in an *in vitro* digestion assay. Thus, these proteins are suitable model antigens to test our hypothesis that antacids could promote food allergy induction in an animal model. Balb/c mice were fed with caviar proteins with or without concomitant application of antacids, and their *in vitro* and *in vivo* immune responses were analyzed. We used the H₂-receptor blocker ranitidine hydrochloride, which directly affects acid production by parietal cells.¹⁸ Moreover, we investigated the effect of sucralfate, a drug known to bind and neutralize HCl,¹⁹ and we included the broadly used proton pump inhibitor omeprazole in our study. From our data, we conclude that antacids might cause digestion insufficiency, converting normally degradable dietary proteins into potential food allergens.

METHODS

Animals

Eight-week-old female Balb/c mice (Institute for Laboratory Animal Science and Genetics, University of Vienna, Austria) weighing 20 to 26 g were used. All experiments were performed according to European Community rules of animal care and with permission GZ 66.009/211-Pr/4/2001 of the Austrian Ministry of Science.

Protein preparations and pepsin digestion

Extracts were prepared from Beluga caviar (Schenkel, Vienna, Austria) and from mouse daily diet (Tagger Koch, Amstetten, Austria). The materials were ground in liquid nitrogen and extracted in PBS under continuous shaking for 24 hours at 4°C. After centrifugation, supernatants were dialyzed, lyophilized, and stored at -20°C. Recombinant carp parvalbumin, rCyp c 1, was manufactured as described previously.²⁰ The protein concentration was determined according to the method of Bradford.²¹

For peptic digestions, protein preparations were incubated at 37°C with 0.87 g/L pepsin (Sigma, St Louis, Mo) at pH 2.0 or pH 5.0 under continuous agitation.^{3,6} The enzymatic reaction was terminated by neutralizing the pH and by cooling the samples on ice.

SDS-PAGE and IgE immunoblots

Protein digestion was evaluated by SDS-PAGE according to Laemmli,²² using Coomassie brilliant blue staining. The molecular weights were calculated using prestained protein standards (RPN 800, Amersham, Buckinghamshire, UK). Digested Beluga caviar protein extracts were separated by SDS-PAGE and blotted onto nitrocellulose.²³ Blot strips were saturated in blocking buffer and incubated overnight at 4°C with sera from patients or healthy controls. Bound IgE antibodies were detected with ¹²⁵I-labeled anti-human IgE antibodies (IBL, Hamburg, Germany) and visualized by autoradiography.

Oral immunization of Balb/c mice

Mice were immunized intragastrically (ig) or intraperitoneally (ip) with 150 μ L caviar extracts. The amounts of antacids given

were calculated for the mouse body weight and correlated to human dosages. Group A (n = 5) was pretreated with 2 μ g Ranitidine (Ulsal, Gebro, Fieberbrunn, Austria) intramuscularly (im) followed by ig gavage of caviar extract (20 mg/mL) 2 hours later. Group B (n = 5) received 2 mg sucralfate (Ulcogant, Merck, Vienna, Austria) ig together with caviar extract (20 mg/mL). Group C (n = 5) was immunized ip with 50 μ L caviar extract (40 mg/mL) and 100 μ L aluminium hydroxide (Serva, Heidelberg, Germany). Group D (n = 5) was fed with caviar alone, and control group N was left naive. Immunizations were performed on day 0 and 56. Mice were subjected to oral provocation and skin tests and put to death on day 92.

Three mouse groups (n = 8 each) received ig parvalbumin (200 μ g/mL). Mice of group 1 were injected im with 2 μ g ranitidine, followed 2 hours later by ig gavage of parvalbumin. Mice of group 2 were medicated with 11.6 μ g omeprazole (Losec, AstraZeneca, Vienna, Austria) im, followed by ig gavages of parvalbumin again 2 hours later. Mice of group 3 were fed with parvalbumin alone. Immunizations were performed on day 0 and 56. Blood samples were taken from the tail vein on days 0, 28, 49, and 84.

