



Anti-acids lead to immunological and morphological changes in the intestine of BALB/c mice similar to human food allergy

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Abstract

We have shown that anti-acid medication for treating dyspeptic disorders can block protein digestion and induce a higher risk for food sensitization. This mechanism was confirmed in human and animal studies on the humoral as well as the cellular level. Here we aimed to investigate the outcome of the treatment with the anti-acid drug sucralfate on the intestine in our murine model, assuming that morphological and immunological changes will occur. BALB/c mice were fed codfish extract plus sucralfate. Antibodies were examined in ELISA, RBL assay and Western blot. Quantitative morphological analysis of the intestine was performed by design-based stereology, focussing on epithelium, lamina propria, smooth muscle, eosinophils and CD3⁺ cells. Histological analyses were performed after H&E-, PAS- and Congo red-staining, while immune histochemistry was done for detection of CD3⁺ cells. Codfish-specific IgE and its activity in RBL assay confirmed the Th2-response after treatment with sucralfate. The reactivity pattern of murine IgE in Western blot was similar to allergic patients' IgE. Histological examination showed more slender villi in the duodenum, and increased goblet cell mucus in the cecum after sucralfate treatment. Stereological analyses of the intestine revealed higher eosinophil/CD3⁺ ratios, decreased mean thickness of the epithelium of duodenum and cecum, and thinner smooth muscle cell layer in the colon of food allergic mice. Anti-acid treatment with sucralfate induces changes in the structure of epithelium and villi, and an increase in eosinophils and mucus-producing cells in the intestine. Therefore, this medication leads to sensitization against food with changes typical for food allergy also in the intestine.

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Keywords: Anti-acids; Eosinophils; Food allergy; Intestine; Sucralfate

Abbreviations: Alum, aluminum hydroxide; ANOVA, analysis of variance; EDTA, ethylene-diamine tetra-acetic acid; ELISA, enzyme-linked immunosorbent assays; GIT, gastrointestinal tract; H&E, hematoxylin and eosin; HEPES, 4-(2-hydroxyethyl)-1-piperazine-ethane-sulfonic acid; HRP, horseradish peroxidase; O.n, overnight; PAS, periodic acid Schiff; PBS, phosphate-buffered saline; RBL, rat-basophile leukemia cells; RT, room temperature; SURS, systematic uniform random sampling.

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Introduction

The increase of allergies, especially asthma and hay fever, may have reached a plateau lately (Braun-Fahrlander et al., 2004; Grize et al., 2006). Regarding food allergies, an average of 2% of the adult population (Sicherer et al., 2004) and about 6% of the pediatric population are affected (Venter et al., 2006). A novel factor responsible for the induction of food allergy was detected in our working group. We could show that anti-acid drugs inhibit the gastric protein breakdown and increase the risk for sensitization against persisting food proteins (Schöll et al., 2005; Untersmayr et al., 2004, 2003). With the anti-acid drug sucralfate, this effect could be due to the elevation of the gastric pH and the adjuvant activity of the contained aluminum component (Brunner et al., 2007). This Th2-inducing influence of anti-acids was confirmed in human patients (Schöll et al., 2005; Untersmayr et al., 2005) and in murine models (Schöll et al., 2007; Untersmayr et al., 2003). Furthermore, the established allergic milieu of BALB/c mouse mothers fed with anti-acids and fish protein was stable enough to impact a subsequent immune response in their offspring (Schöll et al., 2007). The allergic immune status in these mice was confirmed by high levels of allergen-specific IgE- and IgG1-antibodies, positive skin tests and oral provocation test reactions (Schöll et al., 2005; Untersmayr et al., 2003), as well as increased numbers of mast cells and eosinophils in the gastric mucosa (Untersmayr et al., 2004). A detailed characterization of changes at the presumed induction site of food allergy, the intestine, has not been performed in our mouse studies yet.

Several papers show that food allergy in mice is characterized by villous atrophy and goblet cell hyperplasia, as well as infiltration of IgE-positive mast cells performing degranulation in the jejunum (Nakajima-Adachi et al., 2006). Elevated levels of IL-4 and histamine were also found in the intestinal tissue. Furthermore, a loss of body weight, jejunal edema, eosinophil infiltration, mast cell hyperplasia and increased mucus production were observed in food allergic mice (Saldanha et al., 2004). However, it is important to note that these studies either used transgenic mouse strains (Nakajima-Adachi et al., 2006) or the parenteral route in combination with adjuvants for sensitization (Saldanha et al., 2004). The aim of our current work was to characterize the effect of sucralfate on the intestine in a murine model. Due to the effective Th2-response induced in our earlier murine and human studies, we suggest that sucralfate may induce changes in morphology and immunology. In contrast to previous work, sensitization in our study is achieved by the oral route, comparable to the human situation.

