Fecal IgE Analyses Reveal a Role for Stratifying Peanut-Allergic Patients

Czolk R^{1,2}, Codreanu-Morel F³, de Nies L^{2,4}, Busi SB^{4,5}, Halder R⁴, Hunewald O¹, Boehm TM^{1,2}, Hefeng FQ¹, De Beaufort C^{2,4,6}. Wilmes P^{2,4}. Ollert M^{1,7}. Kuehn A¹

¹Department of Infection and Immunity, Luxembourg Institute of Health, Esch-sur-Alzette, Luxembourg

²Department of Life Science and Medicine, Faculty of Science, Technology and Medicine, University of Luxembourg, Esch-sur-Alzette, Luxembourg

³National Unit of Immunology and Allergology, Centre Hospitalier de Luxembourg, Luxembourg, Luxembourg ⁴Luxembourg Centre for Systems Biomedicine, University of Luxembourg, Esch-sur-Alzette, Luxembourg

⁵UK Centre for Ecology and Hydrology, Wallingford, Oxfordshire, United Kingdom ⁶Diabetes and Endocrine Care Clinique Pédiatrique, Clinique Pédiatrique, Centre Hospitalier de Luxembourg, Luxembourg ⁷Department of Dermatology and Allergy Center, Odense Research Center for Anaphylaxis, Odense University Hospital, University of Southern Denmark, Odense, Denmark

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Abstract

Background: Peanut allergy (PA) is an IgE-mediated food allergy with variable clinical outcomes. Mild-to-severe symptoms affect various organs and, often, the gastrointestinal tract. The role of intestine-derived IgE antibodies in gastrointestinal PA symptoms is poorly understood. Objective: This study aimed to examine fecal IgE responses in PA as a novel approach to patient endotyping.

Methods: Feces and serum samples were collected from peanut-allergic and healthy children (n=26) to identify IgE and cytokines using multiplex assays. Shotgun metagenomics DNA sequencing and allergen database comparisons made it possible to identify microbial peptides with homology to known allergens.

Results: Compared to controls, fecal IgE signatures showed broad diversity and increased levels for 13 allergens, including food, venom, contact, and respiratory allergens (P<.01-.0001). Overall, fecal IgE patterns were negatively correlated compared to sera IgE patterns in PA patients, with the greatest differences recorded for peanut allergens (P<.0001). For 83% of the allergens recognized by fecal IgE, we found bacterial homologs from PA patients' gut microbiome (eg, thaumatin-like protein Acinetobacter baumannii vs Act d 2, 109/124 aa identical). Compared to controls, PA patients had higher levels of fecal IgA, IL-22, and auto-IgE binding to their own fecal proteins (P<.001). Finally, levels of fecal IgE correlated with abdominal pain scores (P<.0001), suggesting a link between local IgE production and clinical outcomes. Conclusion: Fecal IgE release from the intestinal mucosa could be an underlying mechanism of severe abdominal pain through the association between leaky gut epithelia and anticommensal T_H2 responses in PÁ.

Key words: Abdominal pain. Gut microbiome. IgE. Phenotype. Peanut allergy.

Resumen

Antecedentes: La alergia al cacahuete (AC) es una alergia alimentaria mediada por IgE de presentación clínica variable. Los síntomas varían de leves a graves y afectan a diferentes órganos y, a menudo, al tracto gastrointestinal. No se conoce bien el papel de los anticuerpos IgE de origen intestinal en los síntomas digestivos de la AC.

Objetivo: Estudiar las respuestas IgE fecales en la AC, como un enfoque novedoso en el endotipado de pacientes.

Métodos: Se recogieron muestras de heces y suero de niños alérgicos al cacahuete y de niños sanos (n=26) para identificar IgE y citoquinas mediante ensayos multiplex. La secuenciación metagenómica del ADN y las comparaciones con bases de datos de alérgenos permitieron identificar péptidos microbianos con homología a alérgenos conocidos.

