

Food Intolerance of Unknown Origin: Caused by Mucosal Inflammation? A Pilot Study

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INTRODUCTION: The prevalence of patients with food intolerance (FI) has increased significantly. Immunoglobulin (Ig)E-mediated food allergies (FAs) are detected by determining IgE antibodies and skin prick test. Carbohydrate malabsorptions are clarified with breath tests. However, these diagnostic measures cannot capture all intolerances and have limitations in case of gut-mediated FI. The aims of this pilot study were to evaluate different methods to determine intestinal mucosal IgE in patients with FA and to characterize the intestinal mucosa in patients with FI of unknown origin (FH).

METHODS: Patients with FA and FH were compared with healthy controls. To determine the IgE antibodies and the cytokines tumor necrosis factor (TNF)- α and interferon (IFN)- γ of the intestinal mucosal, a lavage was performed as part of an ileocolonoscopy and samples were taken using the cytobrush and biopsy forceps. In a subgroup, mucosal samples were also taken from the duodenum.

RESULTS: Data in homogenates of intestinal mucosal samples yielded the highest sensitivity for IgE antibody titers compared with lavage and cytobrush. Patients with FA presented increased intestinal TNF- α and low IFN- γ values. This was in contrast to FH patients, who showed low intestinal IgE antibodies and TNF- α levels, but increased IFN- γ values.

DISCUSSION: The determination of IgE antibodies to diagnose intestinal IgE-mediated FA is most reliable in intestinal mucosal samples. Increased TNF- α and low IFN- γ levels in patients with FA characterize an allergic reaction. Decreased TNF- α and increased IFN- γ levels in patients with FH indicate an inflammation-related intolerance reaction (see Visual Abstract, Supplementary Digital Content 1, <http://links.lww.com/CTG/A520>).

SUPPLEMENTARY MATERIAL accompanies this paper at <http://links.lww.com/CTG/A520>

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INTRODUCTION

The prevalence of food intolerances (FIs) has been increasing significantly in the past few years. Patients describe gastrointestinal and extraintestinal complaints that considerably reduce the patients' quality of life. Up to 20% of the Western population believe to have a food allergy (FA) (1). In reality, only 4%–5% adult patients suffer from FA (2,3). FIs not mediated immunologically, e.g., carbohydrate malabsorption, are notably more frequent (15%–50% of population) (1). Serological immunoglobulin (Ig)E determinations and prick testing are the standard tests to detect IgE-mediated FA and cross-reactions in pollen allergies. However, the sensitivity of these tests is only between 60% and 80% (4). The gold standard to prove FA is a double-blind provocation (5). As this, however, implies a lot of time and organization and is very expensive, this provocation is rarely

performed. In addition, it is not possible to differentiate between an intolerance caused by an IgE-based allergic reaction or a nonimmunological reaction, e.g., due to biogenic amines.

In particular, FIs that affect primarily the gastrointestinal tract often cannot be reliably detected with the previously established diagnostic tools, e.g., skin reactions, so that diagnostic techniques for clarifying FIs focus increasingly on the gastrointestinal tract (6).

Within the past few years, several working groups examined biomarkers and diagnostic procedures to detect an FA in the gut that is independent of systemic IgE-triggered allergic mechanisms. It is suggested that mast cells, eosinophils, and antigen-presenting cells are involved in FA-mediated enteropathy by releasing IgE, leukotrienes, prostaglandins, cytokines, or activating inflammatory responses (7–9). In this context, raised cytokine reactions in the nasal lavage (10) and further allergic

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mediators in the intestinal lavage (11,12) have been suggested as possible diagnostic tool to detect patients with FA. In the clinical routine, however, the use of these procedures is limited because of their complexity.

However, in addition to immunological and nonimmunological FIs, there are FIs of unclear origin. There are hints that a disorder of the intestinal barrier may secondarily lead to FIs, which cannot be diagnosed reliably as diagnostic measures are limited (13).

In this study, we examined and compared the intestinal mucosa of patients with IgE-induced FA, patients with FIs of unknown origin (FH), and healthy controls (HC). Our aims were to detect surrogate parameters in patients' intestinal mucosa to better differentiate FH from FA patients and to find suitable and practical diagnostic tool to distinguish between both patient groups.

METHODS

Patient recruitment

Patients were recruited in our outpatients' department for FI between 2016 and 2018. As part of the medical examinations, we performed an esophagogastroduodenoscopy (EGD) and an ileocolonoscopy to exclude celiac disease, inflammatory bowel diseases, esophagitis, or gastritis. Furthermore, patients with even minor mucosal inflammation, e.g., elevated intraepithelial lymphocytes, or patients with intolerances to carbohydrates were excluded from the study. Individuals without gastrointestinal symptoms who presented for a preventive ileocolonoscopy were included as HC. The EGD was also a part of the preventive medical check-up, e.g., in case of family cancer history.