Materials and methods

Preparation of codfish extract

Commercially available frozen codfish was used to prepare an extract as described previously (Untersmayr et al., 2003). The protein content was determined according to the method of Bradford (1976), using a Bio-Rad Protein Assay (Bio-Rad, Munich, Germany).

Protocol of sensitization of mice

Female BALB/c mice (8 weeks) were purchased from Harlan Winkelmann (Borchen, Germany) and treated according to the European Community rules of animal care (Waldegrave, 1986) with the permission of the Austrian Ministry of Science (GZ 66.009/0033-BrGT/2005). Mice were treated intragastrically with PBS (110 µL), or codfish extract (2 mg in 110 µL PBS) plus 2 mg sucralfate (Ulcogant[®], Merck, Vienna, Austria) on day 0, 4, 18, 32, 108 and 116. The control group was immunized on day 0, 14 and 28 intraperitoneally with codfish extract (20 µg) adsorbed to 2 mg aluminum hydroxide (100 µL; Inject[®] Alum, Pierce, Rockford, USA). Blood was drawn before (preimmune serum) and after immunizations.

Colonoscopy and sampling of intestinal tissues for quantitative histology

On day 38, mice were anesthetized with ketamine/xylazine intraperitoneally (Ketaminol[®]/Rompun[®]; 67 mg/kg body weight for ketamine, 5 mg/kg for xylazine) and rectally challenged with 200 µL of codfish extract (20 mg/mL + 50% ethanol 1:1) or PBS (PBS + 50% ethanol 1:1, control group). After 24 h, colonoscopy was performed under anesthesia with a high-resolution mouse video endoscopic system (Becker et al., 2005).

On day 125, 24 h after another rectal allergen challenge, mice were killed and the intestine was prepared into segments of duodenum, jejunum, ileum, cecum, colon and rectum. Each segment was transversely cut into pieces with approximately equal length, opened longitudinally, rinsed with saline, flattened, and fixed with phosphate buffered 4% PFA. A collection of specimen representative of each segment was obtained by systematic uniform random sampling (SURS). To generate vertical sections, the horizontal plane of each flattened specimen was fixed whereas the vertical plane was randomized perpendicular to the horizontal plane using an orientator clock (Baddeley et al., 1986; Mayhew, 1991). A central cut was performed parallel to the randomized vertical plane, running through the center of each specimen. Specimens were embedded into

paraffin with the cut face placed towards the bottom of the embedding mold.

Antibody detection in serum by enzyme-linked immunosorbent assays (ELISA)

Microtiter plates (Maxisorp, Nunc, Roskilde, Denmark) were coated with codfish extract (5 µg per well in 100 µL 50 mM NaHCO₃, pH 9.6). Samples were diluted 1:100 for IgG1, IgG2a, IgG2b and IgG3, or 1:10 for IgE. Further, ELISA procedure was performed as described previously (Schöll et al., 2002).

Beta (β)-hexosaminidase secretion assay from rat-basophile leukemia (RBL-2H3) cells

RBL-2H3 cells were generously provided by Dr. Arnulf Hartl, Institute of Biochemistry, University of Salzburg, Austria. RBL assay was performed as described previously (Schöll et al., 2007), with pooled sera from PBS-, alum- or sucralfate-treated mice (5 µL/well), stimulated with codfish extract (relevant allergen), hazelnut extract (irrelevant control allergen), Tyrode's buffer without allergens (spontaneous release) and Triton-X (Sigma; total release, 100%). No statistical calculation was performed due to the usage of pooled sera.

Specificity analysis of immune response in Western blot

Codfish extract (2 mg/mL) was separated on a 12% SDS gel under reducing conditions and blotted onto a nitrocellulose membrane. Individual strips were incubated with pooled mouse sera (1:20) from groups treated with PBS, codfish + sucralfate or codfish + alum, or with pooled sera from 2 codfish-allergic patients (1:20). IgE was detected with anti-human IgE conjugated to horseradish-peroxidase (HRP, 1:1000; AbD Serotec, Düsseldorf, Germany) or with rat anti-mouse IgE (1:500; BD Pharmingen, Franklin Lakes, USA) followed by anti-rat IgG-HRP (1:1000; GE Healthcare). The reaction was developed with an enhanced chemiluminescence kit (GE Healthcare, Buckinghamshire, UK).

Staining of histological samples of the intestine

H&E staining

Dewaxed, rehydrated paraffin sections were stained with Mayer's hemalaun (Merck, Darmstadt, Germany) for 5 min, rinsed under running tap water for 5 min, followed by staining with alcoholic Eosin (Sigma-Aldrich, Munich, Germany) and glacial acetic acid (Merck) for 30 s.