Measurement of specific antibodies in ELISA

Microtiter plates (NUNC, Roskilde, Denmark) were coated with caviar extract or parvalbumin as described.²⁴ Plates were washed and incubated with rat anti-mouse antibody (PharMingen, San Diego, Calif) for 1 hour at 37°C and 1 hour at 4°C. After washing, plates were incubated with peroxidase-labeled mouse anti-rat IgG (Jackson ImmunoResearch, West Grove, Pa) for 1 hour at 37°C and 1 hour at 4°C. The reaction was developed by adding 2,2'-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) in citric acid and 0.1% hydrogen peroxide. The optical density was measured at 405 to 490 nm with an ELISA-reader.

Food challenge and skin test

Five weeks after the last immunization, 100 μ L of 0.5% Evans blue (Sigma) was injected into the tail vein. Subsequently, 125 μ L of caviar extract (40 mg/mL in PBS) was applied orally, and skin tests were performed.²⁵ Caviar extract and mouse food extract (50 μ g/mL each) were injected intradermally into the shaved abdominal skin. The mast cell degranulation compound 48/80 (20 μ g/mL; Sigma) was injected as positive control and rBet v 1 (Biomay, Vienna, Austria) (2.5 μ g/mL) as negative control. After 20 minutes, mice were put to death, skinned, the diameter of the color reaction was evaluated on the inside of the abdominal skin, and the reactivity of the soft and hard palate was examined.

Electron microscopy

Samples from the small stomach curvature (lower third) were fixed and embedded.²⁶ Sections oriented perpendicular to the surface showing mucosa and submucosa were used for evaluation. Mast cells and eosinophils in entire sections were counted. The section size was measured using a light microscope. The appropriate grids were placed on a glass slide, and the section sizes were measured using an eyepiece equipped with a graticule that had been equilibrated using a Zeiss equilibration slide. The number of mast cells or eosinophils per square millimeter was counted.

Proliferation assay

Spleens were removed under sterile conditions, minced, and passed through sterile nylon filters. Erythrocytes were lysed in 0.75% ammonium chloride; the remaining cells were washed and resuspended in RPMI medium (Gibco Ltd, Paisley, Scotland) supplemented with 10% FCS, 0.1 mg/mL gentamicin, 2 mmol/L glutamine, and 50 μ mol/L 2-mercaptoethanol. Cells (100,000/well) were plated into 96-well round-bottomed tissue culture plates (NUNC),

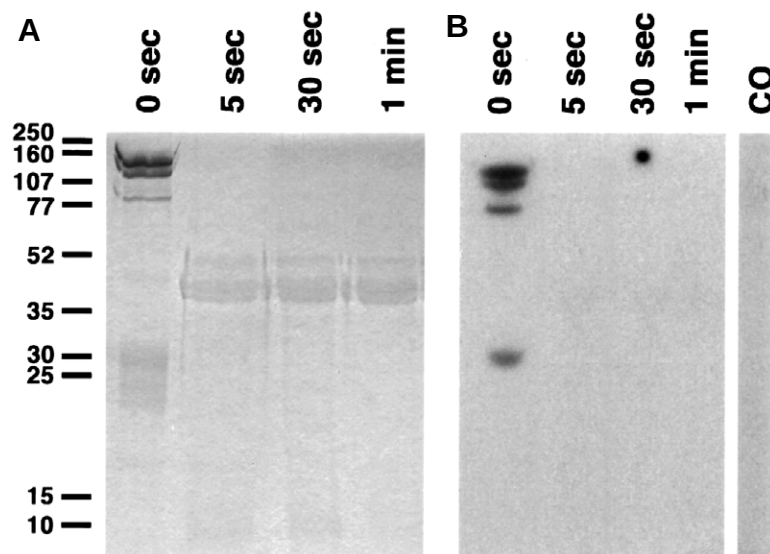


FIG. 1. Time kinetics of in vitro digestion of caviar proteins by pepsin at pH 2.0. **A**, The effects of protein digestion were analyzed by Coomassie-stained SDS-PAGE. **B**, Protein extracts of untreated or pepsin-digested caviar extracts were blotted onto nitrocellulose and tested for IgE reactivity using serum of an allergic patient. Bound IgE was detected by ^{125} I-labeled anti-human IgE-antibody.

and cultured with rBet v 1 (2 μ g/well), caviar extract (20 μ g/well), mouse food extract (20 μ g/well), or concanavalin A (0.2 μ g/well; Sigma) for 4 days at 37°C. Thereafter, the cultures were pulsed with 0.5 μ Ci/well ^{3} H-thymidine (Amersham) for 16 hours. Cells were harvested, and ^{3} H-thymidine incorporation was measured in a liquid scintillation counter.

