

The FASEB Journal express article 10.1096/fj.04-3170fje. Published online January 25, 2005.

Anti-ulcer drugs promote IgE formation toward dietary antigens in adult patients

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ABSTRACT

Recently, we have demonstrated that anti-ulcer drugs, such as H₂-receptor blockers and proton pump inhibitors, promote the development of immediate type food allergy toward digestion-labile proteins in mice. The aim of this study was to examine the allergological relevance of these findings in humans. In an observational cohort study, we screened 152 adult patients from a gastroenterological outpatient clinic with negative case histories for atopy or allergy, who were medicated with H₂-receptor blockers or proton pump inhibitors for 3 months. IgE reactivities to food allergens before and after 3 months of anti-acid treatment were compared serologically. Ten percent of the patients showed a boost of preexisting IgE antibodies and 15% de novo IgE formation toward numerous digestion-labile dietary compounds, like milk, potato, celery, carrots, apple, orange, wheat, and rye flour. Thus, the relative risk to develop food-specific IgE after anti-acid therapy was 10.5 (95% confidence interval: 1.44–76.48). The long-term effect was evaluated 5 months after therapy. Food-specific IgE could still be measured in 6% of the patients, as well as significantly elevated serum concentrations of ST2, a Th2-specific marker. An unspecific boost during the pollen season could be excluded, as 50 untreated control patients revealed no changes in their IgE pattern. In line with our previous animal experiments, our data strongly suggest that anti-ulcer treatment primes the development of IgE toward dietary compounds in long-term acid-suppressed patients.

Key words: food allergy • digestion • epidemiology • skin prick test • oral sensitization • ST2 • simulated gastric fluid

Anti-ulcer medication is taken for a large variety of complaints, such as functional nonulcer dyspepsia, stomach upset, gastro-esophageal reflux, gastritis, and gastric or duodenal ulcer. Most dyspeptic symptoms are dealt with by the patient without seeking medical advice (1–3) by using antacids (4). These drugs act via buffering the gastric pH and inactivating the major gastric protease pepsin (5). Since the beginning of the 1980s, antacids were to a great extent replaced by H₂-receptor blockers (6) and proton pump inhibitors (PPIs) (7). In 2001, the PPI Prilosec was the second biggest-selling drug in the United States. Increasing sale figures are to be expected in the future, as recently this drug has also been approved for over-the-counter sales (8).

The highly potent, long acting PPIs and H₂-receptor antagonists can almost totally abolish acid secretion (9, 10), although antisecretory therapy increasing the gastric pH above 3.0 is not necessary for ulcer healing (11). A recent study revealed that only 5 days of treatment with the PPI omeprazole elevates the gastric pH to an average of 5.0 (12). Increases of gastric pH might interfere with the physiological gate-keeping function of the stomach, e.g., the protective role of gastric acid against bacterial infections (13–15). Moreover, gastric acidity is required for the activation of pepsinogens and the initiation of protein digestion. The optimal pH for proteolysis varies for different pepsins in gastric juices, but total enzyme activity has an optimum in the pH range of 1.8–3.2 (16).

It has been postulated that resistance to peptic digestion is a prerequisite for food proteins to act as allergens (17). Thus, allergens or allergenic epitopes that survive the gastrointestinal transit in an intact form can trigger mucosal sensitization (18). For these reasons, digestion assays with simulated gastric fluid (SGF) have become a standard procedure to predict the allergenic potency of food antigens (19) and are used as a safety criterion in the decision tree approach approved by the FAO/WHO for assessment of the allergenicity of transgenic proteins introduced into food (20).

Paradoxically, digestion-sensitive food proteins are among the most frequent elicitors of food allergy (21, 22). However we could recently demonstrate in an animal model that anti-ulcer drugs turned digestion-labile fish proteins into potent elicitors of type I hypersensitivity by elevating the gastric pH and impairing peptic digestion (23). Consequently, our hypothesis was that anti-ulcer treatment could support food allergy induction also in humans. We present here the results of an observational survey including 152 gastroenterological patients and 50 untreated controls.

