Small Bowel Homing T Cells Are Associated With Symptoms and Delayed Gastric Emptying in Functional Dyspepsia

Tobias Liebregts, MD, MBA\textsuperscript{1,2,8}, Birgit Adam, MD, MBA\textsuperscript{1,2,8}, Christoph Bredack, PhD\textsuperscript{2}, Montri Gururatsakul, MD\textsuperscript{2,3}, Katherine R. Pilkington, PhD\textsuperscript{4}, Stuart M. Brierley, PhD\textsuperscript{2}, L. Ashley Blackshaw, PhD\textsuperscript{2}, Guido Gerken, MD, PhD\textsuperscript{1}, Nicholas J. Talley, MD, PhD\textsuperscript{5} and Gerald Holtmann, MD, PhD, MBA\textsuperscript{6,7}

OBJECTIVES: Immune activation may have an important pathogenic role in the irritable bowel syndrome (IBS). While little is known about immunologic function in functional dyspepsia (FD), we have observed an association between cytokine secretion by peripheral blood mononuclear cells (PBMCs) and symptoms in IBS. Upper gastrointestinal inflammatory diseases are characterized by enhanced small bowel homing $\alpha$4-, $\beta$7-integrin, chemokine receptor 9 (CCR9) positive T lymphocytes. We hypothesized that increased cytokine release and elevated circulating small bowel homing T cells are linked to the severity of symptoms in patients with FD. Thus, we aimed to (i) compare cytokine release in FD and healthy controls (HCs), (ii) quantify "gut homing" T cells in FD compared with HC and patients with IBS, and (iii) correlate the findings to symptom severity and gastric emptying.

METHODS: PBMC from 45 (Helicobacter pylori negative) patients with FD (Rome II) and 35 matched HC were isolated by density gradient centrifugation and cultured for 24 h. Cytokine production (tumor necrosis factor (TNF)-$\alpha$, interleukin (IL)-1$\beta$, IL-6, IL-10) was measured by enzyme-linked immunosorbent assay. CD4$^+$ $\alpha$4$\beta$7$+$ CCR9$^+$ T cells were quantified by flow cytometry in FD, HC and 23 patients with IBS. Gastric emptying was measured by scintigraphy. Symptom severity was assessed utilizing the standardized Gastrointestinal Symptom Score.

RESULTS: FD patients had significantly higher TNF-$\alpha$ (107.2±42.8 vs. 58.7±7.4 pg/ml), IL-1$\beta$ (204.8±71.5 vs. 80.2±17.4 pg/ml), and IL-10 (218±63.3 vs. 110.9±18.5 pg/ml) levels compared with HC, and enhanced gut homing lymphocytes compared with HC or IBS. Cytokine release and CD4$^+$ $\alpha$4$\beta$7$+$ CCR9$^+$ lymphocytes were correlated with the symptom intensity of pain, cramps, nausea, and vomiting. Delayed gastric emptying was significantly associated ($r=0.78$, $P=0.021$) with CD4$^+$ $\alpha$4$\beta$7$+$ CCR9$^+$ lymphocytes and IL-1$\beta$, TNF-$\alpha$, and IL-10 secretion.

CONCLUSIONS: Cellular immune activation with increased small bowel homing T cells may be key factors in the clinical manifestations of H. pylori-negative FD.

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INTRODUCTION

Chronic abdominal pain or discomfort is common and frequently these patients seek health care (1). In >50% of patients presenting with chronic abdominal symptoms, no structural lesion is found after a standard diagnostic work-up that is likely to explain these symptoms (2). Consequently, patients are diagnosed as having a functional gastrointestinal disorder (FGID); the best-recognized disorders are the irritable bowel syndrome (IBS) and functional dyspepsia (FD) (3). Unfortunately, the underlying pathophysiological mechanisms in IBS and FD still remain poorly understood.