Statistical analysis

Statistical comparisons were made using 1-way ANOVA. *t* Tests for homogeneous and inhomogeneous variances, respectively, and the Mann-Whitney *U* test were applied for group comparison. A *P* value < .05 was considered significant.

RESULTS

Digestion of caviar proteins

The sensitivity of caviar protein to digestion was examined in an in vitro assay using pepsin at pH 2.0. As shown by Coomassie-stained SDS-PAGE, after 5 seconds of treatment, the high molecular weight bands at 84, 100, and 118 kDa disappeared (Fig 1, A). We further examined the capability of untreated and digested caviar proteins to bind IgE from the serum of a patient with Beluga caviar allergy. Again, 5 seconds with pepsin digestion were sufficient to abrogate the IgE-binding capacity (Fig 1, B).

Oral immunization of Balb/c mice with caviar

Significant increases of caviar-specific IgE levels were observed in mice treated with ranitidine hydrochloride (group A) or sucralfate (group B) (*P* < .01) compared with control groups C (caviar and Al(OH)₃ ip) and D (caviar alone), which stayed at the baseline level (Fig 2, A). Notably, caviar-specific IgE immediately increased after a

single oral gavage and showed significant levels on day 28. The highest amount of caviar-specific IgG1 and IgG2a antibodies was induced by the ip immunization with Al(OH)₃ as adjuvant (Fig 2, B and C). No increase in the level of caviar-specific antibody was observed in naive mice (data not shown). Furthermore, using protein extracts of daily mouse dietary food for coating ELISA plates, we could not detect IgE antibodies against these proteins in any of the groups (data not shown).

In vivo skin tests and food challenges of Balb/c mice

To show the in vivo relevance of IgE antibodies in type I allergy, testing at skin and mucosal sites was performed. As depicted in Fig 3, panel 1, mice from group B and group C showed specific type I skin reactivity to caviar extracts but not to a control allergen. Groups A and D remained negative in the skin tests. None of the mice showed positive reactivity to protein extracts of daily mouse food. Fig 3, panel 2, illustrates that oral food challenges with caviar induced immediate-type reactivity of the palate mucosa in mice of groups A, B (treated with antacids), and C (caviar ip), but not in group D, which was fed with caviar alone. All 5 mice of groups A, B, and C, but not of group D and N, showed positive reactivity (data not shown).

Mast cells and eosinophils

The murine stomach mucosa does not normally contain appreciable numbers of mast cells and eosinophils (Table I, group D). In contrast, in the gastric wall of mice of groups A, B, and C, an increase in the number of mast cells was observed (Table I). In transmission electron microscopy investigations, mast cells were scattered singly between the stomach glands (Fig 4). Most mast cells had swollen gran-