The study was conducted according to the guidelines of the Declaration of Helsinki, registered with ClinicalTrials.gov (NCT 03151252), and approved by the ethics committee of Friedrich-Alexander-Universität Erlangen-Nürnberg (259_14B); informed written consent was obtained from all patients.

Patients were categorized into 3 groups: (i) proven IgE-dependent FA, confirmed by serum IgE and skin prick tests ($n = 10$); (ii) FH and seronegative IgE status combined with a normal skin prick test ($n = 5$); and (iii) healthy subjects without any gastrointestinal symptoms and without the presence of an FA ($n = 6$).

Sample-taking/specimen collection

To differentiate the genesis of FI, we performed an endoscopically controlled lavage and also took samples by cytobrush and biopsy forceps during ileocolonoscopy. In these samples, the total IgEs were measured and specific IgEs were determined against egg, casein, lactalbumin, lupin seeds, wheat flour, rGly m4 from soy, celery, pork, beef, and nut mixture. In addition, total protein and the cytokines tumor necrosis factor (TNF)- α and interferon (IFN)- γ were determined.

Lavage and mucosal tissue sampling were performed in the ileum, cecum, and rectosigmoid colon. Mucosal samples were taken with a cytobrush (MTW Endoskopie Manufaktur, Wesel, Germany) and biopsy forceps (Boston Scientific, Natick, MA). In patients with indication for an EGD, further mucosal samples were collected from duodenum.

First, the cytobrush was introduced through the channel of the endoscope. The mucosa was brushed 10 times in the ileum, cecum, and rectosigmoid colon, respectively. After retraction, the brush was stripped in 1.5-mL phosphate-buffered saline with

protease inhibitors and samples were immediately cooled down on ice to 4°C.

Subsequently, a lavage was performed through the endoscope and a catheter, and 50 mL of a 0.9% physiological saline solution was applied to the corresponding part of the intestine (ileum, cecum, and rectosigmoid area). After 1-minute exposure time, the fluid was aspirated and collected in a special tube containing a protease inhibitor cocktail (aprotinin, Pefabloc, and EDTA) and put on ice.

Finally, tissue samples were taken through biopsy forceps from the respective intestinal segment. Biopsies were frozen immediately in cryo tubes in liquid nitrogen and stored at -150°C . Tissue samples were also taken from the duodenum in patients and controls who underwent an EGD.

Processing of samples

Lavage samples. The lavage was processed as described in Schwab et al. (14). In brief, the lavage fluid was centrifuged at 4,000 rpm at 4°C for 15 minutes to remove rough particles. Afterward, the supernatant was filtered (Whatman, FP 30 CN, 5 μm). Approximately 15 mL of the filtered solution was transferred to Vivaspin 50,000 centrifuge vials and further centrifuged at 400 rpm at 4°C until its volume was reduced at least to 1/10 (≤ 1.5 mL) of the starting volume. This concentrated fluid was used to measure IgE, TNF- α , and IFN- γ .

Cytobrush samples. Samples collected with cytobrush were homogenized for 20 seconds at 4°C with Ultra-Turrax. The homogenate was centrifuged at 4,000 rpm at 4°C for 15 minutes. The supernatant was used to determine IgE, TNF- α , and IFN- γ .

Biopsy samples. The frozen tissue samples were transferred into 1.5-mL phosphate-buffered saline containing protease inhibitors (aprotinin, Pefabloc, and EDTA) and were homogenized for 20 seconds at 4°C with Ultra-Turrax at full speed. The samples were processed as described for the cytobrush samples.

Measurement of IgE, TNF- α , and IFN- γ

In all samples, the IgE antibodies were measured with ImmunoCap Assays (Phadia 250 Instrument; ThermoFisher Scientific, Waltham, MA). The total amount of protein was calculated with the BC Assays (Interchim, Montluçon, France).

TNF- α was determined with IDKTNF α ELISA (Immundiagnostik AG, Bensheim, Germany), according to manufacturer's instructions. In brief, microtiter wells were precoated with monoclonal antibodies against TNF- α by the manufacturer. The frozen supernatants derived from lavage, cytobrush, or homogenized biopsy samples were thawed, and 100 μL of undiluted supernatants or standard samples were transferred in duplicates to the wells and incubated for 2 hours at room temperature. Bound antibodies against TNF- α were subsequently detected according to the manufacturer's specifications. We measured the optical density with a BioRad iMark Microplate Reader and used a 4-parameter algorithm for analysis.