PAS staining

Dewaxed, rehydrated paraffin sections were stained with 1% periodic acid (Merck) for 10 min, briefly rinsed with tap water, stained with Schiff's reagent (Merck), followed by staining with Mayer's hemalaun for 5 min.

Congo-red staining of eosinophils in intestine

Dewaxed, rehydrated paraffin sections were stained in 0.5% Congo red (Merck) in 50% ethanol for 30–40 min, differentiated in 0.2% KOH in 80% ethanol, rinsed and counter-stained with Mayer's hemalaun for 5 min.

Anti-CD3 immuno staining

Paraffin sections were dewaxed and rehydrated prior to blocking of endogenous peroxidase activity with 1% H₂O₂ in methanol (Roth, Karlsruhe, Germany), pH 7.2, for 30 min. Antigen retrieval was performed by microwave treatment in 3% citrate buffer at pH 6.0 (Roth). After washing in PBS, the sections were incubated for 20 min with normal goat antiserum (1:10; Vector Laboratories, Burlingame, USA) and then for 1 h with a polyclonal goat anti-mouse CD3ε antiserum (1:800; Santa Cruz Biotechnology, Santa Cruz, USA). Thereafter, biotinylated anti-goat secondary antibody (1:100; Vector Laboratories) was applied at RT for 30 min. Binding was visualized with 3,3'-diaminobenzidine (Vectastain Elite ABC Kit, Vector Laboratories) following the manufacturer's instructions. Sections were counterstained with Mayer's hemalaun.

Stereological analysis of intestine

To estimate global shrinkage, the length of each intestinal sample was measured before (L_{before}) and after (L_{after}) tissue processing and embedding. Linear shrinkage (LS) was calculated according to $LS = L_{\text{after}}/L_{\text{before}}$. LS was 87% in samples of PBS-treated vs. 85% in samples of sucralfate-treated animals. There were no significant differences in LS, neither with respect to intestinal region nor with respect to treatment.

Design-based stereology was used to analyze vertical sections of duodenum, cecum, colon and rectum of the PBS- and sucralfate-treated groups by point and intersection counting by means of a computer-assisted stereology tool box (CAST-Grid 2.0, Olympus, Denmark). Briefly, the vertical axis was defined in each section, which was then meander-scanned starting at a random position outside the section and proceeding at a constant step width along the x- and y-axis until the section was completely scanned. At each position, the tissue was superimposed with a test system composed of test points and cycloids. At a final magnification of $\times 852$, all test points (P_{toi}) hitting the tissue of interest (epithelium, lamina propria, lymphoid follicles) were counted as well as all intersections (I_{ep}) of the cycloids with a calibrated length (L_{cy}) and the epithelial basal membrane.

For each tissue of interest the volume (V_{toi}) was normalized to unit epithelial basal membrane area (S_{ep}) according to the formula: $V_{toi}/S_{ep} = L_{cy} \times \sum P_{toi}/(2 \times \sum I_{ep})$.

V_{epi}/S_{ep} corresponds to the arithmetic mean height of the epithelium (τ_{ep}), and V_{lp}/S_{ep} corresponds to the arithmetic mean thickness of the lamina propria (τ_{lp}). For quantification of the arithmetic mean thickness of the smooth muscle layer, the intersections of the cycloids with the apical surface of smooth muscle were additionally recorded to calculate τ_{sm} according to the formula: $\tau_{sm} = L_{cy} \times \sum P_{sm}/(2 \times \sum I_{sm})$.

For quantification of eosinophils and $CD3^+$ lymphocytes at a final magnification of $\times 1700$, Congo-red-stained and anti- $CD3$ -stained vertical sections were used for counting all points hitting eosinophils and $CD3^+$ cells (P_{eo} ; P_{CD3}) and all points hitting lamina propria (P_{lp}). The volume density of eosinophils and $CD3^+$ cells within the lamina propria ($V_{V_{eo,lp}}$; $V_{V_{CD3,lp}}$) was calculated according to the formula $V_{V_{eo,lp}} = \sum P_{eo}/\sum P_{lp}$ and $V_{V_{CD3,lp}} = \sum P_{CD3}/\sum P_{lp}$. The volume of eosinophils and $CD3^+$ lymphocytes per epithelial basal membrane area was calculated according to $V_{eo}/S_{ep} = V_{V_{eo,lp}} \times V_{lp}/S_{ep}$ and $V_{CD3}/S_{ep} = V_{V_{CD3,lp}} \times V_{lp}/S_{ep}$.