\textsuperscript{1}Department of Internal Medicine I, University Hospital Schleswig-Holstein, Kiel, Germany; \textsuperscript{2}Nerve-Gut Research Laboratory, Hanson Institute, Adelaide, SA, Australia; \textsuperscript{3}Department of Gastroenterology and Hepatology, University of Adelaide, Royal Adelaide Hospital, Adelaide, SA, Australia; \textsuperscript{4}Deltmol Family Imaging Centre, Institute of Medical and Veterinary Science, Adelaide, SA, Australia; \textsuperscript{5}University of Newcastle, Callaghan, NSW, Australia; \textsuperscript{6}University Hospital of Essen, Essen, Germany; \textsuperscript{7}Department of Medicine, University of Adelaide, Adelaide, SA, Australia; \textsuperscript{8}These authors contributed equally to this study. Correspondence: Tobias Liebregts, MD, MBA, Department of Internal Medicine I, University Hospital Schleswig-Holstein, Kiel, Arnold-Heller-Str, 24105 Kiel, Germany. E-mail: tobias.liebregts@uk-sh.de

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It is now a well-established concept that transient infections can precipitate the onset of lower or upper functional gastrointestinal symptoms in at least a subgroup (4). There is also increasing evidence for an underlying immune activation in IBS, with increased systemic cytokine levels (5,6). Interestingly, higher numbers of intraepithelial T lymphocytes (7) and enhanced expression and release of cytokines at the mucosal level have only been observed in post-infectious IBS (8). Our group has previously identified an association between peripheral blood mononuclear cell (PBMC)-mediated cytokine (TNF-α, interleukin (IL)-1β, IL-6) secretion and symptomatic manifestations in IBS (6). However, in patients with FD relatively little is known about immunologic function and its role in symptom generation.

Expression of α4-integrin (CD49d) and β7-integrin on T lymphocytes delineates a specific cell population that preferentially migrates to the gut (9). Gut-tropic T cells expressing high levels of α4, β7 and the chemokine receptor (CCR9) preferentially migrate to the lamina propria of the small intestine (10). In healthy subjects, circulating CD4+CCR9+ T cells demonstrate characteristics of mucosal T lymphocytes with an activated phenotype and exhibit a Th1 cytokine profile (11). The intestinal ligand of CCR9 is CCL25, and it is mainly expressed in mucosal epithelium. Thus, CCR9 can be considered to be relatively mucosa specific (12). As the frequency of CCR9+ T lymphocytes in the peripheral blood of patients with small bowel inflammatory diseases such as Crohn’s disease and celiac disease is increased (13), characterization of circulating CD4+αββ7+CCR9+ T lymphocytes in FD may serve as an indirect measure of upper gastrointestinal mucosal immune activation.

Based upon our finding in IBS patients, we therefore hypothesized that compared with healthy controls (HCs), patients with FD are characterized by an underlying immune activation with (i) an enhanced production of cytokines, namely TNF-α, IL-1β, IL-6, and IL-10 and (ii) an increased proportion of circulating “small bowel homing” T lymphocytes (CD4+ T lymphocytes coexpressing the gut homing marker α4-integrin (CD49d), β7-integrin, and CCR9).

METHODS

Study population
The following groups were studied after obtaining informed consent:

1. Forty-five consecutive Helicobacter pylori-negative patients with FD (26 females/19 males, median age 47.6 years) defined, according to the Rome II criteria (14), as recurrent abdominal pain or discomfort centered in the upper abdomen for at least 12 weeks within the preceding 12 months.
2. Thirty-five age, gender and body mass index matched healthy asymptomatic subjects (23 females/12 males, median age 41.8 years).
3. Twenty-three patients (14 females/9 males, median age 44.2 years) with IBS (9 diarrhea-predominant, 7 alternating, 7 constipation-predominant) according to Rome II criteria (15).

None of the patients included reported a transient gastroenteritis preceding the onset of their symptoms. Patients were recruited from the Outpatient Clinic of the Department of Gastroenterology and Hepatology at the Royal Adelaide Hospital, while healthy volunteers were recruited by advertisement.