Resultados: En comparación con los controles, las respuestas de IgE fecal mostraron una amplia diversidad y niveles aumentados para 13 alérgenos, incluidos alérgenos de alimentos, inhalantes, himenópteros y látex (P=<0,01-0,0001). En general, los patrones de IgE fecal se correlacionaron negativamente en comparación con los patrones de IgE sérica en pacientes con AC, con mayores diferencias para los alérgenos del cacahuete (P=<0,0001). Para el 83% de los alérgenos reconocidos por la IgE fecal, encontramos homólogos bacterianos del microbioma intestinal de los pacientes con AC (por ejemplo, la proteína A similar a la taumatina, baumannii vs. Act d 2, 109 de 124 aa idénticos). En comparación con los controles, los pacientes con AC presentaron niveles más altos de IgA fecal, IL-22 y auto-IgE ligadora de proteínas fecales propias (P=<0,001). Por último, los niveles de IqE fecal se correlacionaron con las puntuaciones de dolor abdominal (P=<0,0001), lo que sugiere una relación entre la producción local de IgE y los datos clínicos.

Conclusiones: La liberación de IgE fecal desde la mucosa intestinal proporciona un posible mecanismo para explicar el dolor abdominal intenso al conectar el epitelio intestinal permeable y las respuestas Th2 frente a la flora comensal en la AC.

Palabras clave: Dolor abdominal. Microbioma intestinal. IgE. Fenotipo. Alergia al cacahuete.

Summary box

- What do we know about this topic?
 Peanut allergy manifests with patient-specific outcomes such as mild-to-severe gastrointestinal symptoms. Current diagnostic approaches based on serological analyses fail to predict such clinical outcomes in acute episodes.
- How does this study impact our current understanding and/or clinical management of this topic?

The link between fecal IgE and peanut allergy symptoms suggests a possible correlation between local antibody production and antigut commensal $T_{H}2$ immune responses. We provide evidence that measuring fecal IgE is a novel diagnostic approach for the predictive stratification of peanut-allergic patients.

Introduction

Peanut allergy (PA) manifests through patient-individual outcomes [1,2]. Gastrointestinal symptoms (eg, abdominal pain, vomiting, diarrhea) are common in accidental anaphylaxis and oral immunotherapy [3,4]. Such tissue-specific inflammation is difficult to assess for diagnostic purposes, in contrast to serological analyses. Peanut-specific IgE (sIgE) correlates with the diagnosis of PA (sensitivity, 70%-90%; specificity, $\approx 60\%$), yielding results that are similar to those of skin prick and basophil activation tests, although they are of limited value for predicting clinical phenotypes [1]. Recently, we showed that peripheral immune signatures reflect clinical outcome during oral food challenge (OFC) with peanut [5]. We found that gastrointestinal symptoms are associated with novel migratory immune signatures, marked responses for T_H2, memory Tregs, and CD8+ T cells, and chemotactic navigation via CD196/ CCR6 upregulation, further emphasizing the importance of gut tissue inflammation. A recent PA study based on gastrointestinal biopsies revealed that the gut mucosa is enriched with IgE⁺ B lineage cells, correlating with systemic peanut sIgE levels [6]. According to these findings, IgE antibodies are detectable in the feces of food-allergic patients [7,8]. Fecal IgE levels were found to be increased after egg OFC, potentially owing to localized antibody production [9]. Beyond dysbiotic gut microbiota, IgE binding to fecal bacteria was reported in food allergy, pointing to an advanced T_H2 response [10]. In fact, the gut microbiome is a large reservoir of microbial biomass and, therefore, an important source of proteins and peptides [11], which might be potential targets of a pathogenic immune response in food allergy. In food allergy, the absorption of intestinal proteins seems to be facilitated by harmed gut epithelia through the initiation of inflammatory responses via cytokine release [12]. Based on functional remodeling of the epithelia leading to increased gut barrier permeability, defects in intestinal epithelial barriers are not yet well understood in PA patients [13,14]. Several observations in humans point to the role of intestinal epithelial permeability in clinical PA; for example, serum levels of Ara h 6, one of the major peanut allergens, were higher in PA patients than in controls, even though controls ingested higher doses of peanut protein [15]. Another recent study compared serum levels of lipopolysaccharide-binding protein as a surrogate marker of intestinal epithelial cell permeability between

PA patients and age-matched controls, finding that, upon digestion, lipopolysaccharide-binding protein levels were highest in PA, especially in PA patients with low threshold dose reactivity [16]. Animal models of PA also suggested unregulated uptake of gut luminal proteins as a marker of gut permeability, potentially including microbial proteins [17,18]. These could eventually be recognized by IgE via allergen-like structures (molecular mimicry) or novel epitopes [19]. Recent research showing IgE binding to commensal intestinal bacteria in food-allergic individuals provided the first evidence of anticommensal $T_{\rm H2}$ responses [10].