Statistical analysis

Quantitative stereological data are given as mean $\pm$ standard deviation (SD). Two-way analysis of variance (ANOVA) was performed for testing significant differences between intestinal regions (factor a) and treatment (factor b) followed by posthoc multiple

comparisons (Holm–Sidak method). All analyses were performed by means of Sigma Stat 3.1 software program (Jandel Scientific, Erkrath, Germany). Antibody levels were compared by Mann–Whitney– U test with SPSS for Windows. p -Values < 0.05 were considered significant.

Results

Sucralfate treatment of BALB/c mice induces allergen-specific antibodies

Female mice were fed intragastrically with PBS or with codfish + sucralfate. Mice treated with sucralfate showed significantly higher levels of codfish-specific IgE, IgG1, IgG2b, IgG3 and IgA compared to the PBS-treated group (Fig. 1). Antibody levels of IgE and IgG1 after sucralfate treatment were not significantly different from the codfish + alum treatment (IgE 0.8–1.0 vs. 0.8–1.1 $\mu\text{g/mL}$, IgG1 3–116 vs. 37–186 $\mu\text{g/mL}$). The biological relevance of codfish-specific IgE was confirmed in an RBL assay, where cells were passively sensitized with serum from mice and stimulation was performed with buffer, codfish or hazelnut. In this setting, β -hexosaminidase was released only after stimulation with codfish extract in the groups treated with sucralfate (23.5% of total release) or alum (30.4%), but not in the PBS group (6.3%). Confirming allergen specificity, no reaction occurred after stimulation with hazelnut extract or buffer (all groups between 5% and 8%).

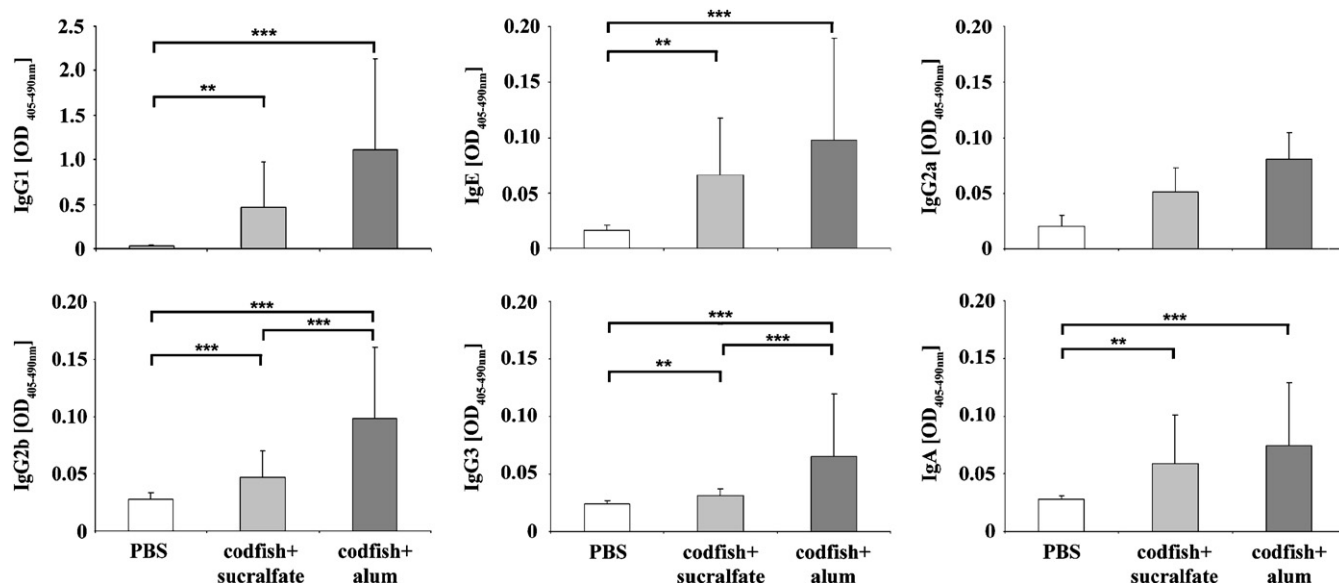


Fig. 1. Gavages of codfish + sucralfate (light gray) to BALB/c mice ($n = 4$ per group) lead to an increase of allergen-specific antibodies. Control groups were fed with PBS (white) or immunized intraperitoneally with codfish + alum (dark gray). Results of sera after the last immunization (day 120) are depicted, preimmune sera gave no signal above background (data not shown). ** p -values < 0.01 , *** p -values < 0.001 .