A comprehensive diagnostic work-up including upper endoscopy had not revealed structural lesions or any evidence for acute infection or celiac disease as the cause of symptoms. Absence of H. pylori was confirmed by histology and rapid urease test. In all subjects, after symptom assessment (described below) and before endoscopy procedure, a blood sample was taken between 0800 and 1000 hours and a full blood count, renal and liver function, fibrinogen, serum IgE, C-reactive protein, and Hba1c measured to exclude subjects with medical conditions that may have confounded the study aims. All subjects with overlap of FD and IBS or concomitant chronic fatigue syndrome, fibromyalgia or a history of anti-inflammatory, analgesic or immunosuppressive medication (nonsteroidal anti-inflammatory drugs, steroids, etc.) within the last 3 months were excluded. The study was approved by the Royal Adelaide Hospital Human Ethics Committee.

Symptom assessment
In patients with FD, abdominal symptoms were assessed utilizing the gastrointestinal symptom score. The gastrointestinal symptom score is a valid and reliable instrument that assesses symptom intensities in patients with FD consisting of 10 items graded on 5-point Likert scales (16). The following were considered key symptoms and preselected before the data analysis: upper abdominal pain, cramps, fullness, satiety, nausea, and vomiting.

Psychological comorbidity
Utilizing the standardized Hospital Anxiety and Depression Scale two subscales of anxiety and depression were calculated. Responders who score ≥11 on either subscale were considered as having a clinically relevant psychological disorder (17).

Gastric emptying
The overnight fasted subject was prepared for the test by sitting the person comfortably in front of a large field of view γ camera. Thereafter, subjects were asked to drink 300 ml Ensure mixed with radiopharmaceutical 99mTc-Sulfur colloid (20 MBq). Ensure is a readily available liquid nutritional food supplement, which is pleasant tasting and has a defined calorific content.

With the subjects positioned, the large field of view γ cameras captured dynamic images of the stomach and distribution of the test meal contained for a total of 4h. Time 0 (t = 0 min) was defined as the time of completion of the drink. A region of interest was drawn around the total stomach (18). Data were corrected for subject movement, radionuclide decay, and γ-ray attenuation (19). The retention of the drink in the total stomach was derived for t = 0–240 min at 15 min interval and expressed as percentage of the counts in the total stomach, where 100% immediately followed ingestion of the drink. Delayed gastric emptying was defined as $T_{95} > 95\%$ confidence interval in healthy volunteers (116 min).

Cell isolation and culture conditions
For the following cell culture, enzyme-linked immunosorbent assay and flow cytometry experiments investigators were blinded
with regard to study subject details including diagnosis, age, and gender. PBMCs were freshly isolated by density gradient centrifugation. Diluted blood (1:2 in RPMI 1640) was layered onto Ficoll-Hypaque (Sigma, Castle Hill, NSW, Australia) and centrifuged at 400 g for 15 min. PBMC were washed twice with sterile phosphate-buffered saline and viability was assessed by trypan blue exclusion. PBMC were re-suspended to 1 × 10^6 cells/ml in complete medium (RPMI 1640 medium (Gibco, Karlsruhe, Germany), supplemented with 10% fetal calf serum, 100 U/ml penicillin, 0.1 mg/ml streptomycin, and L-Glutamine). PBMC were cultured in 24-well plates for 24 h at 37°C in a humidified 5% CO₂ atmosphere.

**Enzyme-linked immunosorbent assay**

Cell free culture supernatant was collected, diluted 1:1 in RPMI and stored at −20°C until assayed. TNF-α, IL-1β, IL-10, and IL-6 were quantified using ELISA kits (eBioscience, San Diego, CA) according to the manufacturer’s instructions with minor modifications. Optical density was measured at a wavelength of 450 nm and a reference wavelength of 590 nm. Density values were linearly correlated with the concentrations of cytokine standards. The limit of sensitivity of the assays was 5 pg/ml.