MATERIALS AND METHODS

Study subjects

For the observational cohort study, 248 adults from the outpatient clinic of the Department of Gastroenterology, Hetényi Géza Hospital Szolnok, Hungary were assessed for eligibility. Patients were recruited for the study during a 2 month period in January and February 2003. A randomized study could not be conducted due to ethical reasons as patients had to be treated upon diagnosis of dyspeptic disorders. The decision for medical therapy of dyspeptic diseases was left to the gastroenterological specialist. A history of allergic disorders, refusal to participate, any additional medication except anti-ulcer drugs, and treatment for hypertension and diabetes

mellitus were defined as exclusion criteria. The final cohort consisted of 152 adult patients (58 men and 94 women; mean age 65.9 yr). The primary time point of follow-up was defined at the end of anti-ulcer treatment, 3 months after the beginning of the study, and a long-term follow-up evaluation at the 8 month time point was planned ([Fig. 1](#)). No interim analyses were performed. The primary outcome was indication of sensitization to food by diagnosing specific IgE induction. The secondary objective was the characterization of the cellular immune response type by determination of cytokine patterns. Patients were treated orally for 3 months with the H2-receptor blocker Famotidine (Quamatel® 2×40 mg/day; Richter Gedeon RT, Budapest, Hungary) or Ranitidine (Zantac® 2×150 mg/day; Pfizer Inc., New York, NY, or Pylorid® 2×400 mg/day; GlaxoSmithKline, Brentford, UK) or the PPI Omeprazole (Losec® 1×20 mg/day; AstraZeneca, London, UK). Exceptions were three patients, who were continuously taking the respective medication and one patient, who was under anti-acid therapy for 6 months. Serum samples were taken before, and 3 and 8 months after the beginning of therapy. The four patients with prolonged anti-ulcer treatment were not included in the evaluation for specific IgE at the 8 month time point. The medication compliance was checked in monthly control consultations.

To exclude any seasonal effects such as priming or boost by inhaled pollen antigens, 50 untreated and apparently healthy subjects (18 men and 32 women; mean age 59.9 yr) served as a control group. A history of gastrointestinal or allergic disorders and refusal to participate were defined as exclusion criteria. Serum samples were collected at two time points, 0 and 8 months, before and after the pollen season.

All patients gave written informed consent. Patient sera were identified by a code number and the personnel performing the tests were not aware of the case status.

Measurements of total and allergen-specific IgE

Total IgE values in the patients' sera were determined before and after 3 months of anti-ulcer treatment. Total IgE serum levels were measured in a fully automated microplate system (ALLERgen, Adaltis, Italy) following the manufacturer's instructions.

Allergen-specific IgE antibodies were measured by immunoblotting (AllergyScreen, MEDIWISS analytic, Moers, Germany) before, and 3 and 8 months after the beginning of treatment. Standard series of 45 inhalant allergens and 19 food allergens were tested. Specific IgE values of 0.35 kU/l (class 1) and above were considered as elevated IgE levels. All measurements were performed simultaneously after the 3 month evaluation and at the 8 month time point including the same control and standard samples to calibrate the AllergyScreen system. For control purposes, IgE levels of randomly selected serum samples were furthermore confirmed in the UniCAP System (Pharmacia, Uppsala, Sweden).

Digestion experiments

Raw milk, egg white, egg yolk, peanut, walnut, almond, potato, tomato, celery, carrot, apple, orange, wheat flour, rye flour, sesame seed, soy bean, codfish, crab, and casein (Fluka, Buchs, Switzerland) were extracted as described previously (23). Protein concentrations of extracts were determined according to the method of Bradford (24). For peptic digestions, proteins were incubated at 37°C with 0.87 g/l pepsin (Sigma, St. Louis, MO) at pH 2.0, under continuous

agitation. The enzymatic reaction was terminated by neutralizing the pH. Protein digestion was evaluated by SDS-PAGE according to Laemmli (25) using Coomassie brilliant blue staining. For milk proteins, the digestion experiments were performed at pH 2.0, 3.0 and 5.0, and the IgE binding capacity of remaining fragments was tested by immunoblotting (23). The molecular weights were calculated using prestained protein standards (RPN 800, Amersham, Buckinghamshire, UK).

Skin testing

At the 8 month time point, 33 patients with elevated specific IgE levels were subjected to skin prick tests with the 19 food extracts (ALK-Abelló, Horsholm, Denmark). Skin prick tests were performed according to the position paper of the European Academy of Allergology and Clinical Immunology, and a diameter >3 mm was defined as positive (+) (26).