IFN- γ was determined with the DUOSet ELISA development system Human IFN- γ (R&D Systems, Minneapolis, MN) according to manufacturer's instructions. In brief, each well of a 96-well plate was coated with 100- μL capture antibody solution overnight at room temperature and followed by 2 wash steps with wash buffer. Afterward, the wells were blocked with 300- μL reagent diluent at room temperature for 1 hour and washed with

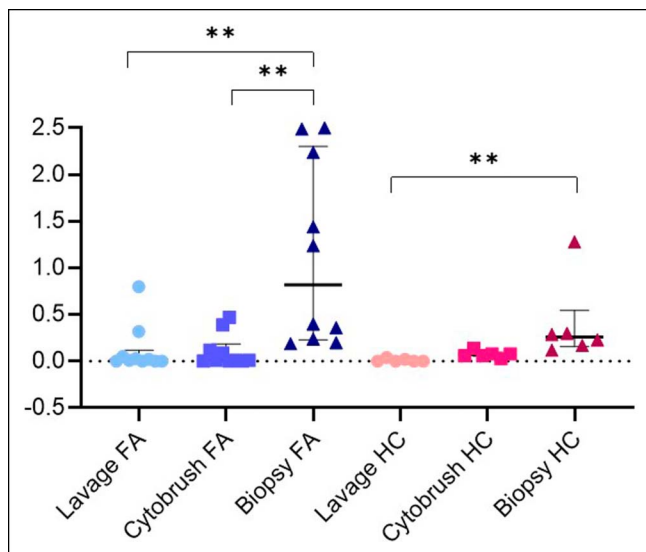


Figure 1. Total immunoglobulin (IgE) concentrations in lavage, cytobrush, and mucosal samples from the terminal ileum of patients with food allergy (FA; $n = 10$) and healthy controls (HC; $n = 6$). Medians with interquartile range are shown. In patients with FA, the IgE concentrations were significantly higher in biopsy samples compared with extracts from lavage or cytobrush of the same patients. The Friedman test was applied and followed by the Dunn's multiple comparisons test to calculate differences between the varying isolation methods. Significant differences are shown. $**P < 0.01$.

wash buffer twice. Frozen supernatants of lavage, cytobrush, or biopsy samples were thawed, centrifuged by 17,000g for 10 minutes at 4°C, and 100 μ L of the undiluted supernatants was transferred in duplicates to the coated wells and incubated for 2 hours at room temperature. Bound antibodies against IFN- γ were detected according to manufacturer's protocol. The optical densities were measured at 450 nm with BioRad iMark Microplate Reader. A standard curve was created with standard samples provided by the test kit, and the IFN- γ concentrations of biopsy and lavage samples were determined by point-to-point calculation.

Statistics

The demographical data are summarized as mean values \pm SDs. Interpretation and graphics were performed with GraphPad Prism 8.3.0. Differences between isolation methods or localizations were determined by the Friedman test and followed by the Dunn's multiple comparisons test. Differences between patient groups were analyzed by the Kruskal-Wallis test and followed by the Dunn's multiple comparisons test if all pairwise comparisons were of interest. The Mann-Whitney test was used if only selected pairwise comparisons were of interest. All tests were 2-sided with a significance level of 0.05.

RESULTS

Demographical data

Overall, we included 5 men and 16 women in our study. The age of the patients and controls ranged between 21 and 77 years with mean age of 48 ± 15 years. Ten patients were included who suffered from FA (all women, aged 46 ± 13 years), 5 patients had

FH (2 men, 3 women, aged 35 ± 9 years), and 6 HC (3 men, 3 women, aged 64 ± 7 years) were included.

Evaluation of diagnostic methods to detect IgE antibodies

To capture the most sensitive diagnostic method for the analysis of mucosal IgE, the measurement in the lavage, cytobrush, and intestinal mucosal samples derived from the terminal ileum was performed in patients with FA and compared with data from HC.

The sensitivity was 10-fold higher when analyzing IgE from samples taken with the cytobrush compared with the concentrated lavage solutions. However, comparing the 3 methods, the highest IgE values were found in the homogenates of intestinal mucosal samples taken through biopsy forceps.

Patients with FA showed significantly higher IgE concentrations in the mucosal samples that were taken with biopsy forceps compared with lavage and cytobrush samples. The same results could be detected in HC, but with lower IgE levels that have no clinical relevance. Comparing the IgE levels of patients with FA with HC, we observed that patients with FA have higher levels in all 3 methods. However, these values did not reach significance probably because of the small sample numbers (Figure 1).

When measuring the mucosal IgE levels against specific food allergens, we found no considerable differences within the 3 sample taking procedures. Figure 2 shows exemplary the specific IgE concentrations against wheat flour. Again, we found the highest specific IgE values in the intestinal biopsies, although all levels were below clinically relevant limits (Figure 2).