**Flow cytometry**

To identify small bowel homing T cells freshly isolated PBMC (10^5) were labeled with previously determined optimal concentrations of monoclonal antibodies (Becton-Dickinson, North Ryde, NSW, Australia) directed against human CD4 (APC-Cy7), CD49d (α4-integrin) fluorescin (FITC), β7-integrin (PE), and CCR9 (Alexa647). Matching IgG1 and IgG2a-conjugated antibodies served as isotype controls. Cells were incubated with the antibodies for 30 min on ice and washed three times in FACS buffer between each step. Nonspecific binding of antibodies was minimized by a 10-min incubation of cell suspensions with pooled human serum (10%) in FACS buffer before addition of the first antibody. After washing twice, cells were re-suspended in 100 μl of 1% paraformaldehyde in PBS and analyzed utilizing a Beckman Coulter FC500 (Beckman Coulter, Gladesville, NSW, Australia). Lymphocyte populations were gated based on forward scatter/side scatter properties. A total of 3 × 10^4 events was routinely collected and analyzed.

**Data analysis**

The two hypotheses tested were that there is a difference with regard to cytokine release from isolated PBMCs and a difference in the proportion of circulating “small bowel homing” T lymphocytes between patients and controls. Analyses of variance with age, gender, and BMI as covariables and for the circulating gut homing cells, the total number of cells were used. In addition, the relationship between the cytokine levels, percentage of gut homing cells, and the intensity of symptoms and gastric emptying was assessed utilizing Spearman rank correlations. For the primary outcome parameters (cytokines and circulating gut homing T cells), P values ≤ 0.025 were considered significant. For all other secondary comparisons, a P level of 0.05 was used as the significance level. Data are presented as mean ± standard error of the mean. For the statistical analysis SAS Version 6.12 and SPSS Version 12 (Statistical Package for Social Sciences, Chicago, IL) were used.

**Results**

**Cytokine levels**

Patients with FD showed significantly (P < 0.025) higher PBMC-mediated cytokine secretion for TNF-α (107.2 ± 42.8 vs. 58.7 ± 7.4 pg/ml; P = 0.02 (Figure 1)), IL-1β (204.8 ± 71.5 vs. 80.2 ± 17.4 pg/ml; P = 0.003 (Figure 2)), and IL-10 (218 ± 63.3 vs. 102 ± 42.8 pg/ml; P = 0.003) (Figures 1 and 2).
110.9±18.5 pg/ml; P = 0.007 (Figure 3) compared with HC subjects. There was a trend towards increased IL-6 (1768±682.3 vs. 938±184.3; P = 0.033 (Figure 4)) secretion, which failed to reach statistical significance. Correlations between cytokine levels in FD were found for TNF-α with IL-1β (r = 0.50, P = 0.015), TNF-α with IL-10 (r = 0.55, P = 0.01), IL-1β with IL-10 (r = 0.81, P < 0.001), and IL-10 with IL-6 (r = 0.49, P = 0.02).

In accordance with our previously published data (6) patients with D-IBS showed significantly increased TNF-α (172.3±26.3 pg/ml, P < 0.001), IL-1β (289.9±58.6 pg/ml, P = 0.003), IL-6 (1701.9±171.6, P = 0.02), and IL-10 levels (285.5±54.2 pg/ml, P = 0.011) compared with HC. No significant differences were observed for C-IBS (TNF-α: 37.6±5.8, P = 0.904/IL-1β: 75.4±30.2, P = 0.05/IL-10: 113.8±36.8, P = 0.52/IL-6: 615.9±104.2, P = 0.36) or A-IBS (TNF-α: 29.5±7.8, P = 0.63/IL-1β: 25.1±6.3, P = 0.66/IL-10: 141.5±73.9, P = 0.52/IL-6: 402.9±129.9, P = 0.17).