Sucralfate treatment elicits an IgE-reactivity pattern comparable to human allergic patients

In Western blot, codfish extract was incubated with pooled sera of mice treated with PBS, codfish + sucralfate or codfish + alum, or pooled sera of human codfish allergic patients. Bound IgE revealed that the reactivity pattern of mice was comparable to that of allergic patients (Fig. 2). The main proteins recognized by mice as well as by humans have a molecular weight of 12 and 24 kDa, corresponding to parvalbumin (Gad c 1) and its dimeric form. Other predominant bands were at 17–18 kDa and at around 30–33 kDa. Reactivity to proteins of higher molecular weights was mainly detected by human sera, but not by murine sera.

Colonoscopic and histological examination of intestine

In sensitized mice immunized intraperitoneally with codfish + alum, thickened intestinal walls could be observed in the colon after colonoscopy (data not shown). However, in the mice fed with codfish + sucralfate, no difference to the PBS group was found. Intestine samples of these groups were therefore subjected to further histological investigations.

In pre-screenings of Congo red-stained histology samples of different intestinal regions, a substantial

number of eosinophils were observed in duodenal, cecal, colonic and rectal parts (data not shown). Therefore, only these parts were further subjected to H&E-, PAS- and anti-CD3 immunostaining and subsequent stereological analysis.

In H&E-stained sections villi were thinner in the duodenum of codfish + sucralfate-treated mice (Fig. 3A vs. E). This was confirmed by stereological analysis, which revealed a significantly reduced epithelial volume in the duodenum as well as in the cecum (Table 1). An increase in stored mucus was seen in the cecum of these mice compared to the sham-treated group (Fig. 3B vs. F). Mucus occupied $29.9 \pm 10.5\%$ of the epithelial volume of crypts in codfish + sucralfate-treated mice, which was significantly different ($p = 0.014$) from control mice with only $14.7 \pm 3.7\%$. No significant differences with respect to the epithelium were found in colon or rectum specimens (Fig. 3C, D vs. G, H).

Immunostaining for CD3 revealed slightly decreased CD3⁺ lymphocytic infiltration of the lamina propria of duodenum, cecum and rectum in the sucralfate-treated group vs. the PBS-treated group (Fig. 4A, B vs. E, F). Although this was confirmed by stereology, the differences in the quantitative data were not significant (not shown). No differences were seen in the colon (Fig. 4C vs. G).

Irrespective of treatment, Congo red-stained eosinophils were more frequent in lamina propria of the rectum compared to the duodenum (Fig. 4D vs. H). Although the stereological measurements were in line with the qualitative findings, the differences failed to reach significance due to the large interindividual variability. Remarkably, in the sucralfate-treated group the volume density of eosinophils in the lamina propria of the duodenum was 0.51% compared to only 0.21% in the control group ($p = 0.029$ when tested by Mann–Whitney rank sum test). As a consequence, the eosinophil/CD3⁺ lymphocyte ratio was significantly higher in the duodenum of allergic mice (Table 2).

Differences in lamina propria thickness and volume of lymph follicles were not significant, neither within regions nor between treatment groups (data not shown). The arithmetic mean thickness of the smooth muscle cell layer was significantly lower in the colon of sucralfate-treated compared to PBS-treated mice (Table 3).

Discussion

In earlier studies, we could show that anti-acids induce food allergy and a Th2-bias at the humoral and cellular level. To characterize the effect of these drugs on the presumed induction and effector site of food allergy, we investigated the intestine in the present study.

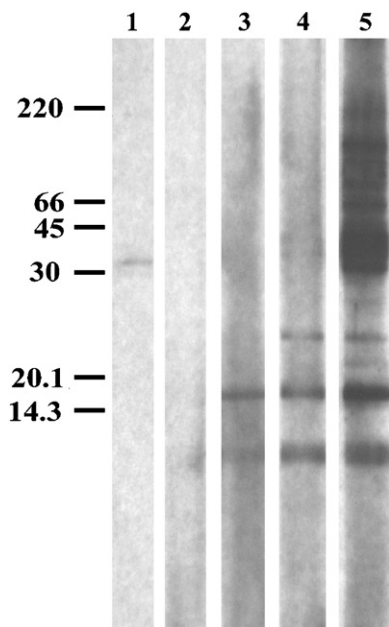


Fig. 2. Reactivity pattern of mouse IgE is similar to human IgE with main reactivity to parvalbumin at 12 kDa. Blotted codfish extract was incubated with buffer (1), pooled serum of PBS-treated mice (2), sera of codfish + sucralfate-treated mice (3), sera of codfish + alum-treated mice (4) or sera of codfish allergic patients (5).