Evaluation of different intestinal locations to detect IgE antibodies

As the homogenates of mucosal samples yielded the most sensitive results to detect local IgE, all further determinations were performed using this method.

Next, we examined the most suitable intestinal area to analyze the IgE levels. Biopsies were taken from the duodenum, terminal ileum, cecum, and sigmoid colon. In general, sensitivity to detect total IgE was highest in duodenal tissue followed by the terminal

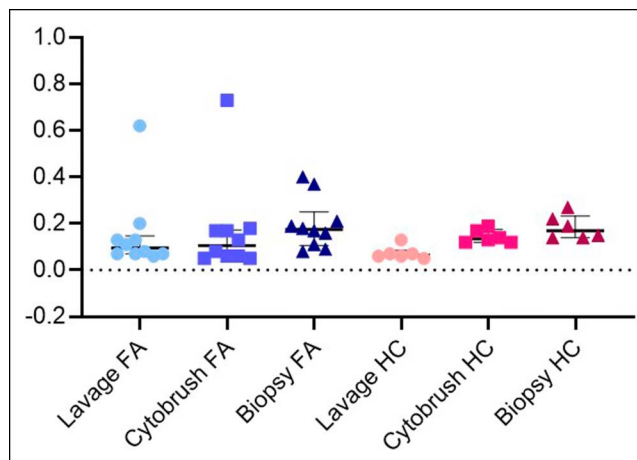


Figure 2. Determination of wheat-specific immunoglobulin (IgE) concentrations in lavage, cytobrush, or mucosal biopsies of patients with food allergy (FA; $n = 10$) and healthy controls (HC; $n = 6$). Medians with interquartile range are shown. The Friedman test showed no significant differences in wheat specific IgE between isolation procedures. Specific IgE against wheat allergen is below clinical relevant limit in both groups.

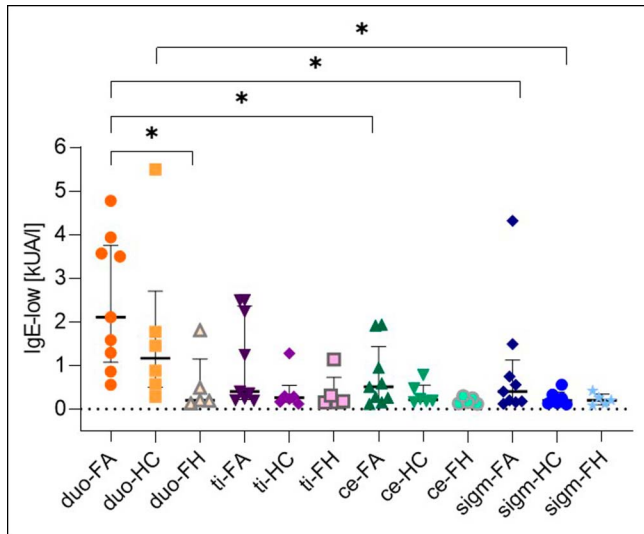


Figure 3. Total immunoglobulin (IgE) antibodies in homogenates of mucosal samples derived from the duodenum (duo), terminal ileum (ti), cecum (ce), and sigmoidal region (sigm) of patients with food allergy (FA; $n = 9$), healthy controls (HC; $n = 6$), and patients with food intolerance of unknown origin (FH; $n = 5$). Shown are medians with interquartile range. The Friedman test was followed by the Dunn's multiple comparisons test and used to study differences between localizations within each patient group. The Kruskal-Wallis test followed by the Dunn's multiple comparisons test was used to analyze differences between all groups. Significant differences were shown between duodenal, cecal, and sigmoidal samples in patients with FA ($P < 0.05$). Significant differences were also noted between duodenal and sigmoidal samples in HC and between duodenal samples derived from patients with FA and FH. * $P < 0.05$.

ileum. Patients with FA showed highest IgE levels in homogenates of all intestinal locations. HC showed distinct increased IgE titers in mucosal homogenates of all 4 locations, whereas patients with FH displayed only very low baseline values. The significantly highest IgE levels were detected in duodenal mucosal samples from patients with FA and even in HC (Figure 3).

Cytokine determination in intestinal biopsy samples

We compared the concentrations of TNF- α and IFN- γ in the homogenates of mucosal samples from different localizations between patients with FA, FH, and HC.

Highest TNF- α concentrations were found in mucosal samples from the terminal ileum, and the cecum of patients with FA, and TNF- α levels were significantly higher in these samples compared with HC.