**Circulating gut homing T cells**

The percentage of circulating CD4+ T cells within the PBMC population did not differ between patients with FD, HCs, or IBS (data not shown). CD4+ T lymphocytes coexpressing the gut homing marker α4-integrin (CD49d), β7-integrin, and CCR9 (CD4+α4β7+CCR9+) (Figure 5) were significantly increased in patients with FD (Figure 6) compared with healthy subjects (5.6±3.5% vs. 1.6±1.0% (P = 0.001)) and IBS (2.1±0.56% (P<0.001)). No significant differences for CD4+α4β7+CCR9+ lymphocytes were observed between IBS subgroups.

**Symptom association**

In patients with FD symptom intensity measured by the gastrointestinal symptom score was closely linked to cytokine levels. IL-1β (Figure 7) and IL-10 (Figure 8) levels were positively correlated with upper abdominal pain, cramps, nausea, and vomiting. Furthermore, TNF-α
levels were significantly \((P<0.05)\) associated with cramps and vomiting while IL-6 was associated with higher scores of abdominal pain. Increased percentage of small bowel homing \(CD4^+\alpha4\beta7^+CCR9^+\) lymphocytes was correlated with higher symptom intensity of pain, cramps, nausea, and vomiting (Figure 9). In contrast, the symptoms fullness and early satiety were not associated with cytokine levels or small bowel homing T lymphocytes (Table 1).

**Psychological comorbidity association**
Anxiety scores were significantly higher in patients with FD compared with HCs \((11.5\pm0.3\ vs.\ 5.3\pm0.6,\ P<0.001)\). Clinical relevant depression was more frequent in FD than in HC \((11.8\pm0.4\ vs.\ 5.5\pm0.7,\ P<0.001)\). No correlation was observed between cytokine secretion and psychological comorbidity.

**Gastric emptying association**
Gastric emptying was significantly delayed in patients with FD \((164.5\pm28.5\ min)\) compared with HCs \((101\pm10.4\ min)\). Overall delayed gastric emptying was significantly associated with increased IL-1\(\beta\) \((r=0.97,\ P=0.032)\), TNF-\(\alpha\) \((r=0.98,\ P=0.003)\), and IL-10 \((r=0.91,\ P=0.032)\) levels. In addition, small bowel homing \(CD4^+\alpha4\beta7^+CCR9^+\) lymphocytes were correlated (Figure 10) with delayed gastric emptying \((r=0.78,\ P=0.021)\).

**DISCUSSION**
Our study has demonstrated an association of systemic cellular immune activation with symptom manifestations in patients fulfilling the Rome II criteria for FD. Secretion of pro- and anti-inflammatory cytokines (IL-1\(\beta\), TNF-\(\alpha\), IL-10) by PBMCs was significantly increased in patients with FD compared with HC subjects. In contrast to IBS, the proportion of \(CD4^+\alpha4\beta7^+CCR9^+\) gut homing T cells was markedly enhanced in FD. Overall delayed gastric emptying and higher intensity of pain, cramps, nausea, and vomiting but not fullness or satiety were associated with increased levels were significantly \((P<0.05)\) associated with cramps and vomiting while IL-6 was associated with higher scores of abdominal pain. Increased percentage of small bowel homing \(CD4^+\alpha4\beta7^+CCR9^+\) lymphocytes was correlated with higher symptom intensity of pain, cramps, nausea, and vomiting (Figure 9). In contrast, the symptoms fullness and early satiety were not associated with cytokine levels or small bowel homing T lymphocytes (Table 1).

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cytokine levels and CD4+α4β7+CCR9+ small bowel homing T lymphocytes.