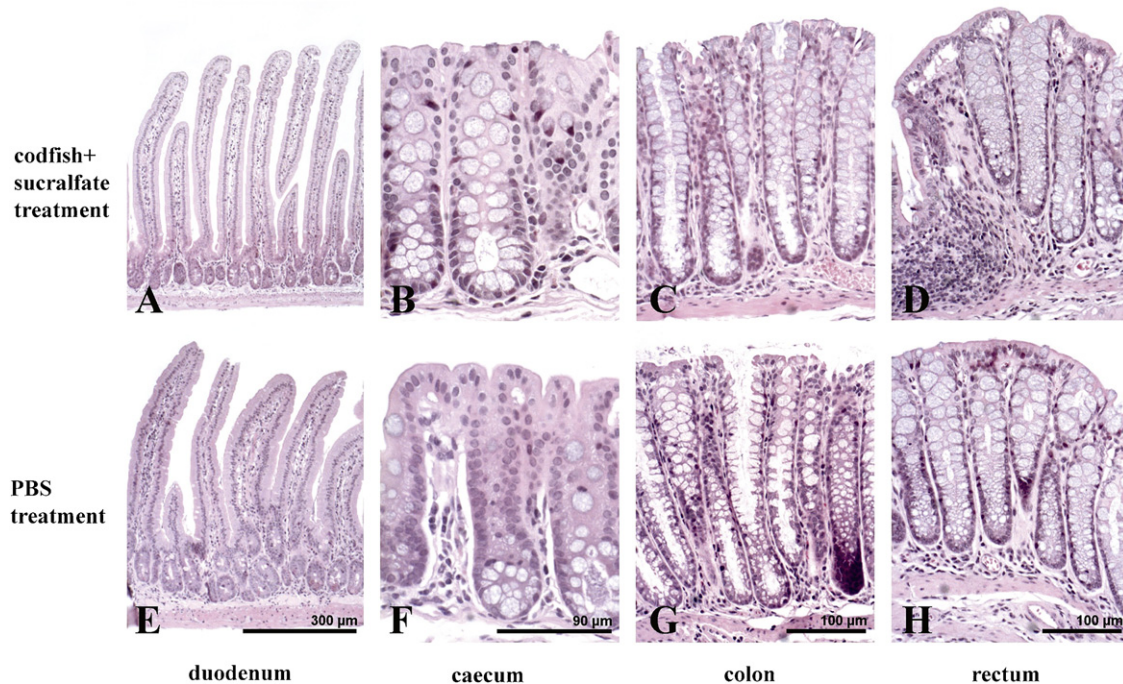


Fig. 3. Representative photomicrographs of H&E-stained intestinal regions from mice treated with codfish + sucralfate (A–D) or PBS (E–H). Villi of duodenum (A, E) are more slender and epithelium is thinner after sucralfate treatment. The cecum (B, F) exhibits an increase in mucin stores in sucralfate-treated mice. There are no obvious alterations of the colon (C, G) or rectum (D, H).

Table 1. Epithelial volume per epithelial basal membrane area [$\mu\text{m}^3/\mu\text{m}^2$]

Intestinal region	Treatment		Results of posthoc test ^a	
	PBS	Codfish + sucralfate	Treatment within region	Regions
Duodenum	21.7 ± 3.1	18.2 ± 1.4	Yes	vs. colon and vs. rectum
Cecum	21.7 ± 3.2	17.5 ± 3.6	Yes	vs. colon and vs. rectum
Colon	16.8 ± 0.5	16.6 ± 1.0	No	vs. duodenum and vs. caecum
Rectum	16.5 ± 0.7	15.3 ± 1.3	No	vs. duodenum and vs. caecum

Means ± SD.

Results of ANOVA: differences between treatments, $p = 0.007$; differences between intestinal regions, $p = 0.001$; interaction between treatment and region, $p = 0.234$.

^aResults of posthoc pairwise multiple comparisons (Holm–Sidak method) if differences between groups were significant at $p < 0.05$ tested by 2-way ANOVA; Yes indicates $p < 0.05$; No indicates $p > 0.05$.

Using anti-acid treatment with sucralfate and codfish as model allergens, we were able to induce significantly elevated allergen-specific IgE and IgG1 in BALB/c mice. This humoral immune response confirmed again the ability of anti-acid drugs to induce sensitization against food proteins. Other antibody isotypes were also significantly elevated in sucralfate-treated animals in comparison with the sham-treated control group, and were comparable to the positive control group immunized intraperitoneally with codfish plus alum. This revealed that the potency of sucralfate to induce an immune response as high as that of an established Th2-adjuvant.