Cytokine assays

Circulating IL-4, IFN- γ , IL-13, and ST2 levels in the patients' sera were determined by commercial enzyme linked immunosorbent assay (ELISA) using monoclonal antibodies against human IL-4 and IFN- γ (Bioscience, San Diego, CA), IL-13 (Cytimmune, College Park, MD), and polyclonal anti-human IL-1R4/ST2 antibodies (R&D Systems, Minneapolis, MN). The assays were performed following the manufacturers' instructions.

Data and statistics

Total IgE was compared between groups by Mann-Whitney U test. Differences between specific IgE before, and 3 or 8 months after onset of treatment were tested by Wilcoxon's matched-pairs test. No correction was made for multiple testing. A P value <0.05 was considered significant. Risk of development or increase of specific IgE against food allergens was estimated based on Poisson regression and results were expressed as relative risks and 95% confidence intervals.

RESULTS

Three months of medication with anti-ulcer drugs induces a boost or de novo IgE formation toward dietary antigens in gastroenterological patients

In [Fig. 1](#) the study design and subject progress through the phases of the observational cohort study can be seen. Before a 3 months course of anti-ulcer medication 15 of 152 gastroenterological patients (10%) had elevated specific IgE to at least 1 of 19 tested food allergens. Similarly, in control subjects 5 of 50 (also 10%) had preexisting food-specific IgE. After therapy, 40 patients (26%) were positive for food-specific IgE. The characteristics of these 40 patients demonstrating a positive specific IgE response are given in [Table 1](#). No statistically significant differences on quantity or quality of sensitization to food proteins between the three drug types used were observed. Hence, the relative risk for an increase of an IgE response to food allergens after a 3 months of anti-acid therapy is 10.5 (95% confidence interval: 1.44–76.48; $P=0.0203$).

Based on the results of serological allergy tests, the 40 “reactive” patients were classified into three groups: Group 1 ($n=5$, 13%) already had preexisting IgE before therapy mostly toward

inhalative antigens, such as ragweed, mugwort, house dust mites, fungi, and grass antigens, and three of them had IgE to food antigens; Group 2 ($n=12$, 30%) had preformed food-specific IgE in their serum; Group 3 ($n=23$, 57%) had neither preexisting IgE to food nor to inhalative allergens before the beginning of the anti-ulcer treatment. The results depicted in [Fig. 2](#) show the effects of anti-ulcer therapy on allergen-specific IgE formation toward 19 different food antigens in the 3 groups of reactive patients. When compared with the values before onset of therapy, the increases of IgE were statistically significant for peanut ($P=0.028$), walnut ($P=0.027$), almond ($P=0.043$), potato ($P=0.003$), tomato ($P=0.027$), celery ($P=0.003$), carrot ($P=0.023$), orange ($P=0.028$), wheat flour ($P=0.002$), and rye flour ($P=0.042$).

Classification of 50 untreated healthy controls into groups of subjects with IgE 1) to inhalative allergens ($n=8$, 16%), 2) to food ($n=5$, 10%), or 3) without preexisting specific IgE ($n=37$, 74%) showed that their sensitization profile was comparable to that of the patients.

Elevation of total IgE in anti-ulcer patients

Before therapy, total IgE levels in control subjects did not differ from those observed in patients ($P=0.142$; median, Q1-Q3: 32, 9-98 kU/l). Three months after the beginning of anti-acid therapy, overall total IgE levels (median, Q1-Q3: 146, 86-320 kU/l) were significantly ($P<0.001$) higher in the patients, when compared with the values before therapy (46, 18-128 kU/l).

Digestion experiments

SGF experiments are a means for predicting the allergenicity of food antigens through their resistance to gastric digestion. When subjecting the 19 regular food antigens included in the study to SGF, potato, celery, carrots, apple, orange, wheat flour, rye flour, codfish, and crab proteins were degraded within 1 min as evaluated by SDS-PAGE, whereas casein, egg white, egg yolk, almond, and tomato proteins resisted enzymatic digestion up to 30 min. The most resistant proteins were found in peanut, walnut, sesame seed, and soy, which were still detectable after 2 h of digestion. That means that potato, celery, carrots, orange, wheat and rye flour, according to the SGF test, would not harbor gastric sensitization properties but still induced de novo IgE in 11.8% of all patients. Cross-reactivity phenomena could be ruled out as these patients did not exhibit concomitant inhalative sensitization.

