Anti-food and anti-microbial IgG subclass antibodies in inflammatory bowel disease

Anke Jansen, Ana D. Mandić, Eveline Bennek, Lisa Frehn, Julien Verdier, Irene Tebrügge, Holger Lutz, Konrad Streetz, Christian Trautwein and Gernot Sellge

Department of Internal Medicine III, University Hospital RWTH Aachen, Aachen, Germany

ABSTRACT

Objectives: Inflammatory bowel disease (IBD), particularly Crohn's disease (CD), is associated with increased microbial-specific IgG and IgA antibodies, whereas alterations of anti-food antibodies are still disputed. The knowledge about IgG subclass antibodies in IBD is limited. In this study we analysed IgG subclass antibodies specific for nutritional and commensal antigens in IBD patients and controls.

Methods: Serum IgG1, IgG2, IgG3 and IgG4 specific for wheat and milk extracts, purified ovalbumin, Escherichia coli and Bacteroides fragilis lysates and mannan from Saccharomyces cerevisiae were analysed by ELISA in patients with CD (n = 56), ulcerative colitis (UC; n = 29), acute gastroenteritis/colitis (n = 12) as well as non-inflammatory controls (n = 62).

Results: Anti-Saccharomyces cerevisiae antibodies (ASCA) of all IgG subclasses and anti-B. fragilis IgG1 levels were increased in CD patients compared to UC patients and controls. The discriminant validity of ASCA IgG2 and IgG4 was comparable with that of ASCA pan-IgG and IgA, whereas it was inferior for ASCA IgG1/ IgG3 and anti-B. fragilis IgG1. Complicated CD defined by the presence of perianal, stricturing or penetrating disease phenotypes was associated with increased ASCA IgG1/ IgG3/ IgG4, anti-B. fragilis IgG1 and anti-E. coli IgG1 levels. Anti-food IgG subclass levels were not different between IBD patients and controls and did not correlate with food intolerance. In contrast to anti-microbial Abs, food-specific IgG responses were predominately of the IgG4 isotype and all food-specific IgG subclass levels correlated negatively with age.

Conclusion: Our study supports the notion that the adaptive immune recognition of food and commensal antigens are differentially regulated.

Abbreviations: AGE: acute gastroenteritis/colitis; Ab: antibody; ASCA: anti-Saccharomyces cerevisiae antibodies; AU: arbitrary units; CD: Crohn's disease; HBI: Harvey Bradshaw Index; IBD: inflammatory bowel disease; SCCAI: Simple Clinical Colitis Activity Index; UC: ulcerative colitis

Introduction

Inflammatory bowel diseases (IBD) and in particular Crohn’s disease (CD) are associated with increased levels of antibodies (Ab) against microbial antigens of the commensal flora.[1–3] Anti-Saccharomyces cerevisiae Abs (ASCA) of the IgG and IgA type, directed against specific oligomannose components found on cell wall of Saccharomyces and other yeasts such as Candida albicans, were first identified and have a diagnostic utility as their serum titres are significantly higher in CD patients in comparison to ulcerative colitis (UC) patients and healthy controls.[4,5] It has been suggested that increased anti-microbial Abs are associated with dysfunctions of the intestinal barrier and an exaggerated immune response to luminal antigens. Whether these antibodies are triggering IBD or are only a consequence of gut inflammation without a disease-aggravating role remains elusive.[3]

Food-specific IgGs and IgAs are frequently found in IBD patients and healthy controls and are most likely not associated with food intolerance by contrast with food-specific IgE.[6–8] although this issue is still a matter of debate.[9–11] Regarding anti-food antibodies in IBD, conflicting results have been published. Different studies found either higher or equal amounts of food-specific IgGs in IBD patients compared to controls.[7,12–15] We recently reported that specific pan-IgGs and IgAs to ovalbumin as well as wheat and milk extracts were not increased in CD and UC patients compared to controls. However, we found a slight increase in serum anti-food IgA levels in patients with complicated CD defined by the presence of perianal, stricturing or penetrating disease phenotypes compared to CD patients without these complications.[8]