The biological relevance of the induced codfish-specific IgE was proven in an RBL assay, where

sera of sucralfate-treated mice showed a release of β -hexosaminidase as high as the positive control group. The same assay confirmed the specificity of the immune response, as no reaction to the control allergen hazelnut was observed.

The reactivity patterns of murine compared to human patients' sera were very similar, with main IgE binding to proteins at 12, 17–18, 24 and around 30–33 kDa. These proteins were already described by other groups to react with human patients' sera (Sten et al., 2004; Van Do et al., 2005). The relevance of our intragastric immunization scheme for comparison with human allergy is further confirmed by this result.

To examine the gastrointestinal reactions, mice were rectally challenged with codfish extract or PBS after four

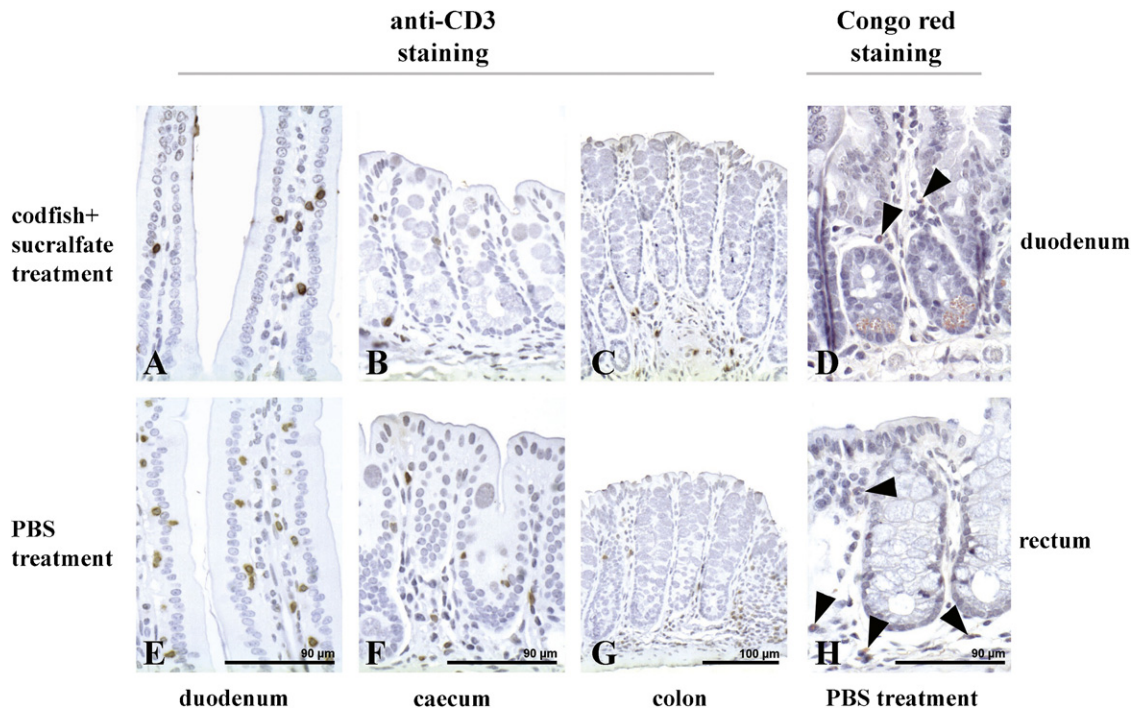


Fig. 4. Anti-CD3- (A–C, E–G) and Congo red- (D, H) stained sections of intestine from mice treated with codfish + sucralfate (A–C) or PBS (D–H). The duodenum (A, E) shows fewer CD3⁺ lymphocytes (brown labeling) in the epithelium and lamina propria after sucralfate treatment (A) compared to PBS treatment (E). Few CD3⁺ cells are seen in the cecum (B, F) and colon (C, G). Congo red staining revealed more eosinophils (arrowheads) in the rectum (H) than in the duodenum (D).

oral immunizations, and colonoscopic investigations were performed with a high-resolution mouse endoscopic system. In these experiments we were not able to reveal a macroscopic difference in groups fed with codfish + sucralfate or PBS; however, we found thickened intestinal walls in the colon of mice treated with codfish + alum intraperitoneally.

To examine possible differences at the microscopic level, investigations were performed via stereological measurements in groups treated with PBS or codfish + sucralfate. The most impressive result is the significantly lower arithmetic mean thickness of the epithelium in duodenum and cecum after sucralfate treatment. Confirming this observation the H&E staining showed partial loss of villous architecture in these animals, again in line with other mouse food allergy studies (Nakajima-Adachi et al., 2006). We assume that this might be due to hypotrophic rather than degenerative changes in epithelial cells; the latter is found in human cow's milk allergic children (Arvola et al., 2006). The events observed in children further resulted in intestinal villous atrophy, which was closely correlated with IgE-mediated and non-IgE-mediated food allergy in children (Kokkonen et al., 2004; Kokkonen and Karttunen, 2002).