Underlying immune activation with increased cytokine production has been postulated to have a key role in the pathophysiology of FGIDs (6,20). In IBS, increased serum (20,21) and PBMC-mediated pro-inflammatory cytokine secretion (5,6) has been reported and there is some limited data that IBS patients have a genetic tendency to excessive immune response but this is based on very small numbers and needs confirmation in much larger studies (22). While we confirmed our previous results of increased TNF-α, IL-1β, and IL-6 levels, we also found increased PBMC-mediated anti-inflammatory IL-10 levels in IBS. In contrast, O’Mahony et al. (5), reported decreased IL-10 levels while others demonstrated increased (23) or unchanged serum IL-10 (21,24) necessitating further exploration. With regard to FD, recent findings indicate altered antigen-induced cytokine production in (n=23) patients with FD (23). In accordance with our results of enhanced spontaneous immune cell-mediated cytokine secretion, it might be speculated that low-grade immune activation reflects a common pathophysiological mechanism in both IBS and FD.

IBS is characterized by increased mucosal T lymphocytes (7) and mast cells (25) while alterations of circulating T cells are limited to a few T-lymphocyte subsets such as CD4+CD69+ and CD4+β7-integrin+ T cells (26,27). There are conflicting data on mucosal immune cell infiltration in FD. A higher mast cell frequency has been reported in gastric biopsies of patients with H. pylori positive and negative dyspepsia (28) while no difference in mast cell or lymphocyte infiltration was found in the duodenum of FD patients (29). Conversely, Talley et al. (30) identified duodenal but not gastric eosinophilia in FD. A recent study by Kindt et al. (31) showed increased duodenal intraepithelial CD8+, but decreased CD4+ cells. Despite altered mucosal CD4+ T-cell numbers in FD and IBS, in line with our current results, circulating CD4+ T-cell numbers seem to be unchanged (23,27).

In our study, we could not correlate minor mucosal pathologic changes with cellular immune activation but we identified an increased number of circulating CD4+ T cells coexpressing the gut homing marker α4-integrin, β7-integrin, and CCR9, which delineates a subset of T cells preferentially migrating to the lamina propria of the small intestine (10). The intestinal ligand of CCR9, CCL25, is selectively expressed in the thymus and small intestine but not the colon (32). Although our findings are somewhat indirect and do not necessarily allow, in the absence of biopsies, to conclude that chronic upper gastrointestinal inflammation is present in patients with FD, the results support mucosal immune activation probably in the small intestine. We identified enhanced PBMC-mediated cytokine secretion in FD and confirmed our previous findings of increased cytokine secretion in patients with diarrhea-predominant IBS (6).

We acknowledge that determination of circulating and mucosal cytokine levels has frequently resulted in conflicting data (33). In patients with inflammatory bowel disease PBMC-mediated cytokine levels are markedly different from lamina propria mononuclear cell-induced cytokine levels with enhanced circulating (34) and even suppressed mucosal cytokine secretion.
clinical activity indices remains controversial (39,41). While we have previously demonstrated an association of mainly diarrhea-associated symptoms and PBMC-mediated cytokine secretion in IBS (6), the present study revealed a correlation of cytokine secretion by circulating immune cells and symptom severity in patients with FD. It is noteworthy that the various cytokines were positively correlated, indicating a heterogeneous population with some patients showing (35). In contrast, PBMCs can display similar responses with regard to proliferation and cytokine secretion as lamina propria mononuclear cells when they are exposed to bacterial antigens, such as *H. pylori* (36). PBMC migrating towards such an antigen have been demonstrated to express a higher level of CCR9 (37). In addition, circulating CD4 + CCR9 + T lymphocytes demonstrate characteristics of mucosal T cells with an activated phenotype and a Th1 cytokine profile (11). Interestingly, there appears to be an inverse association with reduced CCR9 + T cells within inflamed small bowel mucosa but enhanced peripheral blood small bowel homing T cells in patients with small bowel inflammatory diseases such as Crohn’s disease and celiac disease (13,38). Against this background it is noteworthy that reduced duodenal CD4 lymphocyte infiltration has been reported in post-infectious FD (31) while we were able to demonstrate increased circulating small bowel homing CD4 + T cells. Thus, our findings of a higher frequency of CD4 + α4β7 + CCR9 + small bowel homing lymphocytes in patients with FD suggest an ongoing upper gastrointestinal immune activation although direct extrapolation from circulating immune cells to mucosal inflammation needs to be viewed very cautiously.