In humans, IgG is composed of four different subtypes, IgG1, IgG2, IgG3 and IgG4, which are named in order of their abundance in serum (IgG1 > IgG2 > IgG3 > IgG4). Differences in the constant region are responsible for their distinct biological properties, although the exact functional differences remain still poorly understood.[16] All subclasses can act as
opsonins while IgG1 and IgG3 have higher affinities to Fcγ receptors than IgG2 and IgG4.[16,17] IgG1 and IgG3 efficiently activate the classical complement pathway, whereas IgG2 has only weak and IgG4 no complement activating capacity.[18] IgG1 and IgG3 are predominately formed against protein and viral antigens,[19] while polysaccharide antigens trigger preferentially IgG2.[20] IgG4 is often found upon chronic antigen exposure as in allergies.[21] Patients with isolated IgG subclass deficiencies are often asymptomatic but increased risk of infections and lack of vaccine response have been reported in all types of IgG subclass deficiencies, yet most frequently in IgG1 and IgG2 deficiencies.[22] Furthermore, several autoimmune diseases and allergies have been associated with IgG subclass deficiencies.[22]

IgG subclass analyses in serum and intestinal biopsies of IBD patients showed preferentially increased IgG1 in UC and increased IgG2 in CD patients, suggesting a distinct regulation of IgG subclasses in the two IBD entities.[23–28] Elevated numbers of IgG3- and IgG4-producing cells have been detected in the colon of UC patients,[29] whereas most studies found unaltered serum IgG3 and IgG4 concentrations in UC and CD.[23,25] One study detected increased serum IgG4 levels in UC patients in comparison to CD patients.[26]

Oshitani et al. investigated ASCA IgG subclasses and found a significant augmentation of ASCA IgG4, but not IgG1, IgG2 or IgG3 in IBD patients compared to controls.[30]

Except for ASCA, IgG subclasses specific for commensal and food antigens have not been analysed in IBD. In this study we investigated serum IgG subclasses specific for three different food and microbial antigens, respectively, in IBD patients and controls.

### Methods

**Ethical considerations**

Written informed consent was obtained from all study participants. The study is in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments and was inspected and approved by ethics committee of the University Hospital Aachen (RWTH-University, Aachen, Germany, reference number EK 049/12).

**Study participants**

Patients were recruited at the Gastroenterology Department of University Hospital Aachen. Diagnosis of CD and UC was confirmed by a combination of clinical aspects, endoscopic/histological, sonographic/radiographic and biochemical findings. Patients with diagnosis “colitis unclassified” were excluded. No paediatric patients were included. Non-inflammatory control group was formed by two subgroups: (a) control patients with diagnoses without intestinal inflammation and (b) a group of healthy volunteers. Patients suffering from other forms of gastrointestinal inflammation (acute gastroenteritis and colitis [AGE], disease duration <4 weeks) served as controls with intestinal inflammation. For further information, see Table 1. Clinical disease activity of IBD patients was measured by the Harvey Bradshaw Index (HBI) for CD and

| Table 1. Patient’s characteristics. |
|------------------------------------|
| **Controls (Con)  | **A** | **B** |
| (a) Control patients | 36 | 35 (33–67) | 18 (50) |
| (b) Healthy volunteers | 26 | 25 (23–27) | 16 (62) |
| **Crohn’s disease (CD)** | 56 | 35 (26–46) | 29 (52) |
| **Ulcerative Colitis (UC)** | 29 | 35 (27–52) | 11 (38) |

| **Inflammatory bowel disease** |
|--------------------------------|
| **Controls (Con)** | 62 | 38 (25–57) | 34 (55) |