We could further show that the mucus stores were increased in the cecum in the food-sensitized animals after sucralfate treatment, with a statistically significant

elevation in the crypts of the epithelium. Goblet cell hyperplasia with an increase in stored mucus is a well-known feature of respiratory allergic diseases (Prescott, 2006). However, to our knowledge it has not yet been shown in human patients with food allergy. In contrast, the participation of the cecum in food allergy with the higher expression of germ-line Fc-epsilon and higher IL-4 mRNA in human food allergy is well known (Coeffier et al., 2005).

In human food allergic patients it has also been shown that eosinophilic granulocytes play a critical role during an inflammatory response (Croese et al., 2003; Murch, 2005; Rothenberg et al., 2001). In our model, a trend towards higher eosinophilic cell mass infiltrating the duodenum was seen in the sucralfate-treated group, and a statistically significant increase in the density of eosinophils per lamina propria volume unit was found, as well as an elevation in the ratio of eosinophils/CD3⁺ cells. It has already been shown in intestine samples of humans that CD3⁺ cells may not differ between healthy and food allergic patients (Veres et al., 2001, 2002). Therefore, we assume that the ratio of eosinophils/lymphocytes may be an appropriate measure for differences in inflammatory cell infiltration. The increased number of eosinophils in the lower intestine vs. the upper part (Fig. 4H vs. D), also in healthy individuals, was already described by DeBrosse et al. (2006).

Table 2. Ratio of eosinophilic granulocytes to interstitial CD3⁺ lymphocytes

Intestinal region	Treatment		Results of posthoc test ^a	
	PBS	Codfish + sucralfate	Treatment within region	Regions
Duodenum	0.13 ± 0.07	0.50 ± 0.25	Yes	vs. all others
Cecum	0.69 ± 0.49	0.82 ± 0.29	No	vs. duodenum
Colon	0.79 ± 0.44	1.19 ± 0.48	No	vs. duodenum
Rectum	0.98 ± 0.60	1.23 ± 0.53	No	vs. duodenum

Means ± SD.

Results of ANOVA: differences between treatments, $p = 0.011$; differences between intestinal regions, $p < 0.001$; interaction between treatment and region, $p = 0.020$.

^aResults of posthoc pairwise multiple comparisons (Holm–Sidak method) if differences between groups were significant at $p < 0.05$ tested by 2-way ANOVA; Yes indicates $p < 0.05$; No indicates $p > 0.05$.

Table 3. Arithmetic mean thickness of smooth muscle cell layer (μm)

Intestinal region	Treatment		Results of posthoc test ^a	
	PBS	Codfish + sucralfate	Treatment within region	Regions
Duodenum	52 ± 017	40 ± 02	No	vs. colon
Cecum	73 ± 014	81 ± 06	No	n.s.
Colon	183 ± 105	93 ± 26	Yes	vs. duodenum
Rectum	135 ± 072	77 ± 05	No	n.s.

Means ± SD.

Results of ANOVA: differences between treatments, $p = 0.032$; differences between intestinal regions, $p = 0.004$; interaction between treatment and region, $p = 0.168$.

^aResults of posthoc pairwise multiple comparisons (Holm–Sidak method) if differences between groups were significant at $p < 0.05$ tested by 2-way ANOVA; Yes indicates $p < 0.05$; No and n.s. indicate $p > 0.05$.

A contrasting result to the existing literature is the decreased thickness of the smooth muscle layer in the duodenum and colon of our allergic mice. The previous studies have shown that inflammation, e.g. with nematodes, induced hypercontractility and hyperplasia of smooth muscle cells in the jejunum of mice and rats (Akiho et al., 2002; Blennerhassett et al., 1999). However, this work was performed on isolated muscle cells ex vivo. Furthermore, these changes were observed after a period of 6 days post-infection. When examining the mRNA level for gamma-smooth muscle, an increase was seen after 6 days, but importantly was reversed after 35 days. The observed differences in our model may therefore be due to the long observation period. To the best of our knowledge, there is no existing literature covering the topic of smooth muscle cell layer thickness in the intestine of food allergic mice or humans. It only was shown that chronic inflammation of airways leads to smooth muscle hypertrophy and hyperplasia (Vieira et al., 2007).

We conclude from our data that anti-acid medication can induce a substantial sensitization against food with the involvement of the whole intestine in the allergic events. These results strengthen our previous observations and confirm that these drugs should be taken with caution and only after doctoral prescription.

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