It is largely unknown whether such immune cross-talk between gut microbiota and hosts plays a role in acute episodes of PA by governing clinical reaction patterns. In order to investigate the role of fecal IgE in PA, we examined fecal IgE levels and fecal sIgE signatures to correlate them with clinical outcome, threshold dose reactivity, and gastrointestinal symptoms.

Methods

Unless otherwise stated, all chemicals were from Sigma-Aldrich (Merck).

Study Cohort and Human Sample Collection

Recruitment of participants, including sample and data handling, processing, and storage were approved by the Luxembourg Comité National d'Éthique de Recherche (CNER). Written and informed consent was obtained after a detailed consultation and prior to specimen collection. Samples from children with PA were collected during an ongoing trial on peanut anaphylaxis (ethics committee approval number: CNER No. 201710/02, ClinicalTrials.gov Identifier: NCT04604912). Sixteen patients were eligible and gave their consent to provide fecal samples. The mean age was 6.4 years (range, 3-11 years). The cohort had already been profiled in depth on the basis of immune cell patterns [5]. In our previous study comparing 11 patients who had experienced a clinical reaction and 8 patients who did not reach the threshold dose for clinical reactivity during OFC, we successfully detected significant differences in blood immune responses, which we used as historical data to inform the current study [5]. Available data on those patients were obtained from the clinical assessment, history, skin reactivity testing, serum total/specific IgE determination, and OFC (Table E1). Fecal samples were collected at home, at the earliest 2 months after OFC with peanut protein to allow for a baseline measurement. All fecal samples were collected, flash frozen directly after collection, and stored at -80°C until further use. The exclusion criteria for fecal sampling were use of antibiotics within the month before sampling and recent or current gastrointestinal symptoms (eg, diarrhea, Bristol scale 5-7) at the time of collection. Control samples of healthy children were enrolled in a pediatric cohort (ethics committee approval number: CNER No. 201110/06) [20]. Ten children from this cohort were included (C1-C10; mean [SD] age, 6.4 [2.6] years). Samples were collected and stored in the same way as in the allergy cohort. Samples from an adult population were used for comparison (ethics committee approval number: CNER No. 201710/02; ClinicalTrials.gov Identifier: NCT02931955) [21]. These were 7 healthy controls (mean age, 33.1 years; 14% male). Serum samples were taken at the clinics, as for children with PA, and fecal samples were collected at home (Omnigene Gut kit, DNAgenotek) and frozen at -80°C until further use. Regarding controls, child controls were used wherever possible, and the data are provided are those recorded from these controls. Only when specifically mentioned were data from adult controls shown.

Preparation of Fecal Extracts

Details can be found in the Supplementary Material [22,23].

Enzyme-Linked Immunosorbent Assay

Enzyme-linked immunosorbent assay (ELISA) kits were used for the detection of total IgE and IgA antibodies in fecal extracts (IgE/IgA Human Uncoated ELISA Kit, 88-50610-88/88-50600, Invitrogen). All assays were performed according to the manufacturer's instructions. Assays were repeated with differently diluted samples when the values did not fit into the calibration curve. Fecal extracts were generally diluted in the range of 1:10-1:30 for IgE and 1:10-1:4000 for IgA. In order to control the recovery rates of IgE and IgA in fecal extracts, extracts were spiked with known amounts of pure IgE or IgA in a range of 60-300 ng/mL. Next, the assays were performed according to the manufacturer's protocol (cut-off for positivity, 0.1 ng/L). We also converted ELISA values into kU/L using a 2.44-ng/mL IgE baseline corresponding to 1 kU/L [24]. Mean total IgE (tIgE) levels were around 100-fold higher in feces from patients with PA than in those from controls, namely, 42.13 μ g/L (0.0002-264.13 μ g/L) and 0.41 μ g/L (0.0002-1.98 µg/L; 60% fecal IgE positivity), respectively. This corresponded to a mean IgE level of 17 267.13 kU/L (0.08 kU/L-108 251.4 kU/L) in patients' feces compared with 170.07 kU/L (0.08-813.29 kU/L) in controls. ELISA experiments based on patient sera were used to test binding of patients' IgE to autologous fecal extracts. ELISA-based IgE was quantified as described earlier, with the modification of diluting patient sera 1:2 in 3% BSA/TBST [25,26]. Finally, fecal calprotectin was assessed as a measure of intestinal inflammation. Details can be found in the Supplementary Material [27,28].