There is still limited knowledge about the role of immune activation in driving the symptom manifestations in patients with FGIDs. In patients with inflammatory bowel disease, cytokine secretion has been frequently reported to reflect the severity of inflammation (39) and in particular TNF-α, IL-1β, and IL-6 are believed to reflect disease activity (40). However, the correlation of cytokine levels with clinical activity indices remains controversial (39,41). While we have previously demonstrated an association of mainly diarrhea-associated symptoms and PBMC-mediated cytokine secretion in IBS (6), the present study revealed a correlation of cytokine secretion by circulating immune cells and symptom severity in patients with FD. It is noteworthy that the various cytokines were positively correlated, indicating a heterogeneous population with some patients showing
more severe immune alterations while others appear to be less affected. Furthermore, we were able to demonstrate a correlation of small bowel homing T cells with symptom manifestations. Thus, our data support a pathophysiological role of enhanced immune cell migration in FD. Consistent with these observations, others have observed a link between mucosal mast cell infiltration and upper abdominal symptoms including abdominal pain and nausea (42), while Talley et al. (30) identified an association between duodenal eosinophilia with pain, nausea, retching, and early satiety in community subjects with FD.

Importantly, the direction of association between immune activation and symptom manifestation remains to be further elucidated. In particular, psychological factors, such as anxiety and depression are known to be associated with symptom severity in patients with FGIDs (43,44). Psychological stress itself can induce depression are known to be associated with symptom severity in patients with FGIDs (43,44). Psychological stress itself can induce stress can trigger bacterial endotoxin-stimulated peripheral and mucosal cytokine release (48). In our study, psychological disorders were more frequent in FD than in HCs. However, anxiety and depression were not correlated with baseline cytokine levels. In line with our previous findings of anxiety-associated LPS-induced TNF-α secretion in IBS (6) recent data demonstrate increased antigen induced but not baseline cytokine secretion in patients with FD and concomitant anxiety and depression compared with FD without clinically relevant psychological disorder (23). Thus, it is reasonable to speculate that both immune activation and symptom manifestation could be centrally or psychologically driven.

On the other hand, inflammation may induce psychological disorders. Clinical observations suggest a higher prevalence of psychological disorders in patients with inflammatory bowel disease during exacerbation compared with inflammatory bowel disease in remission. Based on animal models, inflammation is known to induce anxiety-like behavior (49) by modulating autonomic and central nervous system processing (50). Cytokines have been identified to stimulate the hypothalamic–pituitary–adrenal axis either directly or via activation of nociceptive, visceral, and somatosensory afferents (51). Indeed in IBS, increased serum cytokine levels are associated with an overactive hypothalamic–pituitary–adrenal axis (20). Although association with symptom manifestation remains controversial (52), immune activation is likely to influence complex behavioral processes, as well as affective state (53).

Based on animal models, cytokines are known to inhibit gastric emptying (54). TNF-α may induce gastroparesis by modulating intrinsic vago-vagal reflex pathways (55) and prolonged IL-1β exposure affects neuronal transmission with profound effects on gastrointestinal motility (56). As we identified a correlation of increased TNF-α, IL-1β, and IL-10 levels with delayed gastric emptying, it is reasonable to speculate that persisting underlying immune activation may have a role in altered motility in patients with FD.

The potential immune cell–gut interactions involved in the manifestation of symptoms in FGIDs remain to be further elucidated. Based on animal models, it is known that acute gastrointestinal inflammation alters visceral sensory function with lowered thresholds for visceral stimuli (57). Furthermore, mucosal inflammation may trigger persisting alterations of visceral sensitivity (45,58), with an association between severity of the inflammatory stimulus and symptom intensity (59). In IBS, Barbara et al. (25) showed a potential modulation of abdominal pain by mast cells located in close proximity to visceral afferents, possibly mediated via histamine and tryptase release. Our finding of a correlation between symptom severity and cytokine release may also point towards the role of mediators released by circulating immune cells in generating gastrointestinal symptoms. This hypothesis is further supplemented by our recent findings demonstrating a direct stimulatory effect of PBMC supernatants on visceral sensory afferents (60).

