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MAJOR ARTICLE

Prolonged Duodenal Mucosal Lymphocyte Alterations in Patients With and Without Postinfectious Functional Gastrointestinal Disorders After *Giardia* Infection

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Background. Persisting low-grade inflammation is suggested to play a role in postinfectious functional gastrointestinal disorders (PI-FGIDs). The present study examined alterations in duodenal mucosal lymphocytes during and after *Giardia* gastroenteritis in patients who did, or did not, develop PI-FGIDs.

Methods. Duodenal mucosal intraepithelial lymphocytes (IELs) and lamina propria CD3, CD4, CD8, and CD20 lymphocytes were quantified in 28 patients with chronic giardiasis (CG), 66 patients with persistent abdominal symptoms after acute *Giardia* infection (PI-FGID), 19 recovered controls (RCs), and 16 healthy volunteers (HCs). Associations with illness duration, abdominal symptoms, and histology grade were assessed.

Results. Duodenal CD4 IELs were significantly elevated in CG, then decreased, followed by an upward trend after 1 year in both the PI-FGID and RC groups. Duodenal lamina propria crypt CD4 T cells were decreased in CG, and stayed low for about 14 months before normalizing in both the PI-FGID and RC groups. Lamina propria CD20 cells were persistently elevated in all 3 *Giardia*-exposed groups. Biopsies with microscopic inflammation showed increased lamina propria CD20 levels.

Conclusions. Duodenal mucosal lymphocyte alterations were prolonged after *Giardia* infection, but similar in patients who developed PI-FGID and recovered asymptomatic controls.

Keywords. duodenal mucosa; *Giardia*; functional gastrointestinal disorders; PI-IBS; histology; B cell.

Giardia lamblia (synonyms *duodenalis*, *intestinalis*) is an intestinal protozoan parasite that infects the small intestine causing giardiasis, resulting in a variable spectrum of abdominal symptoms. It often causes a gastroenteritis with prolonged diarrheal illness and abdominal cramping, but may also be asymptomatic. It is commonly seen in returning travelers from low-resource settings and a frequent cause of waterborne outbreaks.

Giardiasis has been recognized as a risk for developing postinfectious functional gastrointestinal disorders (PI-FGIDs) [1–4]. Irritable bowel syndrome (IBS) is the most common of these conditions and occurs in 3%–36% of individuals after infectious gastroenteritis [5, 6]. Follow-up studies of laboratory-confirmed *Giardia* infection after an outbreak in Bergen, Norway, found a high prevalence of IBS after 3, 6, and 10 years [4, 7, 8].

The mechanisms behind development of FGID are not known, but are regarded to be multifactorial. Several studies suggest that persisting low-grade inflammation, with increased numbers of mucosal B and T lymphocytes, could be an important contributing factor [6, 9, 10].

Giardia infection is known to elicit both B- and T-cell–dependent immune responses [11–13]. Animal studies have shown that microvillous injury, disaccharidase deficiencies, and increased crypt/villus ratio are mediated by CD8 cells, while CD4 cells contribute to parasite clearance [14]. Mucosal lymphocyte kinetics during and after giardiasis, and their potential association with development of PI-FGID, have not been examined before.

The aim of the present study was to evaluate lymphocyte alterations in the duodenal mucosa in giardiasis and to examine whether such alterations were associated with persisting abdominal symptoms following *Giardia* infection.

MATERIALS AND METHODS

Study Subjects

This study is based on the clinical and research data and specimens obtained during a structured workup and follow-up of

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patients referred to Haukeland University Hospital in Bergen, Norway [15]. Patients were referred due to persisting abdominal symptoms after the *Giardia* outbreak in Bergen in 2004. They were examined and biopsied between January 2005 and April 2006 (Figure 1), at which time they all had been ill for 3 months or more. Biopsies were available from 28 patients with chronic giardiasis (CG) and from 72 patients (66 of these randomly selected for this study) in whom *Giardia* had been successfully eradicated (diagnosed by at least 3 microscopy-negative samples and later verified with negative polymerase chain reaction). A detailed workup including upper endoscopy with duodenal biopsies, serum antiendomysial antibodies, antitissue transglutaminase antibodies, routine blood screening tests, immunoglobulins, immunoglobulin E, and fecal calprotectin did not reveal other organic disease in the *Giardia*-negative patients, who were diagnosed as having PI-FGID. To evaluate possible coinfection, *Helicobacter pylori* was analyzed in frozen stool samples by stool antigen test [16] in 20 randomly selected PI-FGID patients.