Patients with FH showed the lowest TNF- α parameters, with significantly lower concentrations compared with FA and also significantly lower compared with HC in the terminal ileum, cecum, and rectosigmoid area. TNF- α determination in duodenal mucosal samples also showed slightly increased concentrations in patients with FA, although without statistical significance between the patient groups (Figure 4).

IFN- γ concentrations were in clear contrast to TNF- α parameters. FA had significantly lower IFN- γ concentrations as compared to patients with FH in all examined intestinal areas. In general, patients with FH showed highest levels of IFN- γ in all

intestinal locations, although statistically significant differences compared with HC were only proven for duodenal derived samples (Figure 5).

DISCUSSION

The increasing prevalence of FIs has led to a growing scientific interest toward the underlying pathomechanisms (1,5,15,16). Since diagnostic methods are very limited for patients with predominantly gastrointestinal symptoms, several attempts have been made to detect a locally mediated FA and intolerance by nasal (17–19) or intestinal lavage (12), Microarray-RNA technique (20), IgE-detection in immunohistochemistry (3,7,21), or measurement of TH2-mediated cytokines (22).

The importance of detecting local mucosal IgE antibodies has been demonstrated in several studies showing that serum IgE very often does not correlate with clinical symptoms in patients suffering from gut-mediated allergy. A study among children with atopic eczema and gastrointestinal symptoms showed increased numbers of duodenal IgE-positive cells. Children with high local mucosal IgE-positive cells responded better to an elimination diet, in contrast to children with low intestinal IgE. In comparison, a skin prick test and serum IgE antibodies did not represent an adequate follow-up parameter (23). Fuiano et al. could also show that the correlation of the nasal IgE expression with clinical symptoms was significantly higher than that of the skin prick test (24,25).

The intestinal lavage has been claimed as a suitable tool to detect IgE in the intestinal mucosa and gut-mediated allergy (14). Mucus and adhering immunoglobulins are dissolved by short-term incubation of the intestinal mucosa with a saline solution. Subsequently, IgE antibodies can be detected in the lavage solution. This method, however, only allows to measure free IgE

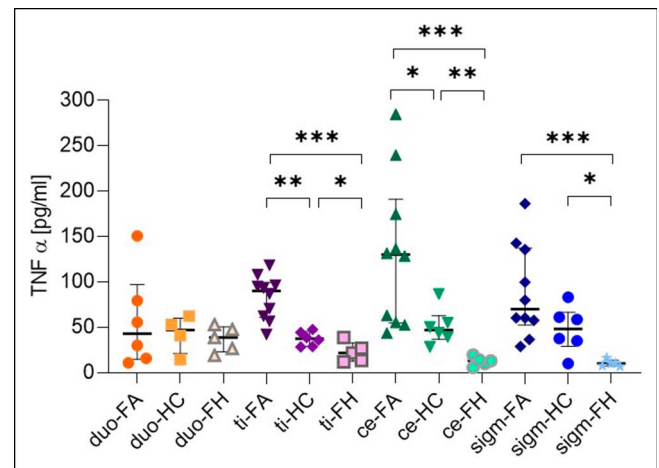


Figure 4. Tumor necrosis factor (TNF)- α concentrations in mucosal samples taken from the duodenum (duo), terminal ileum (ti), cecum (ce), and rectosigmoid area (sigm) of patients with food allergy (FA; $n = 10$; $n = 6$ for duodenal tissue), healthy controls (HC; $n = 6$; $n = 4$ for duodenal tissue), and patients with food intolerance of unknown origin (FH; $n = 5$). The differences between the patient groups were analyzed by the Mann-Whitney test. Patients with FA showed significantly increased TNF- α levels in the terminal ileum and cecum compared with HC. Patients with FH presented significantly lower TNF- α levels compared with patients with FA and HC in the terminal ileum, cecum, and rectosigmoid area. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

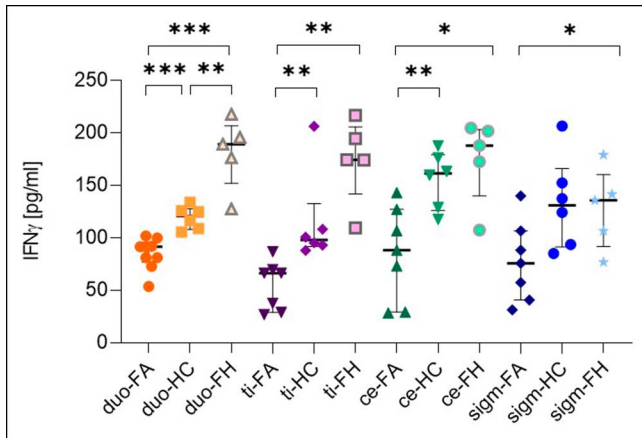


Figure 5. Interferon (IFN)- γ concentrations in mucosal samples taken from the duodenum (duo), terminal ileum (ti), cecum (ce), and rectosigmoid area (sigm) of patients with food allergy (FA; $n = 7$; $n = 9$ for duodenal samples), healthy controls (HC; $n = 6$), and patients with food intolerance of unknown origin (FH; $n = 5$). Given are medians with interquartile range. Patients with FH showed highest IFN- γ levels. Patients with FA showed distinct lower IFN- γ values compared with patients with FH or HC. Differences between the patient groups were determined by the Mann-Whitney test, and significant variations are shown. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