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| Table 2. Detailed characteristics of IBD patients. |
|---------------------------------------------|
| **Crohn’s disease (CD)** |
| **A** | **B** |
| Inactive disease | 32 (HBI <5) | 12 (SCCAI <3) |
| Active disease | 24 (HBI >5) | 17 (SCCAI >3) |
| **Localization** |  |  |
| L1 (ileal) | E1 (proctitis): 1 |
| L2 (colonic) | E2 (left side): 16 |
| L3 (ileocolonic) | E3 (pancoatitis): 10 |
| L4 (additional upper GI disease) | Pouchitis: 2 |
| **Behaviour** |  |  |
| B1 (non-stricturing/penetrating): 18 |  |
| B1p (B1 + perianal disease): 5 |  |
| B2 (stricturing): 7 |  |
| B2p (B2 + perianal disease): 9 |  |
| B3 (penetrating): 10 |  |
| B3p (B3 + perianal disease): 6 |  |
| **Age at diagnosis** |  |  |
| A1 (<16 years): 7 | A1: 7 |
| A2 (17–40 years): 40 | A2: 14 |
| A3 (>40 years): 9 | A3: 8 |
| **Duration** | 8.6 (3.3–15.7) years |

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Antigens were prepared as recently published.[7] Albumin from chicken egg white (Ovalbumin, Sigma, Grade V) and non-fat dried milk powder (Sigma) were mixed with carbonate buffer pH 9.6. Wheat flour was mixed with sodium acetate buffer pH 3.8 (sodium acetate 6 mM; acetic acid 88 mM; pH 3.8). Mixtures were centrifuged at 27,000g by ultracentrifuge for 30 minutes to remove non-soluble contents. Escherichia coli K12 DH5α were cultured in LB medium overnight under aerobic conditions and Bacteroides fragilis ATCC 25285 were cultured in thioglycolate medium for 72 hours in anaerobic environment. Cultures were centrifuged in a swinging rotor centrifuge at 3600 rpm for 5 minutes four times to wash out medium proteins. For bacterial lysis, tubes were shaken for 30 minutes in a shaking disruptor (Disruptor Genie, Scientific Industries, Inc., NY) at 2.850 rpm with glass beads (0.3 μm diameter, Sigma). After repeated centrifugation (9000 rpm, 10 min) supernatants were filtered through a 0.2 μm syringe filter (Millex GP). Protein content of all antigen preparations were measured by the BCA method (Pierce).

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Simple Clinical Colitis Activity Index (SCCAI) for UC.[31,32] Disease phenotypes were classified according to the Montreal classification.[33] (Table 2) Adverse reactions to foods were analysed by standardised questionnaires and interview.

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apart from mannan oligosaccharide was measured by the Bradford method. Purified mannan from *Saccharomyces cerevisiae* (Sigma) was solubilised in carbonate buffer pH 9.6. Aliquots were stored at −80 °C.

**ELISA measurement**

ELISA measurement was performed as recently published.[7] Microtitre plates (96 wells, Nunc Maxisorp, Sigma-Aldrich, Munich, Germany) were coated with 50 μl of above described antigen preparations in defined concentrations (mannan 100 μg/ml, all other antigens 10 μg/ml protein content) and incubated overnight at 4 °C. Plates incubated with carbonate buffer pH 9.6 devoid of antigens were used for analysis of background binding. All following incubations steps were intermitted by washing four times with PBS/TWEEN 0.05% using an ELISA-washer (Nunc). Unless stated otherwise, all steps were performed at room temperature and 50 μl of respective solution per well was used. Blocking was performed with 200 μl PBS/BSA 5% per well for 60 min. Plates coated with bacterial antigens were treated with avidin/biotin blocking reagent (Vector laboratories) for 30 minutes to prevent unspecific streptavidin binding. Plates were loaded with patients’ and control’s sera diluted in PBS/BSA 1%. Each serum was tested at 3–4 different dilutions. The lowest dilution was 1:6.25 or 1:12.5 and the subsequent dilutions were 4-fold higher, respectively. If all measurements were above the upper detection limit, ELISA for these particular samples was performed again using higher serum dilutions. A 2-fold serial dilution curve of a serum with known high reactivity for the respective antigen served as standard. Sera were incubated overnight at 4 °C. Treatment with 100 μl H2O2 3% per well for 10 minutes was used to block endogenous peroxidases. Detection antibodies for IgG1, IgG2, IgG4 (0.5 mg/ml; BD Pharmingen, NJ; IgG1 Clone G17-1; IgG2 Clone G18-21; IgG4 Clone G17-4, dilution 1:10000 in PBS/BSA 1%) and IgG3 (0.5 mg/ml; Abcam, Cambridge, UK; Clone HP6050; dilution 1:1000 in PBS/BSA 1%) were added and incubated for 2 hours. Streptavidin-horseradish peroxidase solution (R&D Systems, MN, 1:200 dilution in PBS/BSA 1%) was added for 40 minutes. 100 μl TMB (3,3′-5,5′-Tetramethylbenzidine; Sigma) substrate solution (1 mg/ml in 0.05 M phosphate-citrate buffer, pH 5.0) was added and stopped by 50 μl 2 N H2SO4 after 5–20 min as required. Reaction was quantified photometrically at 450 nm (μQuant; Biotec, Bergisch Gladbach, Germany).