Control Groups

Nineteen patients with laboratory-confirmed giardiasis during the outbreak, who had recovered well after treatment, were selected randomly and recruited by invitation letter/telephone and examined 12–19 months after onset of the gastroenteritis [17]. These patients were designated the recovered control (RC) group. Additionally, 16 healthy controls (HCs), with no history of persisting bowel symptoms and not taking immunosuppressive medication, were recruited by advertisements and went through the same investigations as the cases (Figure 1).

All participants provided written informed consent and the study was approved by the Regional Committees for Medical and Health Research Ethics (REC WEST, Norway) number 2016/1632.

Symptoms

Abdominal symptom scores for the last 30 days were recorded on the day biopsies were taken. Nausea, bloating, abdominal pain, diarrhea, and constipation were scored using an ordinal scale from 0 (no symptoms) to 10 (severe symptoms) [18]. Illness duration was defined as the time from the start of acute symptoms of *Giardia* infection until the date of clinical examination with biopsies. At follow-up, PI-FGID patients were asked to complete a validated Norwegian version of the ROME II questionnaire [19].

Duodenal Biopsies and Histology

From all study groups, 3 biopsy specimens were obtained from the second part of the duodenum, embedded in paraffin, and processed for routine hematoxylin and eosin staining and immunohistochemistry. In 11 of the PI-FGID patients examined at 16–19 months of illness duration, biopsies were available from a previous examination 3–7 months after onset of symptoms, when 8 were still *Giardia* positive and 3 were *Giardia* negative.

The severity of duodenal inflammation, as well as the architecture of villi and crypts, was determined by an experienced pathologist in a blinded manner. The routine histological findings were classified as H0 if they were normal and as H1 if there was inflammation with infiltration of leukocytes and increased number of plasma cells in the lamina propria with or without shortening and blunting of intestinal villi (Supplementary Figures 1–3).

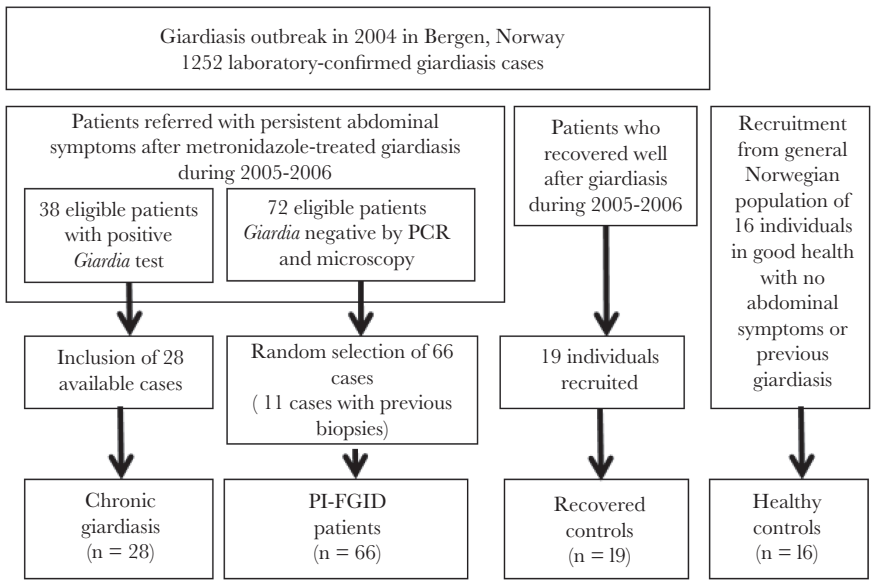

Figure 1. Flowchart of the study population and study design. Abbreviations: PCR, polymerase chain reaction; PI-FGID, postinfectious functional gastrointestinal disorder.

Immunohistochemistry

Formalin-fixed, paraffin-embedded duodenal specimens were cut into 4-μm sections, de-paraffinized in xylene, and rehydrated through graded ethanol series and distilled water.