antibodies that are released into the intestinal lumen and associated with the intestinal mucosa (14), whereas IgE bound to mast cells cannot be measured. Further limitations of the lavage method are the substantial extension of the time of the gastroduodenoscopy/ileocolonoscopy. Impurities with undigested foods or feces can strongly affect the IgE measurement, too.

In our study, we therefore examined the detection of IgE antibodies in the intestinal lavage and in tissue samples of the intestinal mucosa using cytobrush and biopsy forceps. We obtained the best results by determining total and specific IgE in homogenates from mucosal samples collected with biopsy forceps. The highest values were found in the duodenum. Since this method is reliable and easy to perform, we recommend this method for the detection of IgE antibodies in the gut of patients with FA.

In addition, the detection of cytokines was most efficient in the homogenates of intestinal biopsies. We could show that patients with FA and systemic IgE have a clearly higher level of total IgE in the intestinal mucosa compared with HC. In addition, we found significantly higher TNF- α and significantly lower IFN- γ values in intestinal samples from patients with FA compared with HC and patients with FH.

This corresponds with the results of others that found that patients with IgE and not IgE-mediated FA showed increased TNF- α expression in the intestinal mucosa (8,26). In the absence of local and systemic signs of inflammation, the elevated TNF- α levels in our patients are probably due to mast cell degradation. We therefore suppose that the elevated intestinal TNF- α levels are indicative of an allergic reaction.

Lin et al. showed that symptomatic patients with suspected local FA have significantly higher numbers of IgE-positive cells and eosinophils in duodenal mucosa than patients without symptoms and HC. The IgE-positive cells were mostly mast cells,

and only a few were eosinophils. In addition, higher numbers of interleukin-4 (IL-4)-positive cells were found in the mucosa of symptomatic patients, whereas numbers of IFN- γ -positive cells were reduced. There was no difference in serum levels of IgE, IL-4, and IFN- γ between all groups (3). These data are in accordance with our results with significant lower IFN- γ values among patients with FA compared with HC or patients with FH. Increased IL-4 values point to a TH2 activation with an increased secretion of IL-4 and interleukin-13 that causes the suppression of IFN- γ . IFN- γ inhibits the synthesis of IgE, thus downregulating the inflammation in allergic response (3).

In this study, we could also show that apart from patients with systemic and gut-mediated FA, there are additional patients with FIs characterized by increased IFN- γ levels of the intestinal mucosa without detectable systemic or local IgE. These patients with FH showed significantly increased IFN- γ , but low TNF- α values. None of these patients showed a systemic inflammation in blood parameters. All patients with FH had in common that the endoscopic diagnostics were inconspicuous in microscopic and macroscopic assessment. It was only the mucosal examination and the determination of the cytokines within the scope of our study that revealed an unspecific inflammation. We therefore assume that the underlying pathomechanism in these patients is completely different from the allergic response in patients with FA.

It is well known that aging is associated with inflammation (27,28). In this context, it is important to consider that our HC group had a higher average age. This was caused by the fact that these persons had EGD/ileocolonoscopy in the course of a preventive medical check-up that is recommended at the age over 50 years. Our HC showed moderately elevated values for TNF- α and IFN- γ , ranging between data from patients with FA and FH. The increased levels for IFN- γ might be a consequence of low-grade inflammation caused by higher age since no disease could be diagnosed by any of the control subjects.

As neither the laboratory parameters nor the endoscopic diagnostics from patients with FH hinted at an inflammatory reaction, and as the patients are substantially younger than our controls, the patients' significantly increased IFN- γ values are of special interest. Recently, researchers showed a correlation between a dysbiosis and the occurrence of FIs (8). It is possible that the dysbiosis leads to an unspecific mucosal inflammation and a disturbance of the intestinal permeability, thus inducing an FI. Farin et al. were able to demonstrate that IFN- γ is a surrogate parameter of the immune response to intestinal microbiota. The authors showed that the turnover of Paneth cells that regulate gut microbiota through the release of antimicrobial peptides is strongly dependent and mediated by IFN- γ (29). However, the interpretation of the increased IFN- α levels can only be assessed hypothetically in this study. Possible influences of the intestinal flora on the integrity of the intestinal mucosa and FIs will be investigated in a follow-up study.