Multiplex Analysis for Deep IgE Profiling

Reactivity profiles of specific IgE (sIgE) antibodies in participant sera and fecal extracts were assessed using the ALEX2 macroarray with 298 allergens (MADX) [29,30]. The assay was performed according to the manufacturer's protocol (further details in Supplementary Material).

Metagenomic Analyses of Fecal Microbiome Samples

Genomic DNA was extracted using the DNeasy PowerSoil Pro Kit (Qiagen). The purification and preparation of metagenomic libraries and 2×150-bp read Illumina shotgun sequencing were performed in collaboration with the sequencing platform at the Luxembourg Centre for Systems Biomedicine, University of Luxembourg. The Integrated Meta-omics Pipeline (IMP; v3 - commitID #6f1badf7) was used for preprocessing of data, as well as assembly, genome reconstruction, and annotation, as previously described [11,31]. Subsequently, microbiome composition was analyzed using the vegan (version 2.6-4), DESeq2, and Maaslin2 packages in R (version 4.2.3) [32-34]. Bacterial richness was expressed using the ACE-index, and microbial alpha diversity was expressed using the Shannon and Chao1 indices [35].

Prediction of Allergenicity and Sequence Alignments

Amino acid translations of metagenomics data provided by the IMP pipeline were filtered for potential allergen-like sequences using Allerdictor in Python [36]. All predicted sequences were then aligned with databases containing full allergen sequences (provided by the University of Wageningen through the allermatch database, resulting in 2074 sequences on the date of download) [37] or linear epitopes, which were downloaded from IEDB.org using the following settings: Epitope, "linear"; Assay, "T-cell"; Host, "Human"; Disease, "Allergic". The process yielded 9594 sequences on the date of download [38]. Alignments were performed using the program CLC workbench 11, based on a BLOSUM62 matrix with a gap opening penalty of 10 and a gap extension penalty 136 of 0.5, allowing 5 hits per metagenomics sequence. In a first feasibility approach, full-length allergens and allergen epitopes were matched with bacterial sequences in order to identify linear sequence similarity and in silico allergen epitope presence on the microbiota of the intestine. This approach yielded 49 unique bacterial hits against 32 different allergens. The alignment hits led to variable peptides of variable length, such as 13-amino acid (aa) peptides with 100% identity to 39-aa peptides with 66.7% identity. Commensal peptides matching with allergens originated overwhelmingly from the Bacillota phylum (82.5% of all identifiable phyla). Furthermore, most mimicry matches were found against allergens from respiratory sources such as molds and yeasts, especially mites and cockroaches (24.5% of all mimicry matches) and tree pollen (18.4%). In order to increase the specificity of our approach in PA patients, we linked alignments and fecal IgE recognition patterns in a targeted approach. Therefore, we selected the 12 allergens detected by patients' fecal sIgE (Tri a aA TI, Act d 2, Act d 5, Gly m 5, Bla g 2, Bla g 4, Bla g 5, Mala s 6, Aln g 1,



Figure 1. Comparison of fecal slgE patterns in peanut-allergic patients vs controls. A, Fecal slgE diversity by Shannon index in patients (pink) and controls (blue). B, Fecal slgE binding to known allergens by slgE levels in patients and controls. Heatmap: participants in columns; color code by slgE levels (gray, nondetectable, <0.3 kU_A/L; yellow-red, 0.3-12.5 kU_A/L). Asterisks, *P* values (**** <.0001, *** <.001, ** <.01, * <.05, and \geq .05 [ns]). ns indicates nonsignificant; slgE, specific IgE.



Figure 2. Comparison of fecal vs serum sIgE patterns in peanut-allergic patients. A, sIgE diversity by Shannon index in sera (red) and feces (brown). B, Unique fecal sIgE binding to known allergens. C, Unique serum sIgE binding to known allergens. Heatmaps: patients in columns; color code by sIgE positivity (gray, nondetectable, <0.3 kU_A/L; pink, >0.3 kU_A/L). Asterisks, *P* values (**** <.0001, *** <.001, ** <.01, and * <.05). sIgE indicates specific IgE.