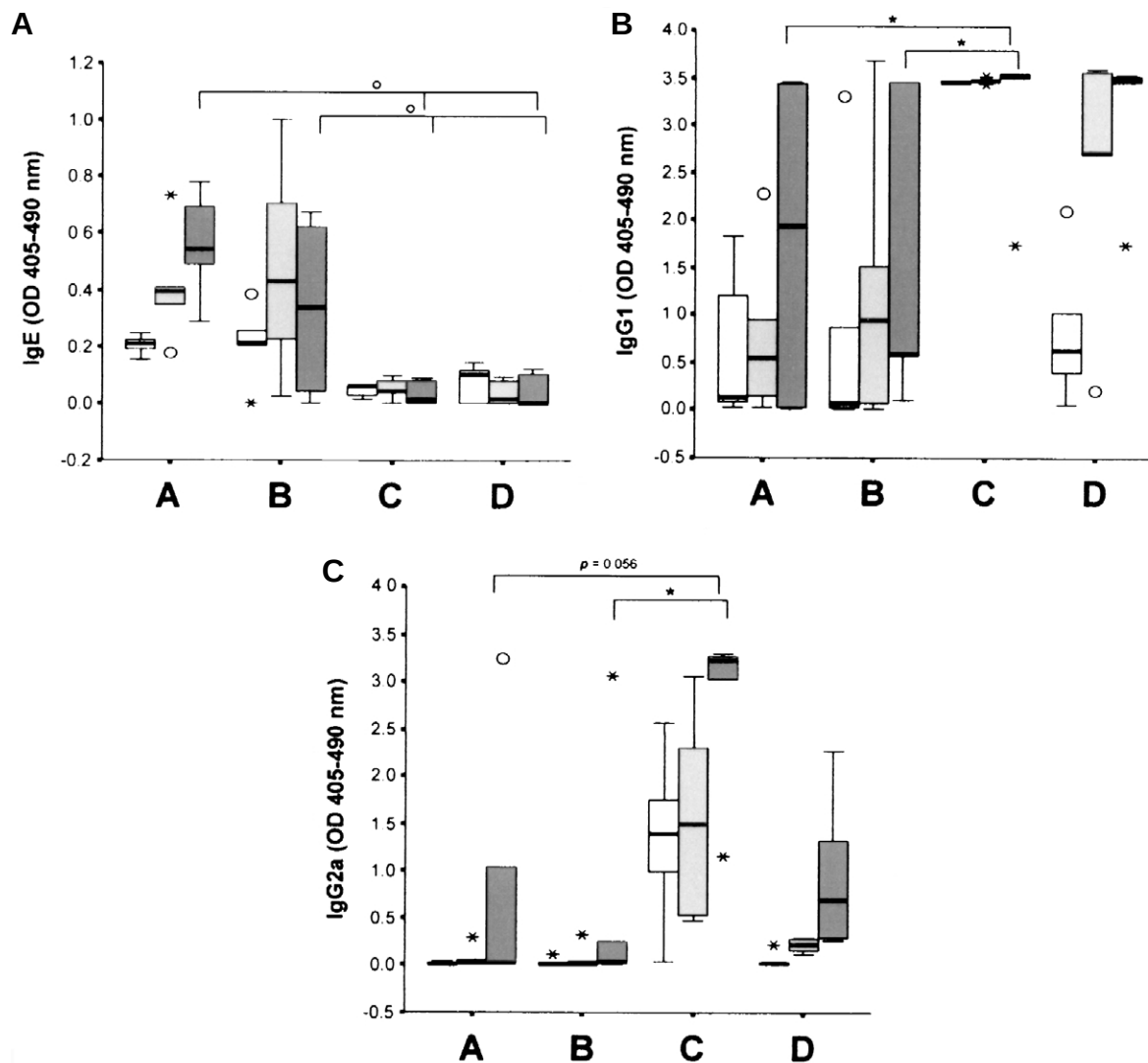


FIG 2. Induction of IgE, IgG1, and IgG2a antibodies to caviar in antacid-treated mice. Balb/c mice were divided in 4 groups (n = 5): group A (ranitidine hydrochloride im and caviar ig), group B (caviar and sucralfate ig), positive control group C (caviar and Al(OH)₃ ip), and in group D (caviar extract ig). Blood was taken from the tail vein on day 0 (preimmune serum), 28 (white boxes), 49 (light gray boxes), and 84 (dark gray boxes). Caviar-specific IgE (A), IgG1 (B), or IgG2a (C) antibodies were measured by ELISA. The background values of preimmune sera were subtracted. The boxes represent the range of the inner quartiles of the samples divided by the median. Sera with signals showing more than 1.5-fold deviation from the end of the box were defined as outliers and marked as circles. Sera with titers lying more than 3-fold away were defined as extremes and marked with asterisks. Brackets indicate the groups being statistically compared (* $P < .01$, * $P < .05$).

ules, indicating an incipient stage of degranulation, and 15% of them had signs of severe degranulation.

As further shown in Table I, a marked increase in eosinophil counts was noticed in the mice of groups A, B, and C, particularly in the mice of group B. The eosinophils tended to occur lined up between the stomach glands, perhaps migrating from submucosal vessels to the basal layers of the mucosa. Margination to and through vessel walls was frequently observed (Fig 4). Approximately 50% of eosinophils from the mice of groups A and B was severely degranulated, whereas eosinophils from groups C and D mice were not.