As a paradigm, in vitro digestion of milk proteins is shown in [Fig. 3A](#). The proteins were mostly degraded within 1 min under physiological conditions (pepsin, pH 2.0). Simulating hypoacidic conditions like during anti-ulcer medication by elevating the pH (3.0 and 5.0) and 1 h incubation in SGF affected the protein patterns only marginally or not at all. Furthermore, the digested milk extract was tested in an IgE immunoblot ([Fig. 3B](#)), demonstrating that after 1 h of regular digestion peptic fragments were left, but with a reduced potential to bind IgE. However, the quality and quantity of IgE binding to samples treated with pepsin at elevated pH were very similar to that of native milk extract.

Anti-ulcer treatment induces long-term sensitization

Five months after discontinuation of anti-ulcer therapy, food-specific IgE could be detected in 6% of the 152 patients' sera, and the IgE levels against potato ($P=0.043$), tomato ($P=0.043$), celery ($P=0.027$), carrot ($P=0.043$), orange ($P=0.046$), and wheat flour ($P=0.043$) were still

significantly elevated. At the same time point (after the pollen season), no statistically significant differences in IgE responses between 0 and 8 months were observed in the control group. Next we calculated the differences between randomly selected patients and controls ($n=20$ each). The patients exhibited significantly elevated specific IgE values ($P<0.05$) for peanut, potato, tomato, celery, carrot, and wheat flour at the secondary time point of follow-up ([Table 2](#)).

Thirty-three patients with elevated food-specific IgE values and 42 controls were also subjected to skin prick tests including 19 food antigens. In the patients 7.02% of the skin tests (44 of 627) were positive. In controls only 1.00% of positive wheal and flare reactions could be elicited (8 of 798 pricks). In the individual patients, the skin reactivity correlated with the sensitization patterns seen with specific IgE in immunoblots, validated by a UniCAP assay where randomly selected sera were tested (data not shown).

The increase of food-specific IgE is paralleled by elevated ST2 levels

To determine whether the production of specific immunoglobulins was accompanied by changes in cytokine levels, we randomly picked 20 treatment patients and 20 controls and measured the concentration of serum IL-4, IFN- γ , and IL-13 at the 8 month time point. No statistically significant differences in cytokine levels were observed between the two groups ([Table 3](#)).

In contrast, major differences between the groups were observed when determining the levels of soluble ST2. As can be seen in [Table 3](#), ST2 levels were significantly higher in the treatment than in the control group ($P=0.016$). To perform a more stringent analysis, Group 3 of control and treatment patients, who had no preexisting allergen-specific IgE before onset of therapy, were compared. Also in this case, ST2 levels were significantly higher in the treatment group ($P=0.013$).

DISCUSSION

In a recently published murine study (21), we reported for the first time that therapy with anti-ulcer drugs correlates with the development of IgE-mediated food hypersensitivity. In the present epidemiological investigation, we provide evidence that also in humans anti-ulcer treatment leads to induction of IgE toward foods. We surveyed 152 gastroenterological patients, who were treated with H2-receptor blockers or PPIs due to dyspeptic complaints. As these drugs are highly effective in suppressing acid secretion (9, 10), our working hypothesis was that reduced acidity might impair peptic digestion, turning essentially harmless, digestion-labile proteins into potent elicitors of sensitization.

The most striking feature in our study was that after 3 months of treatment 25% of the 152 patients revealed changes in the food-specific IgE antibody patterns and 15% de novo IgE synthesis toward foods without concomitant pollen sensitization. In a random sample from the general adult population, 6% had specific IgE to the food allergens tested (27). Therefore, we suggest that the observed increase is most likely associated with impaired digestion during treatment turning digestion-labile proteins into “sensitizing elicitors” (28). The increase was statistically significant when comparing with a nontreated control group, which was followed up to exclude eventual interference and priming effects of the pollen season.

In an in vitro SGF assay, a panel of 19 regular food compounds was tested. As expected, peanut, walnut, sesame seed, and soybean behaved digestion resistant, whereas other antigens like potato, celery, carrots, apple, orange, wheat, and rye flour were easily digested. Such digestion-labile proteins are termed “incomplete” allergens or “nonsensitizing elicitors” and were previously doubted to harbor properties required for oral sensitization (28). Allergic reactions to digestion-labile food compounds often occur in association with inhalant allergies (29), caused by preexisting IgE, which then crossreacts with food antigens (30). Interestingly, our study shows that under anti-acidic therapy, IgE toward digestion-labile food proteins occurs in 11.8% of the cases without concomitant inhalative sensitization to potentially crossreactive allergens. Our experiment using SGF at elevated pH provided an explanation as it demonstrated that peptic digestion of proteins is hindered in times of hypoacidity resulting in conserved IgE binding capacity. Anti-ulcer drugs, which are designed to elevate the gastric pH optimally above 5.0 (9, 10, 12), thus hamper the biological gate-keeping function of the stomach.