Hev b 6, Pol d 5, Ves v 5) and checked them for similarities to microbiome sequences. This approach generated 35 unique "mimicry hits" for 10 allergens, except for Mala s 6 and Aln g 1, with variable matches (alignments, peptide lengths 6-124 aa; sequence identities, 100%-36.8%; similarities, 100%-34.4%). Graphical representations of alignments were created using the EMBOSS needle pairwise alignment tool of EMBL-EBI with default settings [39].

Multiplex Array for Cytokine Analysis

Levels of T_H1 , T_H2 , T_H9 , and T_H17 cytokines in participant sera and fecal extracts were determined using a multiplex platform (Meso Scale Discovery) according to the manufacturer's protocol and as reported previously [5]. The cytokines targeted were IL-4, IL-5, IL-9, IL-10, IL-13, IL-17 A/F, IL-22, IL-23, INF- γ , and TNF- α .

Statistical Analyses and Data Visualization

Cytokines and IgA and IgE (specific, total) were plotted using GraphPad Prism 9 (Graphpad Software Inc) and/or R (version 4.2.3) in R studio (version 2022.02.3) [40]. The correlation matrix for clinical and in vitro data was also calculated in R using the Spearman correlation for non-Gaussian distributed values with the Hmisc package and visualized using the corrplot package (https://CRAN.R-project. org/package=Hmisc; https://github.com/taiyun/corrplot). Nonparametric *t* tests were used for all significance tests comparing 2 groups (Mann-Whitney test). Nonparametric one-way ANOVA was used for comparison of multiple groups (Kruskal-Wallis test). Significant differences in the microbial composition were calculated using the Wilcoxon rank sum test with a Benjamini-Hochberg correction. Statistical significance was set at P < .0001, <.001, <.01, <.05, and $\ge .05$ (nonsignificant).

Results

Fecal slgE Signatures Vary Significantly in PA Patients Compared With Controls

To evaluate IgE responses in the gut, we conducted a prospective study on patients with PA (Table E1). In-depth IgE typing using a large panel of 298 allergenic extracts/allergens revealed that fecal IgE diversity, ie, array data transformed using the Shannon index, was significantly greater in PA



Figure 3. Comparison of fecal slgE to routine parameters and peanut allergy outcome. A, Fecal vs serum peanut slgE. Heatmap: patients in columns; color code by slgE levels (gray, nondetectable, <0.3 kU_A/L; yellow-red, 0.3-35 kU_A/L). B, Correlation matrix between abdominal pain (AP) and IgE. Color code by negative (red) vs positive correlation (blue). Asterisks, *P* values (**** <.0001, *** <.001). slgE indicates specific IgE; tlgE, total IgE.

patients than in controls (P=.0004) (Figure 1A). Unexpectedly, the patients' allergen-specific IgE (sIgE) pattern was only marginally characterized by sensitization to peanut allergens (Figure 1B). Instead, sIgE patterns covered a broad range of sources, comprising food, venom/contact, and respiratory allergens. Levels of sIgE levels against 13 allergens/allergen extracts were significantly elevated in PA patients. Compared with controls (pediatric controls, Figure 1B; adult controls, data not shown), these included food (eg, wheat α -amylase trypsin inhibitor/Tri a aA TI, peanut Ara h 1, chickpea extract Cic a, fish parasite Ani s 1), venom (wasp Pol d 5), contact (latex Hev b 6), and respiratory sources (eg. rat extract Rat n. cockroach Blag 2, Blag 4). Serum sIgE often reveals complex patterns for molecules from the same source (molecular spreading) and/or cross-recognition due to structural homology between allergens (cross-reactivity) [41], although fecal sIgE signatures did not share these known hallmarks. This observation pointed to differential IgE production sites, unlike germinal center reactions, where naïve B cells become activated to undergo affinity maturation by somatic hypermutation [42,43].