T-cell proliferation

The results depicted in Table II show that T cells from mice of groups A, B, and C, but not group D, were able to proliferate after stimulation with caviar antigens. In contrast, no T-cell proliferation could be achieved using protein extract of the daily mouse diet as antigen source.

Parvalbumin, digestion assay, and oral immunization of Balb/c mice

To strengthen our hypothesis that under antacid treatment the caviar proteins might act as allergens, parvalbu-

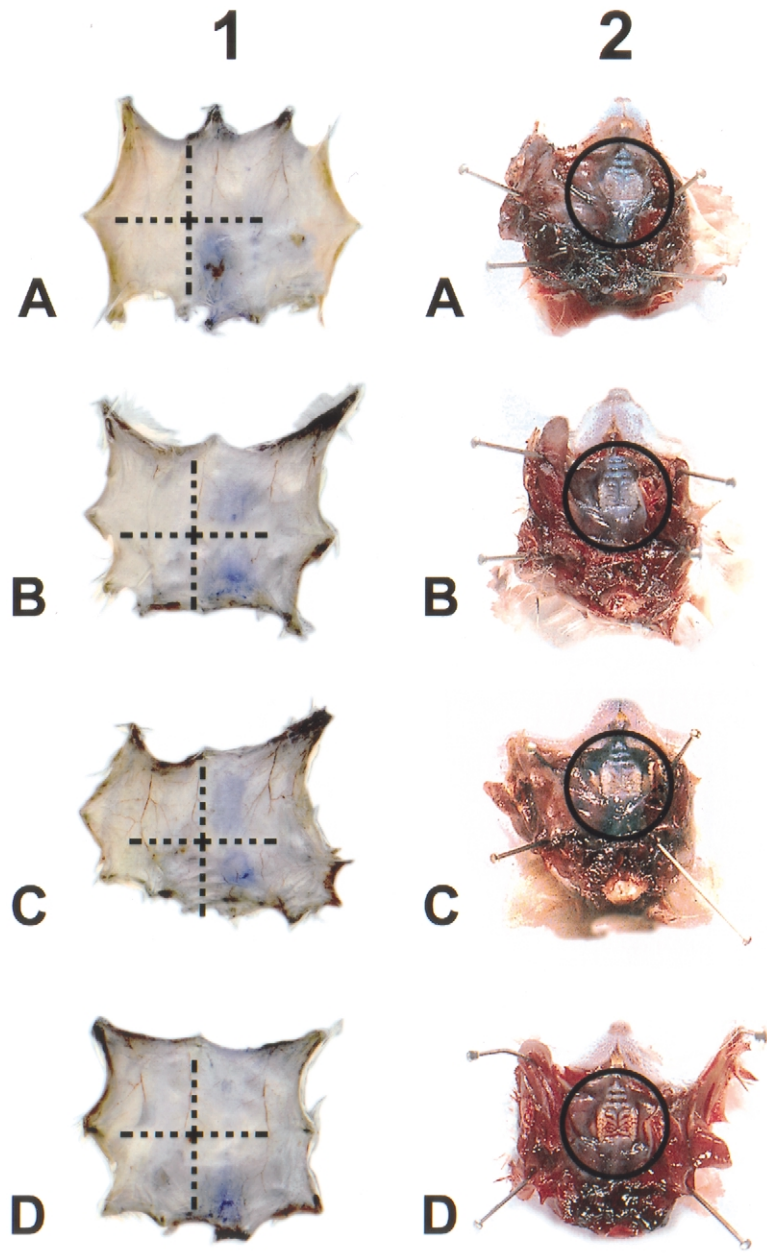


FIG 3. Skin reactivity and mucosa hypersensitivity to Beluga caviar. Four groups of Balb/c mice were treated and immunized as shown in Fig 2. Five weeks after the last immunization, type I skin testing was performed. Evans blue was injected intravenously, then caviar extract was applied intradermally in the upper right quadrant, positive control degranulation compound 48/80 in the lower right, mouse food extract in the upper left, and rBet v 1 in the lower left quadrant. Animals were put to death 20 minutes after injection. Representative tests from groups A, B, C, and D are shown in *panel 1*. In the same mice a food challenge with caviar extracts was performed just before skin tests and 25 minutes before they were put to death. Representative preparations of mice palates are shown in *panel 2*. Circles highlight the color reaction in the palate.

min, the major fish allergen, was subjected to in vitro digestion pepsin assay. After 30 seconds treatment at pH 2.0, parvalbumin was completely degraded, whereas at pH 5.0, the protein was not digested even after 2 hours incubation (data not shown).