Table 1. Correlation of CD4+α4β7+CCR9+ T cells and cytokine levels (mean±s.e.) with symptoms in patients with functional dyspepsia was analyzed using Spearman rank correlations

<table>
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<tr>
<th></th>
<th>Pain</th>
<th>Cramps</th>
<th>Fullness</th>
<th>Satiety</th>
<th>Nausea</th>
<th>Vomiting</th>
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<td>r=0.46</td>
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<td>r=0.40</td>
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<tr>
<td>IL-10</td>
<td>r=0.33</td>
<td>r=0.58</td>
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<td>P=0.003*</td>
<td>P=0.626</td>
<td>P=0.158</td>
<td>P=0.020*</td>
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<tr>
<td>TNF-α</td>
<td>r=0.14</td>
<td>r=0.57</td>
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<td>P=0.520</td>
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<tr>
<td>IL-6</td>
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<td>r=0.23</td>
<td>r=0.14</td>
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<td>P=0.206</td>
<td>P=0.436</td>
<td>P=0.264</td>
<td>P=0.190</td>
<td>P=0.276</td>
</tr>
</tbody>
</table>

IL, interleukin; TNF, tumor necrosis factor.

*P<0.05 was considered significant. Overall cytokine release was correlated with symptom intensity of abdominal pain, cramps, nausea, and vomiting.
There are potential limitations to this study that need to be acknowledged. Subjects were studied at a tertiary referral center, and a larger patient sample needs to be studied to confirm current observations as selection bias cannot be totally excluded. Mucosal biopsy data are lacking and direct extrapolation from peripheral to lamina propria mononuclear cells needs to be viewed cautiously. Despite these limitations, our observations of increased cytokine secretion, enhanced circulating small bowel homing T lymphocytes, and its association with symptom severity and delayed gastric emptying in FD are novel; this finding has partly been reported in patients with upper gastrointestinal Crohn’s disease (13), and our results are most consistent with an inflammatory pathophysiological mechanism being responsible for the manifestations of FD.

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CONFLICT OF INTEREST

Guarantor of the article: Tobias Liebregts, MD, MBA.
Specific author contributions: Planned the study and designed the protocol: Tobias Liebregts, Birgit Adam, L. Ashley Blackshaw, Nicholas J. Talley, and Gerald Holtmann; involved in patient recruitment and symptom assessment: Birgit Adam, Montri Gururatsakul, and Gerald Holtmann; conducted cell culture experiments: Tobias Liebregts, Birgit Adam, Christoph Bredack, and Montri Gururatsakul; conducted flow cytometry experiments: Katherine R. Pilkington and Christoph Bredack; conducted gastric emptying scintigraphy: Birgit Adam and Montri Gururatsakul; provided statistical advice, performed data analysis, and interpretation: Gerald Holtmann, Nicholas J. Talley, and Stuart M. Brierley; participated in clinical data analysis, review, and report writing: Guido Gerken. All authors have contributed to manuscript preparation and have approved the final draft of the manuscript.

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Potential competing interests: None.

Study Highlights

WHAT IS CURRENT KNOWLEDGE

✓ Functional gastrointestinal disorders (FGIDs) are highly prevalent.
✓ Immune activation might have a role in the etiopathogenesis of FGIDs.

WHAT IS NEW HERE

✓ Patients with functional dyspepsia display enhanced baseline cytokine secretion.
✓ Small bowel homing T cells are increased in functional dyspepsia but not irritable bowel syndrome.
✓ Gut homing T cells and increased cytokine levels are associated with symptom manifestations and delayed gastric emptying in functional dyspepsia, suggesting a causal role.
✓ These observations may identify biomarkers and have treatment implications in functional dyspepsia.

REFERENCES