Divergent Fecal vs Serum sIgE Repertoires Point to Distinct IgE-Producing B-Cell Reservoirs

We then compared fecal sIgE with serum sIgE in PA patients. Levels of fecal tIgE levels were unrelated to serum tIgE (Figure E1, A). sIgE recognition patterns were more diverse in sera than in feces (P=.0085) (Figure 2A). Remarkably, fecal sIgE and serum sIgE had nearly inverted patterns. Unique fecal sIgE signatures included wheat, kiwi, soy, mite, mold, alder pollen, latex, and venom allergens (Figure 2B; 12 allergens). Unique serum sIgE patterns were recorded for cereal, peanut, cat dander, mite, and grass/tree pollen allergens (Figure 2C; 35 allergens). Fecal IgE levels for peanut allergens differed significantly from peripheral sIgE levels (Figure 3A). Patients with PA had no sIgE (19%), low sIgE to Ara h 1 (44%; mean, 0.58 kU_A/L), or low sIgE to Ara h 1 and Ara h 2 (37%; mean, 0.89 kU_A/L).

patients with PA, especially for Ara h 1, Ara h 2, Ara h 3, and Ara h 6 (Figure 3A). Fecal peanut-sIgE was considerably lower than serum levels (Ara h 1, 2, 6, P<.0001; Ara h 3, P=.0005). Overall, only serum sIgE was consistent with the clinical diagnosis of PA, that is, all patients had serum sIgE against peanut extract and the allergens Ara h 1, 2, and 6 (Table E1; Figure 3A). These differential IgE recognition patterns in feces compared to serum further substantiated the hypothesis that fecal IgE originates from gut-residing B-cell reservoirs.

High Fecal sIgE Correlates With Gastrointestinal Symptoms in PA Patients

We reasoned that fecal IgE would be a consequence of an extended failure of tolerance, which advanced from the oral to the intestinal epithelia. To investigate whether fecal IgE would play a role in the outcome of PA, we stratified PA patients into subgroups of fecal tIgE, as follows: high, >82 000 kU/L; moderate, 23.6-1432.4 kU/L; and nondetectable, <0.08 kU/L (Table). Interestingly, threshold dose reactivity increased in the subgroups from high to moderate to nondetectable fecal tIgE (mean cumulative eliciting dose [CED], 30 mg, 185 mg, and 700 mg, respectively). The high fecal tIgE subgroup had the strongest abdominal pain scores (8-10/10). The moderate fecal tIgE subgroup had less frequent abdominal pain at moderate intensity (38%, score 6/10), and single patients with nondetectable fecal tIgE had only minor abdominal pain. Routine serological markers, such as serum sIgE to peanut extract, appeared to be similar in those 3 subgroups (median sIgE, 100, 95.5, and 100 kU_A/L, respectively) (Table). Given the low number of patients in the subgroups in the Table, we refrained from performing a statistical comparison. To overcome the limitation through patient numbers, we established the dependence of the variables in the Table by applying an unbiased correlation analysis (Figure 3B). We plotted fecal IgE, serum IgE, and abdominal pain scores in a correlation matrix. Notably, the correlation between fecal tIgE levels and abdominal pain scores was high (P=.0008)

Table. Clinical Characteristics of 3 Subgroups of Patients With Peanut Allergy, Clustered by Levels of Fecal tlgE.		
Patients with high tlgE ^a	Patients with moderate tlgE ^a	Patients with ND tlgE ^a
83 087.7 (82 246.2-108 251.4)	99.45 (23.6-1432.4)	<0.08
5386	41.6	≤1
30/20 (20-50)	185/75 (15-800)	700/100 (100-3000)
100% with AP (8-10/10)	38% with AP (6/10)	40% with AP (2/10)
846 (715-947)	1311 (15-4516)	365 (110-2772)
100 (35-100)	95.5 (0.51-100)	100 (0.65-100)
	Patients With Peanut Allergy, Clustere Patients with high tlgE ^a 83 087.7 (82 246.2-108 251.4) 5386 30/20 (20-50) 100% with AP (8-10/10) 846 (715-947) 100 (35-100)	Patients With Peanut Allergy, Clustered by Levels of Fecal tIgE. Patients with high tIgE ^a Patients with moderate tIgE ^a 83 087.7 (82 246.2-108 251.4) 99.45 (23.6-1432.4) 5386 41.6 30/20 (20-50) 185/75 (15-800) 100% with AP (8-10/10) 38% with AP (6/10) 846 (715-947) 1311 (15-4516) 100 (35-100) 95.5 (0.51-100)

Abbreviations: AP, abdominal pain; CED, cumulative eliciting dose; GI, gastrointestinal (score indicates intensity of abdominal pain [1=low to 10=maximum]); ND, nondetectable; OFC, oral food challenge; sIgE, specific IgE; tIgE, total IgE.

^aPatients stratified by levels of fecal tIgE as high (>82 000 kU/L, n=3), moderate (23.6-1432.4 kU/L, n=8), and nondetectable (ND; <0.08 kU/L, n=5).