With this study, we could show that patients with FA also possess local IgE in the intestinal mucosa. The most reliable and sensitive results to detect mucosal IgE antibodies were obtained with homogenates of mucosal samples taken by biopsy forceps. Therefore, we recommend using homogenates of biopsies as the diagnostic method of choice.

Besides patients with FA, we could identify another patient subgroup with FH that was characterized by very low local IgE titers, low TNF- α , but increased proinflammatory IFN- γ values.

The significance and importance of the increased IFN- γ values and a possible correlation with dysbiosis will be analyzed in a follow-up study.

CONFLICTS OF INTEREST

Guarantor of the article: Walburga Dieterich, PhD.

Specific author contributions: Walburga Dieterich, PhD, and Esther Tietz, MSc, contributed equally to this work. W.D., E.T., M.K., and Y.Z.: designed the conception of the experimental work and performed data analysis. E.T., T.R., and Y.Z.: did patient acquisition and sample collection. W.D., Y.Z., M.K., T.R., P.C.K., and M.F.N.: interpreted the data and written the manuscript. All authors have approved the submitted version and confirm the accuracy and integrity of any part of the work.

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Potential competing interests: None to report.

Study registration: ClinicalTrials.gov (NCT 03151252).

Study Highlights

WHAT IS KNOWN

- ✓ The prevalence of food intolerances (FIs) is increasing.
- ✓ Systemic food allergy (FA) and/or FIs can be proved by determining IgE and/or performing malabsorption breath tests.
- ✓ Diagnostic tools to detect gut-mediated FIs are still missing.

WHAT IS NEW HERE

- ✓ Homogenates of intestinal mucosal samples are most sensitive to detect intestinal IgE antibodies and cytokines.
- ✓ Mucosal samples are the diagnostic tool of choice to detect gut-mediated FA.
- ✓ IgE and cytokine levels differ between gut-mediated FA and FI of unknown origin.
- ✓ Intestinal IFN- γ levels are increased and TNF- α levels are decreased in patients with FI of unknown origin.

TRANSLATIONAL IMPACT

- ✓ IgE measurement from mucosal samples is useful in the diagnosis of gut-mediated FA.
- ✓ Determination of TNF- α and IFN- γ from mucosal samples allows to distinguish between FA and non-IgE-mediated FI.