To test the in vivo relevance of the preceding findings,

we performed oral immunizations of Balb/c mice with parvalbumin, following the same protocol as with caviar. As can be seen from Fig 5, the IgE titers in the sera of mice treated with ranitidine (group 1) or omeprazole (group 2) reached higher levels ($P < .05$) than the control group 3 fed with parvalbumin alone.

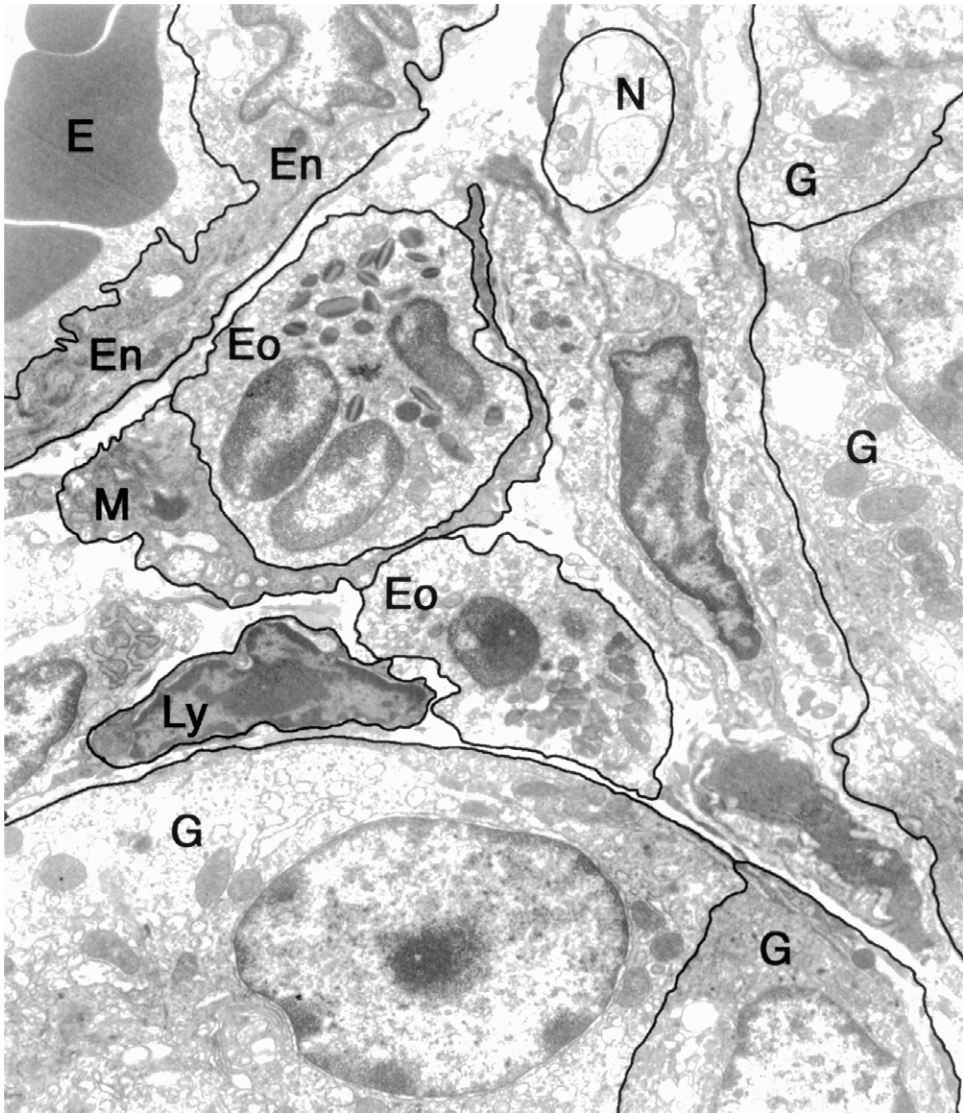


FIG 4. Transmission electron micrograph of a basal portion of the antrum mucosa from a mouse treated with sucralfate and immunized with caviar (group B). Two eosinophils (*Eo*) are located in the lamina propria close to a capillary, composed of endothelial cells (*En*) and erythrocytes (*E*) and cross-sections through gastric glands (*G*). One of the eosinophils is almost completely embraced by a macrophage (*M*); the other is closely attached to that cell. Other stromal cells, a lymphocyte (*Ly*), and a peripheral nerve (*N*) are grouped around the eosinophils in the lamina propria. The cell borders are indicated with *lines*. Stain, uranyl acetate and lead citrate; original magnification, $\times 4000$.

DISCUSSION

In this study we provide evidence that antacid treatment, through interference with peptic protein digestion, leads to IgE induction and food allergy against proteins that are otherwise degraded. In vitro digestion assays have become popular tools for predicting the allergenicity of food proteins. They might reflect the potential of a protein antigen to induce specific IgE by the oral route, or, as shown recently, by the parenteral route.²⁷ Caviar allergens and, surprisingly, the major fish allergen parvalbumin were degraded immediately, although the latter has previously been claimed to be a digestion-stable antigen.²⁸ Thus, both antigens did not fulfill the criteria of typical

TABLE I. Numbers of mast cells and eosinophils in the gastric wall*

Group	Treatment	Mast cells/ mm ²	Eosinophils/ mm ²
A	Ranitidine im and caviar oral	3 ± 2	7 ± 4†
B	Sucralfate and caviar oral	3 ± 3	16 ± 14†
C	Al(OH) ₃ and caviar ip	2 ± 2	3 ± 3
D	Caviar oral	0 ± 0	0 ± 0

im, Intramuscularly; *ip*, intraperitoneally.
*Data from 5 mice per group are presented as mean ± SD.
†*P* < .05.

Food and drug reactions
and anaphylaxis

TABLE II. Proliferation of murine spleen cell cultures stimulated with caviar or mouse food extracts

Group	Treatment	Stimulation index*	
		Caviar	Mouse food
A	Ranitidine im and caviar oral	121.9	3.2