**Analyses and statistics**

Levels of antigen-specific IgG1, IgG2, IgG3 and IgG4 are presented as arbitrary units (AU) as described recently.[7] AU were calculated in relation to standard serum. The AU assigned to the standard serum were defined by the dilution at which the OD was two times above the background OD of buffer control (dilution = AU). Sample measurement was performed at different dilutions (see above) and for AU calculation the OD of the highest dilution above the lower detection limit was selected. Lower detection limit was set as two times background OD of the buffer control. Furthermore, if background OD measured in plates coated without specific antigens (BSA only + incubation with serum) was above 60% of the value measured in the antigen coated plate (antigen + BSA + incubation with serum) the measurement was discarded. For preparation of standard curve and calculation of sample values, the background OD measured in plates coated without specific antigens (BSA only) was subtracted from the matching value measured on respective antigen coated plate.

Analyses of statistical significance were performed by Kruskal–Wallis test followed by a Dunn’s *post hoc* test for comparison of multiple groups and Mann–Whitney *U* test in case of two groups. Correlations were detected by Spearman correlation test. Receiver-operating characteristic (ROC) curve and derived area under the curve were created for comparison of discrimination threshold. All statistical analyses were performed using SPSS (IBM, NY) and GraphPad Prism (GraphPad Software Inc., CA).

**Results**

IgG subclass Abs specific for ovalbumin, wheat, milk, *S. cerevisiae*-derived mannan (ASCA), *E. coli* lysate, and *B. fragilis* lysate were quantified by ELISA in serum of patients suffering from CD, UC and non-IBD acute gastroenteritis/colitis (AGE) as well as age-matched controls without gastrointestinal inflammation. Specific signals for all antigen-specific IgG subclass Abs were detectable in a high number of patients and controls (Figure 1). ASCA IgG1, IgG2, IgG3 and IgG4 as well as anti-*B. fragilis* IgG1 levels were significantly higher in CD patients compared to the amounts found in controls, whereas there was no detectable difference for UC or AGE patients. For all other Abs, we did not detect any significant differences between the groups (Figure 1). Notably, food-specific Abs had a particular high response of the IgG4 subclass and for IgG2 the highest levels were measured for ASCA.

ROC analyses for ASCA IgG subclasses showed that ASCA IgG2 and IgG4 were as effective as ASCA pan-IgG and IgA [7] in differentiating between CD and controls as well as between CD and UC. ASCA IgG1 and IgG3 as well as *B. fragilis* IgG1 showed an inferior discriminant validity than ASCA IgG2, IgG4, pan-IgG and IgA (Figure 2(A,B) and data not shown). Values of all ASCA subclasses correlated significantly, although pan-IgG versus IgG2, pan-IgG versus IgG4 and IgG2 versus IgG4 showed the highest correlation rates (Figure 2(C)).