The in vivo relevance of the induced IgE antibodies was determined in skin tests at the 8 month time point. Although IgE levels had dropped in the patients after discontinuation of anti-ulcer therapy, and only 6% of the treatment group still had elevated food-specific IgE, the percentage of positive skin reaction was still higher than in the controls. The half-life of IgE and the mast cell survival time is prolonged, when IgE occupies its high affinity receptor (31, 32). We suggest that this kind of “tissue memory” was responsible for the positive skin tests at this late time point in our patients.

As an indirect readout for cellular reactivity, the serum cytokine values were determined. No statistically significant difference in cytokine levels was observed between the treatment group and the controls. In a recent study, it was demonstrated that serum levels of soluble ST2 are markedly elevated in patients with exacerbation of asthma (33). The ST2 (T1, Fit-1, DER4) genes are preferentially expressed on activated Th2 cells, but not Th1 cells, and play an essential role in Th2 effector functions (34, 35). Indeed, highly significant increases of soluble serum ST2 levels were seen in patients, who had developed food-specific IgE during anti-ulcer treatment, but not in the control group. Therefore, we suggest that ST2 is a useful marker for monitoring Th2 immunity in food allergy.

The presented epidemiological data strongly suggest that anti-ulcer treatment may lead to the development of IgE toward dietary compounds that are digested by pepsin at normal gastric pH levels. Strikingly, the generated IgE is not directed toward exotic or novel dietary compounds but can obviously target regular constituents of the daily diet. Therefore, continuous acid-suppression may support sensitization and, in consequence, put patients at risk of developing IgE mediated type I food allergic reactions.

ACKNOWLEDGMENTS

We thank Dr. Edit Minker and Ing. Magdolna Vermes for excellent technical support. This work was supported by grants 10326 of the Austrian National Bank “Jubiläumsfond” and SFB-F018#8 of the Austrian National Science Foundation.

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Received September 28, 2004; accepted November 29, 2004.

Table 1**Characteristics of 40 patients with increases of IgE specific to food proteins during anti-ulcer therapy**

	Characteristics	Number (out of total)	Percentage
Gender	male	14 (out of 58)	24%
	female	26 (out of 94)	28%
Disease	gastritis	11 (out of 38)	29%
	dyspepsia	17 (out of 64)	27%
	ulcer	5 (out of 15)	33%
	reflux	4 (out of 17)	24%
	erosions	3 (out of 3)	100%
Treatment	Omeprazole	2 (out of 17)	12%
	Ranitidine	14 (out of 48)	29%
	Famotidine	24 (out of 82)	29%

Table 2**Influence of anti-ulcer therapy on food-specific IgE production**

Allergen	*<i>P</i> value
Milk	0.306
Casein	0.069
Egg white	0.306
Egg yolk	1.000
Peanut	0.014
Walnut	0.142
Almond	0.142
Potato	0.016
Tomato	0.016
Celery	0.018
Carrot	0.011
Apple	0.142
Orange	0.075
Wheat flour	0.016
Rye flour	0.069
Sesame seed	0.306
Soy beans	0.069
Codfish	1.000
Crab	0.306

Levels of antibodies from before (0 months) to after therapy (8 months) between patients ($n=20$) and untreated controls ($n=20$) were compared. The differences are expressed as **P* value calculated from Mann-Whitney U-test. Bold indicates significant difference ($P<0.05$).

Table 3**Cytokine and ST2 levels in serum of treatment and control group at 8 month time point**

	Median (Q1 – Q3) (pg/ml)		<i>P</i> Values
	Treatment group (<i>n</i> =20)	Control group (<i>n</i> =20)	
IL-4	0 (0-0.7)	0.11 (0-0.23)	0.147
IFN- γ	0.1 (0-2.51)	0.65 (0-1.23)	0.147
IL-13	21.8 (12.84-70.92)	20.2 (10.90-36.59)	0.480
ST2	943.3 (309.9-1331.11)	72.08 (17.94-141.73)	0.016
ST2 (groups 3*, <i>n</i> =12)	949.2 (307.4-1478.19)	69.09 (11.68-113.90)	0.013

*Treatment and control patients without preexisting allergen-specific IgE before onset of therapy.