B	Sucralfate and caviar oral	36.5	3.5
C	Al(OH) ₃ and caviar ip	349.9	1.8
D	Caviar oral	7.2	0.6

im, Intramuscularly; ip, intraperitoneally.

*The stimulation index is calculated from mean proliferation with antigen versus medium control mean value. Data from 5 mice per group are presented.

food allergens. It is known that antacids elevate the gastric pH to about 5.0 in patients,²⁹ and, interestingly, at pH 5.0, parvalbumin was no longer digested. In this setting, normally digestible dietary proteins remain intact and potentially adapt the characteristics of food allergens.

To test our hypothesis that antacids might promote food allergy in vivo, we treated several groups of Balb/c mice with or without the most important antacids ranitidine hydrochloride (Ulsal), omeprazole (Losec), and aluminium saccharose hydrogen sulfate (Ulcogant) and fed them with the antigens caviar or parvalbumin. Aluminium-containing compounds (eg, Al[OH]₃) have been long used in the treatment of gastric ulcer. It is worth noting that in experimental immunology Al(OH)₃ is classically used as adjuvant, which induces T_H2-biased immune responses.³⁰ With regard to the possible role of sucralfate as a T_H2 adjuvant, it was important to include in our study ranitidine and omeprazole, which directly interfere with acid production. In our experimental animal model, we demonstrated that high levels of specific IgE antibodies toward dietary proteins are synthesized in antacid-treated mice. In contrast, ip immunizations in combination with Al(OH)₃ showed predominant IgG production, probably because of the relatively large amount of antigen given by this route. Interestingly, feedings of the protein antigen alone induced specific IgG. These findings are in line with previous studies demonstrating that proteins sensitive to gastric digestion are still able to elicit substantial IgG responses after oral ingestion.^{27,31} When IgE was induced, it was specifically directed against the newly introduced food but not toward the daily diet of mice. Accordingly, these allergens but not the daily dietary proteins were able to drive T-cell proliferation in the sensitized mice. This observation is in agreement with data showing that long-time antigen feeding results in immune tolerance in humans.³² Our data indicate that feedings with novel antigens also lead to T-cell tolerance, as long as the proteins are degraded in the stomach.