For the other antigens, the correlation rates between different IgG subclasses specific for the same antigen were also highest between IgG2 and IgG4. The weakest correlations were found between IgG3 and IgG4 (Suppl. Table 1). Correlation rates of identical IgG subclasses specific for different antigens were heterogeneous. We found a generally higher correlation between different anti-food Abs than between different anti-microbial Abs or between anti-food and anti-microbial Abs (Suppl. Table 2).

Several complications in CD such as perianal involvement, intestinal fistulas and strictures and requirement of surgery have been associated with elevated ASCA levels.[3,34,35] We compared CD patients with perianal involvement and/or stricture/penetrating disease to CD patients without these.
complications and found a significant increase of ASCA IgG1/IgG3/IgG4 as well as anti-\textit{E. coli} and anti-\textit{B. fragilis} IgG1 in the first group. Anti-food IgG subclass concentrations were not different in this CD subgroup (Figure 3(A–C)).

Adverse reactions to foods were analysed by standardised questionnaires. As expected, food intolerances were more frequent in IBD patients than in controls (Table 3). The vast majority of IBD patients reported about abdominal pain, constipation, diarrhoea and bloating after ingestion of the culprit food, symptoms that are often associated with non-immune mediated food intolerances. Systemic symptoms such as skin rush and breathing problems or symptoms typical for an oral allergy syndrome were only reported by 7 IBD patients. Six of these patients had been diagnosed with IgE-dependent food allergy (5 pollen-associated, 1 fish). One patient had suggested acid reflux induced asthmatic episodes upon ingestion of milk and garlic. We identified 18 individuals (patients + controls) with milk intolerance and 6 individuals with intolerance towards cereal products including wheat, whereas egg intolerance was not reported. 7/18 and 1/18 patients with milk intolerance had a known lactose intolerance and milk allergy (control), respectively. 10/18 milk intolerant and 6/6 cereal intolerant patients had an unknown aetiology of their food intolerance. We did not find any relation between reported food intolerances and the amount of food-specific IgG subclasses (Figure 4).

Recent studies indicated that anti-microbial and anti-food pan-IgG/IgA values are not influenced by disease activity and inflammatory status in IBD patients\cite{3,7,35,36}. In accordance with these previous data, we found that clinical disease activity scores (HBI for CD and SCCAI for UC) and inflammatory markers (c-reactive protein, white blood cells, platelets, erythrocyte sedimentation rate) show no clear correlation with

Figure 1. Food- and microbial-specific serum IgG1, IgG2, IgG3 and IgG4 levels in IBD patients and controls. Serum IgG1, IgG2, IgG3 and IgG4 specific for ovalbumin, wheat, milk, mannan from \textit{S. cerevisiae} (ASCA), and lysates from \textit{E. coli} K12 and \textit{B. fragilis} ATCC 25285 were quantified by ELISA in controls (Con; \(n = 62\)) and patients suffering from CD (\(n = 56\)), UC (\(n = 29\)) and acute gastroenteritis/colitis (AGE; \(n = 12\)). Boxes indicate median and 25/75 percentiles and whiskers indicate 10/90 percentiles. \(p\) values (*\(< 0.05\); **\(< 0.01\); ***\(< 0.001\)) were determined by the Kruskal–Wallis test followed by Dunn’s post-hoc test. # indicates \(p < 0.05\) in the Kruskal–Wallis test and \(p < 0.05\) in the Mann–Whitney U test between Con and CD but no significance in the Dunn post-hoc test.
anti-food or anti-microbial IgG subclass values (data not shown). These findings are in line with the fact that anti-food and anti-microbial IgG subclass levels were relatively stable over time in patients investigated at different time points. Repeated measurements in 17 patients performed at two different time points (91 ± 44 days between the two visits, mean ± SD) showed a very high correlation for all measured Ab values (median \( r_s = 0.88 \), range 0.64–0.99, data not shown).