(Figure 3B), substantiating initial evidence for local IgE responses in PA. Serum peanut sIgE correlated negatively with threshold dose reactivity (Figure E1, B), confirming previous literature reports [44]. We compared levels of fecal tIgE with sIgE against known allergens, ie, the sum of single sIgE values, as determined by the IgE-typing multiplex array. We found that the high fecal tIgE subgroup also had the broadest gap when tIgE was compared with sIgE sum (ratio of 5386; Table). This gap further decreased for the subgroups with moderate and nondetectable fecal tIgE (ratios of 41.6 and \leq 1, respectively). In the correlation

matrix analysis, we confirmed the statistical significance of this observation. Fecal IgE was positively correlated with the gap between tIgE and sIgE sum (Figure 3B; P<.0001). Large gaps, ie, high levels of fecal sIgE of unknown reactivity, also correlated with local symptom scores for abdominal pain (Figure 3B; P<.001). We concluded that such unknown fecal sIgE would be involved in the immune response underlying peanut-induced abdominal pain. A similar concept of disrupted intestinal homeostasis had been proposed recently in irritable bowel syndrome (IBD) following bacterial infections [45].



Figure 4. Microbiota targets of T_{H2} responses in peanut allergy. A, Homology comparison of allergens vs bacterial sequences. Workflow and overview on sequence hits for all child participants. B, Alignment of homologs, allergen Act d 2 vs patients' microbiome sequence from *Acinetobacter baumannii*. C, Serum IgE reactivity to autologous feces. Workflow and results, patients (pink) vs adult controls (blue). Asterisks, *P* value (*** <.001). ELISA indicates enzyme-linked immunosorbent assay.

Fecal slgE Can Be Directed Against Gut Microbial Targets That Are Similar to Allergens

To further explore whether unknown fecal sIgE could be directed against microbiome targets across damaged gut epithelia, we analyzed intestinal immune factors and gut microbiota. Compared to controls, PA patients had higher levels of fecal IgA and fecal IL-22 (P<.0001 and P=.04 respectively; Figure E1, C. and D.), which are both known players in intestinal diseases [46]. IL-17 A/F, another proinflammatory cytokine of the T_H17 pathway, was higher in feces than in sera from patients with PA (Figure E1, E.). Indeed, T_H17 cells are considered to be involved in the regulation of the intestinal mucosal immune microenvironment [47]. Gut microbiome composition and diversity play a role in food allergy, including PA [48-50]. Illumina shotgun sequencing of fecal DNA confirmed that the gut microbiome of PA children was less diverse than in age-matched controls [51], with variable levels of bacterial phyla and strains (Figure E2). To better understand the allergenic structures of the gut microbiome, we investigated similarities between bacterial peptides and allergen sequences (Figure 4A). Microbial sequences were screened for potential allergenicity, which reduced the sequence pool from over 9.18 million to 2044 sequences. All peptide structures with predicted allergenicity were locally aligned with full allergen sequences. Bacterial peptides with sequence similarity to the allergens of the unique fecal sIgE patterns were considered a hit (Table E2; 10 allergens). This approach generated 35 unique hits for 10 out of 12 allergens with variable alignment matches (alignments: peptide lengths, 6-124 aa; sequence identity range, 100%-36.8%; similarity range, 100%-34.4%). These hits represented a first molecular basis for possible cross-reactivity between allergens and gut microbial antigens. Indeed, the longest alignment was observed for the kiwi allergen Act d 2, which aligned over 124 aa to a bacterial homolog of the PA microbiome (Figure 4B; 88% identity to thaumatin-like protein from Acinetobacter baumannii). Similar peptide structures can also be found in common strains such as C difficile or E coli. Of note, none of the patients was diagnosed for kiwi allergy (Table E1), as corroborated by the overall negativity of serum sIgE to kiwi allergens (Figure 2B). This observation further supported our hypothesis that fecal sIgE recognizing Act d 2 is genuinely directed not to the kiwi allergen but to a similar bacterial protein from the gut microbiome. Finally, the ability of sera from patients with PA to react to autologous fecal samples was tested using ELISA. Compared to controls, 31% of patients with PA displayed marked IgE-binding to fecal proteins, clearly exceeding the positivity cut-off (Figure 4C). This confirms the general principle of the microbiome being able to function as a source for mimicry patterns in IgE-mediated food allergy.