We performed skin and mucosal tests as in vivo readout for type I allergy. Only the mice pretreated with ranitidine showed positive skin reactivity, whereas the naive group and the group fed with caviar alone did not react in these tests. However, in oral provocation tests, all groups except the negative control group showed positive reactivity. These results obviously reflect the situation in human

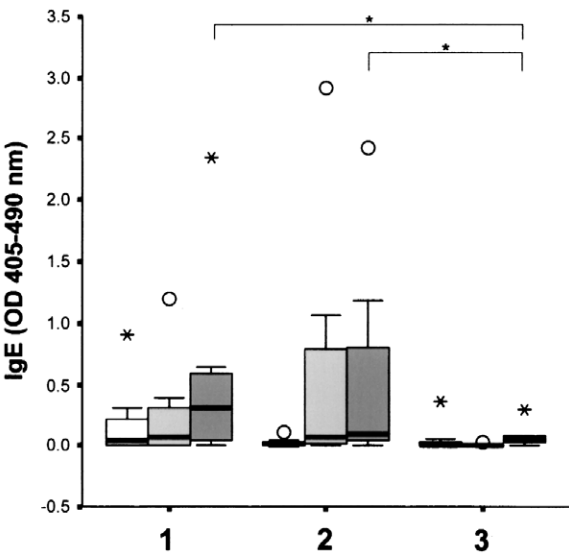


FIG 5. Induction of parvalbumin-specific IgE antibody in antacid-treated mice. Groups of mice (n = 8) were immunized intragastrically with recombinant parvalbumin. Group 1 was premedicated with ranitidine hydrochloride, and group 2 was injected with omeprazole intramuscularly 2 hours before immunization. Control group 3 was fed with parvalbumin alone. Mice were immunized at day 0 and 56. Blood was taken from the tail vein on day 0 (preimmune serum), 28 (white boxes), 49 (light gray boxes), and 84 (dark gray boxes), and parvalbumin-specific IgE antibodies were measured by ELISA. Brackets indicate the groups being statistically compared (*P < .05).

patients, in whom results of skin tests and mucosal reactivity to food allergens often do not correlate.³³ For this reason, the double-blind placebo-controlled food challenge has been suggested as the “gold standard” to diagnose food allergy.³⁴ Alternately, a colonoscopic allergen provocation test might identify the relevant allergen.³⁵

In humans, gastrointestinal hypersensitivity disease syndromes might appear as eosinophilic gastroenteritis³⁶ or esophagitis.³⁷ When mice were fed with the antigen combined with antacids, they had eosinophilia develop in the stomach mucosa but not in the small intestine (data not shown). Moreover, an enhanced number of mast cells was also observed in the stomach of the food-allergic groups. Many of them appeared degranulated, probably because of the oral allergen provocation.

From our experiments with caviar and the major fish allergen parvalbumin, we conclude that antacids critically participate in the pathophysiology of food allergy. They hinder peptic digestion and, thus, preserve the structure of the dietary protein and its B-cell epitopes. It is, therefore, tempting to speculate that conformation of food proteins is a prerequisite for IgE induction. The mechanism possibly involves intestinal $\gamma\delta$ -T cells, which recognize conformational epitopes and have already been proven to directly support IgE production by secretion of IL-4.³⁸ The expansion of specific IgE in our model occurred immediately and independently of other immunoglobulin isotypes.

Our data strongly suggest that medication with antacids puts patients at risk of allergy developing against newly introduced food antigens, a finding that is possibly relevant for about 10% of the adult population who consume antacids.¹¹ We emphasize that this risk should not be underestimated in times of globalization, where eating the most exotic dishes is fashionable and part of an open-minded lifestyle.

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