All anti-food IgG subclass levels correlated negatively with age, whereas for anti-microbial Abs we found only a weak negative correlation between age and ASCA IgG1 as well as anti-\( B. \ fragilis \) IgG1, respectively. Conversely, age and anti-\( E. \ coli \) IgG2 as well as anti-\( E. \ coli \) IgG4 showed a positive correlation (Table 4).

### Discussion

The two major questions in this study were whether the analysis of antigen-specific IgG subclass levels may (i) improve diagnostic accuracy for differentiating between CD, UC and controls and (ii) decipher alterations in specific Ab responses that could not be detected by solely analysing pan-IgG. The latter fact was particular interesting for food-specific antigens, since we recently did not find major alterations of food-specific pan-IgGs in IBD patients compared to controls.[7]

Our study shows that ASCA IgG2 and IgG4 exhibit a similar, yet not superior, diagnostic accuracy in differentiating between CD and UC as well as CD and controls. ASCA IgG1/IgG3 and \( B. \ fragilis \) IgG1 were also found to be increased in CD patients,
but their differential capacity was inferior. Our results extend the findings of a recent study that found only ASCA IgG4 being significantly increased in IBD patients,[30] a conclusion that possibly resulted from the investigation of a smaller cohort than in our study. The fact that ASCA IgG2 was preferentially elevated is most likely related to the fact that IgG2 is predominately formed towards polysaccharide antigens.[16,20] IgG Abs towards multiple polysaccharide antigens have been detected in CD patients.[34,37] Whether polysaccharide-specific Abs are responsible for the enhanced total IgG2 concentrations in CD patients that have been detected in several studies,[23–28] however, remains to be determined. The bacterial lysates used in our study contain most likely multiple protein antigens, therefore, it is not surprising that IgG1 was mainly regulated, a subclass that is preferentially formed against protein antigens.[16,19]

It has been suggested that barrier dysfunctions and loss of immune tolerance are responsible for elevated Ab levels to specific microbial antigens in CD patients. Consequently, complicated CD behaviour that is accompanied with increased mucosal damage and chronic inflammation has also been associated with elevated amounts of anti-microbial IgGs and IgAs.[3,7,35,36] Accordingly, we detected significantly higher ASCA IgG1/IgG3/IgG4, anti- \textit{B. fragilis} IgG1 and anti- \textit{E. coli} IgG1 levels in CD patients with perianal involvement and/or strictureting/penetrating disease to CD patients without these complications. However, the changes are not explained by the presence of acute inflammatory flares. All Ab levels measured in our study were independent of clinical disease activity or surrogate serum markers of inflammatory activity. These results are in line with multiple previous studies analysing the

| Food- and microbial-specific Ab levels in CD patients with or without complications. (A) Differences of specific serum pan-IgG, IgA and IgG subclass levels in CD patients without (n = 18; Montreal classification B1) and with complications (n = 37; perianal, strictureting and/or penetrating lesions; Montreal classification B1p, B2, B2p, B3 and B3p) were analysed by the Mann–Whitney U-test. Table indicates p values. Significant differences are marked in colours as specified in the legend. Boxes and whiskers show levels of (B) ASCA IgG1, IgG2, IgG3 and IgG4 and (C) specific IgG1 for all tested antigens. Significant differences are indicated (*p < 0.05; **p < 0.01). |
|---|
| **Figure 3.** Food- and microbial-specific Ab levels in CD patients with or without complications. (A) Differences of specific serum pan-IgG, IgA and IgG subclass levels in CD patients without (n = 18; Montreal classification B1) and with complications (n = 37; perianal, strictureting and/or penetrating lesions; Montreal classification B1p, B2, B2p, B3 and B3p) were analysed by the Mann–Whitney U-test. Table indicates p values. Significant differences are marked in colours as specified in the legend. Boxes and whiskers show levels of (B) ASCA IgG1, IgG2, IgG3 and IgG4 and (C) specific IgG1 for all tested antigens. Significant differences are indicated (*p < 0.05; **p < 0.01). |
| **Table 3.** Food intolerances. |
| n (%) | All foods | Egg | Cereals | Milk |
| All (n = 153) | 67 (44%) | 0 (0%) | 6 (3.9%) | 18 (12%) |
| Controls (n = 58) | 10 (17%) | 0 (0%) | 1 (1.7%) | 2 (3.4%) |
| Crohn’s disease (n = 55) | 36 (65%) | 0 (0%) | 3 (5.5%) | 7 (13%) |
| Ulcerative Colitis (n = 29) | 17 (59%) | 0 (0%) | 1 (3.4%) | 7 (24%) |
| Non-IBD gastrointestinal inflammation (n = 11) | 4 (36%) | 0 (0%) | 1 (9.1%) | 2 (18%) |
| Food intolerances were not documented for 6 individuals (4 Controls, 1 Crohn’s disease, 1 acute gastroenteritis/collitis). |
| 6/6 without definite diagnosis. |
| 7/18 with diagnosed lactose intolerance, 1/18 with diagnosed IgE-mediated milk allergy, 10/18 without definite diagnosis. |