REFERENCES

1. Zopf Y, Baenkler HW, Silbermann A, et al. The differential diagnosis of food intolerance. *Dtsch Arztebl Int* 2009;106(21):359–69; quiz 69–70; 4 p following 70.
2. Lied GA, Vogelsang P, Berstad A, et al. Dendritic cell populations in patients with self-reported food hypersensitivity. *Int J Gen Med* 2011;4:389–96.
3. Lin XP, Magnusson J, Ahlstedt S, et al. Local allergic reaction in food-hypersensitive adults despite a lack of systemic food-specific IgE. *J Allergy Clin Immunol* 2002;109(5):879–87.
4. Scala E, Villalta D, Meneguzzi G, et al. Comparison of the performance of skin prick and ISAC tests in the diagnosis of allergy. *Eur Ann Allergy Clin Immunol* 2020;52(6):258–67.
5. Worm M, Reese I, Ballmer-Weber B, et al. Guidelines on the management of IgE-mediated food allergies: S2k-Guidelines of the German Society for Allergy and Clinical Immunology (DGAKI) in collaboration with the German Medical Association of Allergologists (AeDA), the German Professional Association of Pediatricians (BVJK), the German Allergy and Asthma Association (DAAB), German Dermatological Society (DDG), the German Society for Nutrition (DGE), the German Society for Gastroenterology, Digestive and Metabolic Diseases (DGVS), the German Society for Oto-Rhino-Laryngology, Head and Neck Surgery, the German Society for Pediatric and Adolescent Medicine (DGKJ), the German Society for Pediatric Allergy and Environmental Medicine (GPA), the German Society for Pneumology (DGP), the German Society for Pediatric Gastroenterology and Nutrition (GPGE), German Contact Allergy Group (DKG), the Austrian Society for Allergy and Immunology (AE-GAI), German Professional Association of Nutritional Sciences (VDOE) and the Association of the Scientific Medical Societies Germany (AWMF). *Allergo J Int* 2015;24:256–93.
6. Hoh RA, Boyd SD. Gut mucosal antibody responses and implications for food allergy. *Front Immunol* 2018;9:2221.
7. Coeffier M, Lorentz A, Manns MP, et al. Epsilon germ-line and IL-4 transcripts are expressed in human intestinal mucosa and enhanced in patients with food allergy. *Allergy* 2005;60(6):822–7.
8. Bischoff SC, Mayer JH, Manns MP. Allergy and the gut. *Int Arch Allergy Immunol* 2000;121(4):270–83.
9. Straumann A, Simon HU. The physiological and pathophysiological roles of eosinophils in the gastrointestinal tract. *Allergy* 2004;59(1):15–25.
10. Krakowiak A, Walusiak J, Krawczyk P, et al. IL-18 levels in nasal lavage after inhalatory challenge test with flour in bakers diagnosed with occupational asthma. *Int J Occup Med Environ Health* 2008;21(2):165–72.
11. Arslan G, Kahrs GE, Lind R, et al. Patients with subjective food hypersensitivity: The value of analyzing intestinal permeability and inflammation markers in gut lavage fluid. *Digestion* 2004;70(1):26–35.
12. Belut D, Moneret-Vautrin DA, Nicolas JP, et al. IgE levels in intestinal juice. *Dig Dis Sci* 1980;25(5):323–32.
13. Wesemann DR, Nagler CR. The microbiome, timing, and barrier function in the context of allergic disease. *Immunity* 2016;44(4):728–38.
14. Schwab D, Raithel M, Klein P, et al. Immunoglobulin E and eosinophilic cationic protein in segmental lavage fluid of the small and large bowel identify patients with food allergy. *Am J Gastroenterol* 2001;96(2):508–14.
15. Lomer MC. Review article: The aetiology, diagnosis, mechanisms and clinical evidence for food intolerance. *Aliment Pharmacol Ther* 2015;41(3):262–75.
16. Muraro A, Werfel T, Hoffmann-Sommergruber K, et al. EAACI food allergy and anaphylaxis guidelines: Diagnosis and management of food allergy. *Allergy* 2014;69(8):1008–25.
17. Cameron LA, Durham SR, Jacobson MR, et al. Expression of IL-4, epsilon RNA, and epsilon RNA in the nasal mucosa of patients with seasonal rhinitis: Effect of topical corticosteroids. *J Allergy Clin Immunol* 1998;101(3):330–6.
18. Cameron L, Christodoulopoulos P, Lavigne F, et al. Evidence for local eosinophil differentiation within allergic nasal mucosa: Inhibition with soluble IL-5 receptor. *J Immunol* 2000;164(3):1538–45.
19. Cameron L, Hamid Q, Wright E, et al. Local synthesis of epsilon germline gene transcripts, IL-4, and IL-13 in allergic nasal mucosa after ex vivo allergen exposure. *J Allergy Clin Immunol* 2000;106(1 Pt 1):46–52.
20. Reisacher WR. Detecting local immunoglobulin E from mucosal brush biopsy of the inferior turbinates using microarray analysis. *Int Forum Allergy Rhinol* 2013;3(5):399–403.
21. Bischoff SC, Schwengberg S, Lorentz A, et al. Substance P and other neuropeptides do not induce mediator release in isolated human intestinal mast cells. *Neurogastroenterol Motil* 2004;16(2):185–93.
22. Novak N, Allam JP, Betten H, et al. The role of antigen presenting cells at distinct anatomic sites: They accelerate and they slow down allergies. *Allergy* 2004;59(1):5–14.
23. Caffarelli C, Cavagni G, Romanini E, et al. Duodenal IgE-positive cells and elimination diet responsiveness in children with atopic dermatitis. *Ann Allergy Asthma Immunol* 2001;86(6):665–70.
24. Fuiano N, Fusilli S, Passalacqua G, et al. Allergen-specific immunoglobulin E in the skin and nasal mucosa of symptomatic and asymptomatic children sensitized to aeroallergens. *J Invest Allergol Clin Immunol* 2010;20(5):425–30.

25. Fuiano N, Fusilli S, Incorvaia C. A role for measurement of nasal IgE antibodies in diagnosis of *Alternaria*-induced rhinitis in children. *Allergol Immunopathol (Madr)* 2012;40(2):71–4.
26. Ozen A, Gulcan EM, Ercan Saricoban H, et al. Food protein-induced non-immunoglobulin E-mediated allergic colitis in infants and older children: What cytokines are involved? *Int Arch Allergy Immunol* 2015;168(1): 61–8.
27. Rea IM, Gibson DS, McGilligan V, et al. Age and age-related diseases: Role of inflammation triggers and cytokines. *Front Immunol* 2018;9:586.
28. Jenny NS. Inflammation in aging: Cause, effect, or both? *Discov Med* 2012;13(73):451–60.
29. Farin HF, Karthaus WR, Kujala P, et al. Paneth cell extrusion and release of antimicrobial products is directly controlled by immune cell-derived IFN-gamma. *J Exp Med* 2014;211(7):1393–405.

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