Discussion

Based on analyses of antibody levels and multiplex antibody binding patterns in a well-characterized cohort of food-challenged patients with PA, our proof-of-concept study revealed a positive correlation between fecal IgE-antibodies and gastrointestinal symptoms in PA [5]. Levels of fecal IgE increased with the intensity of the gastrointestinal symptom abdominal pain, suggesting that local immune responses of the gut contribute to these organ-specific symptoms. In our study, analyses of fecal IgE outperformed serological analyses of peanut sIgE, which correlated with threshold dose reactivity, as reported elsewhere [44,52], but not abdominal pain scores. Indeed, established diagnostics are not predictive of clinical reactivity, such as reaction severity patterns: therefore, many patients continue to require OFC [53,54]. Our study provides the first explanations for gut-related patient reactivity patterns, suggesting fecal IgE as a new target area in predictive patient stratification in PA. Our findings are in line with another recent study of food-induced abdominal pain [45]. As a novel peripheral mechanism described in patients with IBD, colonic IgE⁺ mast cells were involved in local immune responses and aberrant pain signaling following an increase in the mucosal permeability of the gut [45]. Although we were not able to further investigate biopsies from PA patients or perform intramucosal injections of peanut protein, as reported by others [6,45], our data indirectly confirm the gastrointestinal tract as a reservoir for local IgE production. Fecal IgE was clearly differentiated from serum IgE in PA patients. Levels of fecal tIgE levels were unrelated to serum tIgE, in contrast with findings reported for airway allergy and infection by intestinal pathogens [55,56]. Moreover, fecal sIgE recognition patterns differed from those of serum sIgE, further suggesting that different production sites would replenish the fecal and serum sIgE reservoirs [43,57].

As observed in IBD, dysfunction of natural epithelial barriers in the form of altered permeability and dysbiosis of microbiomes appears to play an important role in breakdown of tolerance and progression of PA. Data on fecal immune factors in PA are sparse. As further evidence to disturbed gut barriers and inflammatory features of the intestine in PA, we discovered a positive correlation with several fecal immune factors and levels of IgA, IL-22 (a T_H17 cytokine), and IL-17 A/F (a proinflammatory cytokine). Increased coating of microbiota with IgA has previously been observed in IBD [58]. IL-22 is associated with antimicrobial defense following the disruption of the epithelial barrier [59]. Epithelial barriers, typically the first lines of defense against external elements, are relevant in many diseases if they are harmed and disrupted [60]. Via opened tight junctions, intestinal bacteria can translocate and contribute to inflammatory responses. Such bacterial translocation has been reported for IBD and COVID-19 patients [61-63], while data on food allergy are scarce. We found large amounts of sIgE of unknown specificity in the feces of PA patients (especially in those with >82 000 kU/L fecal tIgE) at the level of the individual patient and reasoned that such unknown sIgE could be directed against translocated microbiome targets. In a subgroup of PA patients, serum IgE self-reacted to autologous feces, corroborating antigut commensal T_H2 responses in PA. Using metagenomics analyses of gut microbiome DNA and sequence comparisons with allergen databases, we showed that peptides from the gut microbiome exhibit marked homology to known allergens (up to 88% identity over 124 aa), thus confirming their role as putative T_H2 targets. The blood of healthy individuals has even been reported to contain bacterial peptides [64]. Further studies will be needed to determine the identity and pathogenic role of translocated bacterial peptides in food allergy.

The major limitation of this study is the relatively low sample size. Despite this limitation, to our knowledge, this is the first study to show that fecal sIgE signatures markedly varied from serum sIgE in PA, revealing spatially different antibody production sites. Our data point to the functional involvement of fecal IgE in gastrointestinal symptoms, likely via anticommensal T_{H2} responses across leaky gut epithelia under temporary proinflammatory signals in acute PA. Further research is needed to determine how such gut T_{H2} responses develop from early to later in life [65], to identify the bacterial targets involved (as shown in other gastrointestinal pathologies such as inflammatory bowel disease [66]), and to examine the potential utility of those immune targets in restoring impaired gut barriers [67] in PA.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Annette Kuehn

Department of Infection and Immunity Luxembourg Institute of Health 29, rue Henri Koch L-4354 Esch-sur-Alzette Luxembourg E-mail: annette.kuehn@lih.lu