![Figure 4.](image)

(A) Anti-milk and (B) anti-wheat IgG, IgA, IgG1, IgG2, IgG3 and IgG4 levels in patients and controls intolerant to (A) milk and (B) cereals. Boxes indicate median and 25/75 percentiles and whiskers show 10/90 percentiles.
relation of anti-microbial pan-IgG and IgA and IBD activity.[3,36]

In contrast to CD patients, our results did not show any differences between the tested IgG subclass levels in UC patients and controls. In general, reports altered anti-microbial Ab levels in UC are less frequent than for CD.[3] UC is often associated with atypical perinuclear antineutrophil cytoplasmic antibodies (p-ANCAs) [5] that recognise the auto-antigen beta-tubulin isotype 5 (TBB-5) but also cross-react with its bacterial homologue FtsZ, which is expressed by almost all commensal bacteria.[38] However, the fact that an elevated immune recognition of commensal antigens might be less prominent in UC compared to CD remains remarkable since UC always affects the terminal colon, the part of the GI tract with highest commensal density. Possibly the involvement small bowel immune system and/or transmural inflammation in the course of CD may lead to a distinct immune recognition of the microbiota.

Food-specific subclass levels showed particular high levels for IgG4, which were in contrast to the low levels of anti-microbial IgG4. IgG4 has the unique property to exchange one half-antibody with a heavy-light chain pair from another microbial IgG4. IgG4 Abs thereby lose their ability to cross-link antigen and to form immune complexes under most conditions.[39] This mechanism together with the lack of complement activation capacity might provide the basis for the anti-inflammatory activity attributed to IgG4. After allergen-specific immunotherapy, titres of antigen-specific IgG4 are often increased and have been associated with successful tolerance induction.[21] It has been suggested that IgG4 and possibly other IgG subclasses mediate anti-allergic functions by blocking IgE epitopes and co-crosslinking of the inhibitory Fcγ receptor IIb.[17,21] The abundant presence of food-specific IgG4 in our study may therefore indicate immune tolerance towards these antigens. However, it remains elusive why only low levels of microbial-specific IgG4 are formed. One possibility would be that studied microbial antigens are generally less present in gut than major food antigens derived from eggs, milk and wheat. Another explanation could be the fact that homeostatic immune responses towards foods and commensals are differentially regulated. Microbial antigens are normally processed and presented by antigen-presenting cells in context of immune-activating molecular patterns (MAMPs), which may have a large influence on IgG subtype formation also in homeostatic conditions. However, this remains yet speculative and requires further investigation. Of note, specific IgG4 is also predominately formed in response to several parasitic infections,[40,41] and elevated IgG4 serum concentrations (with unknown specificity) are the hallmark of a relatively newly defined group of auto-immune diseases, called IgG4-related systemic diseases, including autoimmune pancreatitis and IgG4-related sclerosing cholangitis.[42]

Additional data that point to the assumption that the formation of anti-microbial and anti-food Abs are differentially regulated are the observation that the level of food-specific Abs decrease with age, which was not a common finding for anti-microbial Abs. The inverse correlation of age and anti-food pan-IgG levels has already been previously reported,[7,43] although the mechanism remains poorly defined. A hypothetical explanation would be that during young age the immunological confrontation with food antigens is very present, as food allergies are also more prevalent in children and young adults.[44] At older age tolerance might be induced and due to immunological diversification and confrontation with many different antigens during lifetime, Ab production to harmless food antigens gets diluted.