Fig. 1

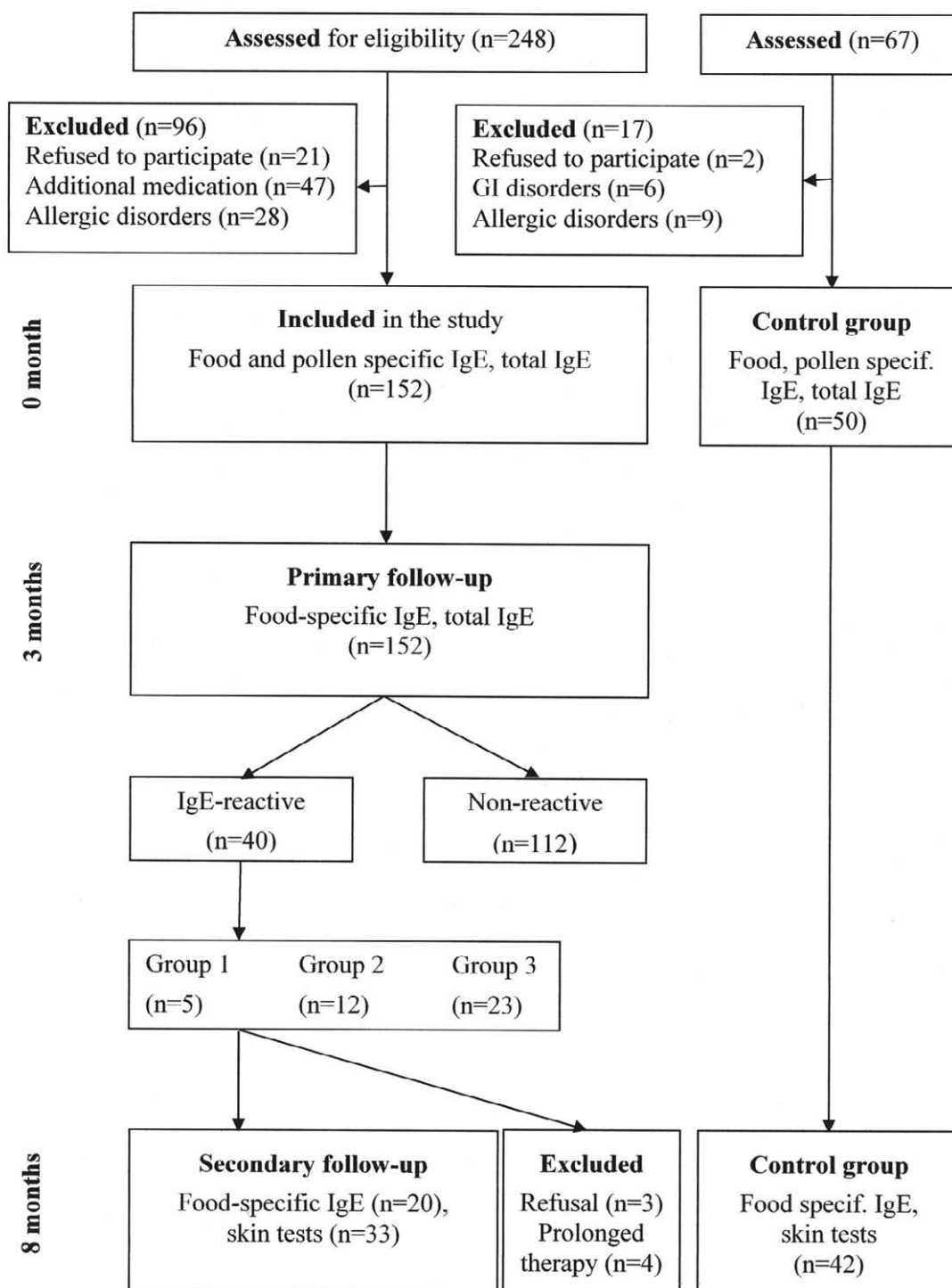


Figure 1. Flow diagram of subject progress through the phases of the study. Before the beginning of anti-ulcer treatment, 248 patients were assessed for eligibility. In the observational study, 152 gastroenterological patients were included, the primary time point of follow-up was defined at 3 months, and a long-term follow-up was performed 8 months after the beginning of therapy. A control group was included to assess any priming effects during the pollen season and evaluated at the 0 and 8 month time point.