The majority of reported food intolerances in adults are non-immune mediated food allergies, e.g. caused by carbohydrate malabsorption. Immune mediated food intolerances are caused by IgE-dependent reactions (classical type I food allergies), cellular reactions (e.g. coeliac disease) or mixed IgE-mediated/cellular reactions (e.g. eosinophilic esophagitis). Previous studies on food-specific IgG in IBD lead to some controversies because different studies have reported divergent results of whether food-specific IgG titres are altered in IBD patients and may predict immune-mediated food intolerance.[7,10,11] Several commercially available assays testing for food specific IgGs are offered to patients implying a diagnostic tool for immune-mediated food intolerances. However, clinical guidelines and reviews generally do not recommend the measurement of food-specific IgG for the diagnosis of food allergies [8,45,46]. As already discussed above, food-specific IgGs, in particular of the IgG4 subclass, are frequently found in healthy individuals and are suggested to reflect the normal immune reaction to foods possibly associated with tolerance induction. In this study, we did not detect any alterations of food-specific IgG subclass levels in IBD patients compared to controls. The presence of adverse reactions to foods in our IBD cohort was very frequent (> 60%); however, the specific foods to which patients (and controls) were intolerant were very heterogeneous. We identified some patients and controls with specific milk and cereal intolerances (11/18 and 6/6 with unknown aetiology, respectively). However, we did not find any correlation of anti-food pan-IgG, IgA [7] or IgG subclass [this study] levels with the presence of general or specific food intolerances.

In summary, this study revealed increased ASCA IgG1, IgG2, IgG3 and IgG4 as well anti-B. fragilis IgG1 levels in CD patients in comparison to controls and UC patients. However, IgG subclass analyses showed no superior diagnostic accuracy than ASCA pan-IgG and IgA. Food-specific IgG subclasses

Table 4. Correlation age and Ab levels (n = 159).

| Correlation coefficient r_s | ≤ -0.2 | ≤ -0.3 | ≥ 0.3 |
|-----------------------------|--------|--------|-------|
| IgG1 | IgG2 | IgG3 | IgG4 |
| Ovalbumin | -0.43*** | -0.32*** | -0.20* | -0.31*** |
| Wheat | -0.29** | -0.31*** | -0.23** | -0.37*** |
| Milk | -0.39*** | -0.42*** | -0.31*** | -0.26** |
| ASCA | -0.28** | -0.15 | -0.03 | -0.15 |
| E. coli | 0.03 | 0.39*** | -0.04 | 0.37*** |
| B. fragilis | -0.20** | -0.10 | -0.03 | -0.10 |

Colours illustrate r_s levels as specified in the legend. Asterisks indicate significant differences (*p < 0.05; **p < 0.01; ***p < 0.001). Colours are only presented in online journal.
were not associated with food intolerance and were not altered in IBD patients. Overall, our study indicates that adaptive immune recognition of food and commensal antigens are differentially regulated in IBD patients.

Acknowledgements

The authors thank Harald Seifert for providing B. fragilis ATCC 25285 and the nursing team of the outpatient clinic of the department of Internal Medicine III at University Hospital Aachen for collecting the patients’ blood samples.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

Funding

This work was supported by the German Research Foundation [DFG SE 1122/1 and SFB985/C3] and the European Union’s Seventh Framework Programme (FP7/2007-2013) under grant agreement n° 305564 (SysmedIBD).

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