Fig. 2

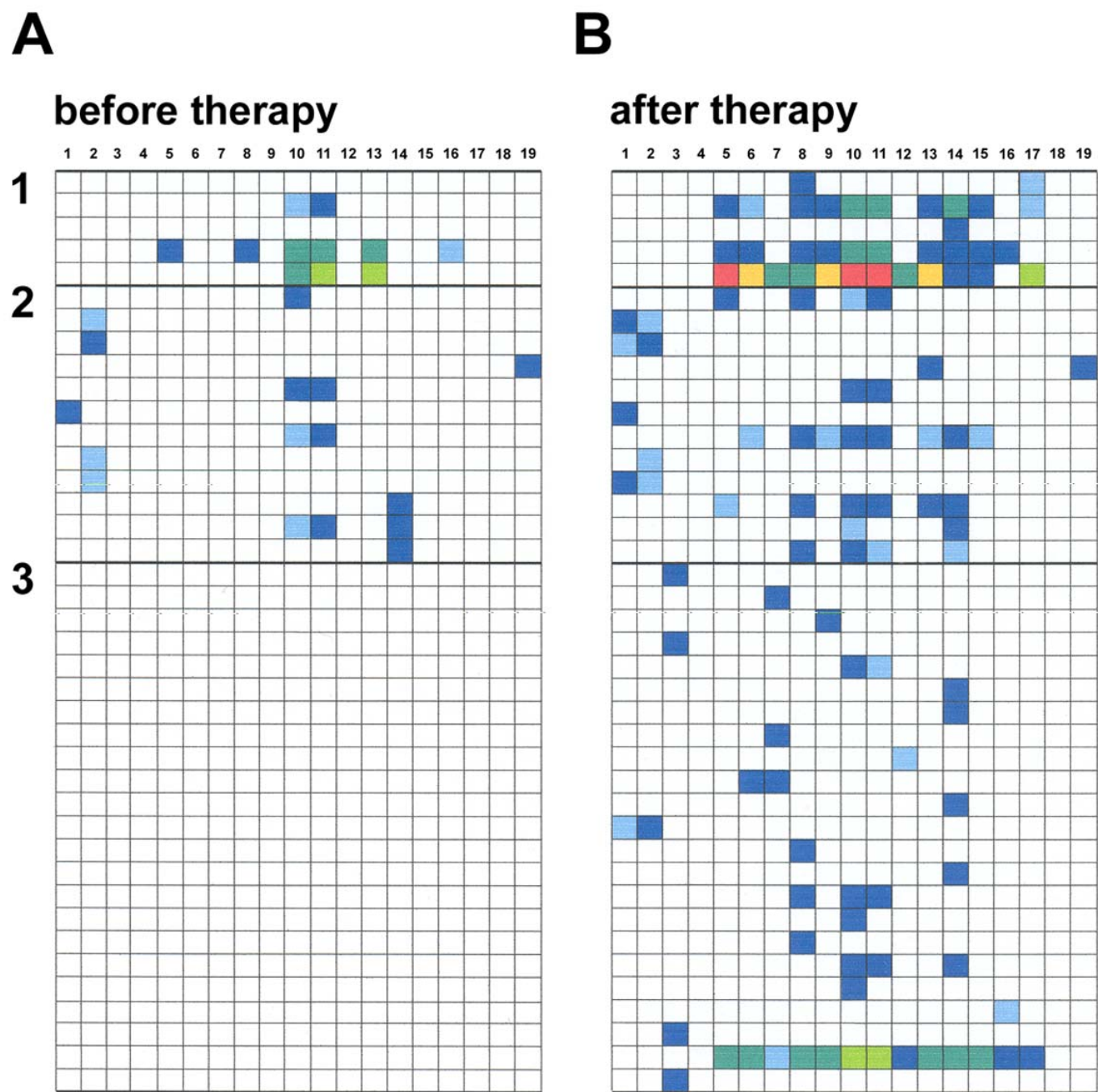


Figure 2. Food-specific IgE antibodies in patients under anti-acid medication. Patients were classified into 3 groups according the results from serological allergy testing before anti-ulcer treatment: Group 1 had preexisting IgE to inhalative as well as food antigens, group 2 had food-specific IgE in their serum, and group 3 showed no preexisting IgE. Specific IgE reactivity toward 19 common food antigens (1: milk, 2: casein, 3: egg white, 4: egg yolk, 5: peanut, 6: walnut, 7: almond, 8: potato, 9: tomato, 10: celery, 11: carrot, 12: apple, 13: orange, 14: wheat flour, 15: rye flour, 16: sesame seed, 17: soy bean, 18: codfish, and 19: crab) was determined in patients treated with H2-receptor blockers or PPIs for 3 months. Antibody levels were given in 6 classes: class 1 (light blue, 0.35-0.75 kU/l), class 2 (dark blue, 0.75-3.5 kU/l), class 3 (dark green, 3.5-17.5 kU/l), class 4 (light green, 17.5-50 kU/l), class 5 (yellow, 50-100 kU/l), and class 6 (red, >100 kU/l). Blood samples were taken before onset of therapy (A) and after intake of the anti-ulcer drugs at 3 month time point (B).

Fig. 3

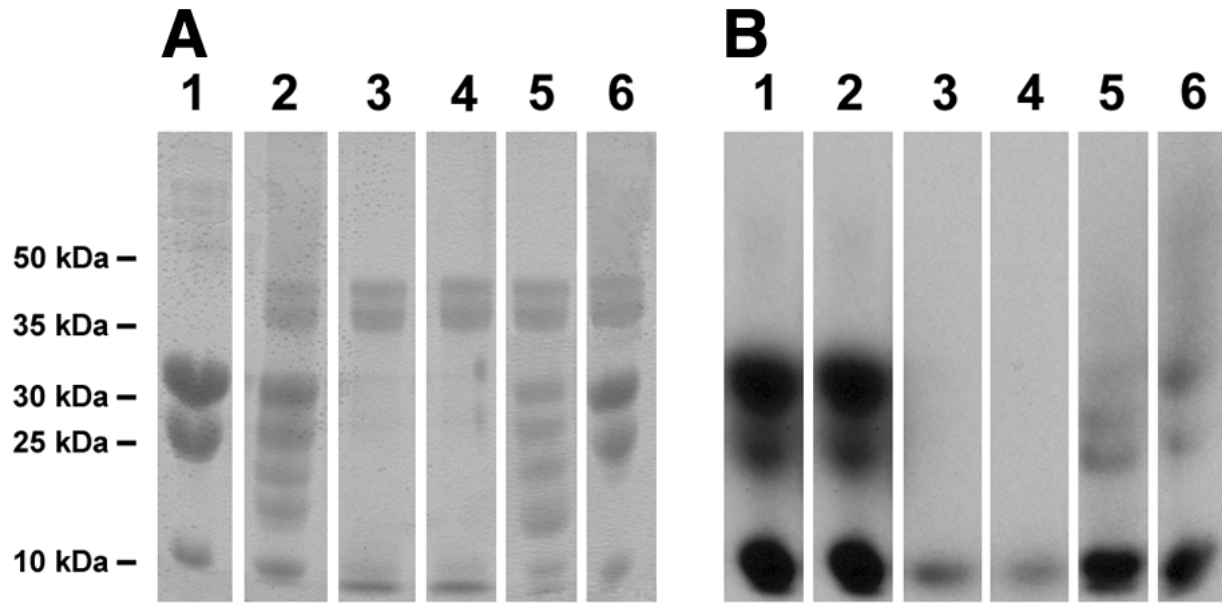


Figure 3. Time and pH dependency of milk protein in vitro digestion. Protein patterns of digested and undigested milk were analyzed by Coomassie stained SDS-PAGE (**A**), and IgE binding capacity was determined by immunoblotting (**B**). Milk extract (lane 1) was subjected to in vitro digestion (pepsin, pH 2.0) for 5 s (lane 2), 1 min (lane 3), and 1 h (lane 4). To simulate gastric hypoacidic conditions, the experiment was also performed with pepsin at pH 3.0 (lane 5) or pH 5.0 (lane 6) for 1 h, respectively. In **A**, lanes 2-5, the pepsin double band can be seen at ~40 kDa.