After heat-induced epitope retrieval in Tris–ethylenediaminetetraacetic acid buffer, pH 9.0 for 15 minutes at 350 W, endogen peroxidase activity was blocked with 0.3% peroxide (Dako) for 5 minutes. Tissue was then incubated with primary antibodies: CD3 (polyclonal rabbit antihuman CD3, 1/400, Dako), CD4 (monoclonal antibody NCL-CD4-IF6, clone IF6, 1/25), CD8 (monoclonal mouse antihuman CD8α, clone C8/144B, 1/100, Dako), and CD20 (monoclonal mouse antihuman CD20, clone L26, 1/1000, Dako).

We used EnVision (Dako 5007) secondary antibody for 30 minutes, with 3,3'-diaminobenzidine as chromogen. Sections were counterstained with hematoxylin (Dako S3301) (Supplementary Figures 4 and 5).

Methods of Cell Counting

Lymphocyte subsets were counted in 3 anatomically defined regions. The numbers of intraepithelial lymphocytes (IELs) located above the basal membrane, per 100 epithelial cells, were counted on 5 well-orientated villi with longitudinal sections and expressed as the number of IELs per 100 epithelial cells [20].

Lamina propria villous (Lpv) lymphocytes located underneath epithelial basal membrane were assessed in 5 villi with results expressed as average cell counts per area (cells/mm^2). Lamina propria crypt (Lpc) lymphocytes were counted per area within 5 consecutive, nonoverlapping ×200 fields of Lpc and the results averaged (cell count/mm^2).

Positive cells in vicinity of lymphoid follicles or clusters were not taken into consideration. Only cells with a visible nucleus were considered as positive and counted.

The cell counts were performed using Olympus program Cell P image analysis software, with a ×200 objective. All measurements were performed in a blinded manner by V. D. and double-checked by a pathologist (O. D. L.). Interobserver agreement (V. D. and O. D. L.) and intraobserver agreement (V. D.) for counting of CD cells was assessed using Bland–Altmann correlations test.

We also analyzed correlation between T- and B-cell data obtained in this study with duodenal enterochromaffin (EC) cell numbers from 19 patients in the PI-FGID group and 19 RCs, which were available from a previous study [21].

Statistical Analysis

Age and illness duration are expressed as median (range). Abdominal symptom score and lymphocyte cell counts are presented as median (interquartile range [IQR]). Differences between groups for age, illness duration, and CD cell counts were assessed using the Kruskal–Wallis test. The Fisher's exact test was used for categorical values. Mann–Whitney unpaired test was used for comparisons of histology and lymphocyte counts. The Wilcoxon paired test was used to compare lymphocyte counts in repeated biopsies. Correlation analyses were performed using the Pearson (parametric data) or Spearman rank test (nonparametric data). All data were analyzed using Graph Pad Prism 4 software. Due to multiple comparisons across 4 groups and 11 locations of lymphocyte subsets, we set an arbitrary P value of <.01 as the level of significance.

RESULTS

As shown in Table 1, there was no significant difference in age or sex between study groups. No correlation was found between participant age or sex and T- and B-cell subsets, except that PI-FGID females had higher levels of CD3 IELs (16 [13–23] vs 11 [8–19]; P = .002) and lower Lpc CD8 (245 [182–349] vs

Table 1. Patient Characteristics by Study Group

Characteristic	Chronic Giardiasis (n = 28)	PI-FGID (n = 66)	Recovered Controls (n = 19)	Healthy Controls (n = 16)	P Value
Age, y, median (range)	27 (19–48)	28 (18–51)	29 (22–45)	36 (22–56)	NS[a]
Female sex, No. (%)	17 (61)	40 (61)	9 (48)	11 (69)	
Time since onset of giardiasis, mo, median (range)	6 (3–14)	10 (3–19)	15 (12–19)	NA	< .0001[a]
Duodenal inflammation, No. (%)					
Macroscopic	5 (17.9)	4 (6.1)	0	0	.05[b]
Microscopic	24 (85.7)	19 (28.8)	3 (15.8)	0	< .0001[b]
Symptom score	(n = 22)	(n = 50)	(n = 19)	(n = 16)	
Abdominal pain/discomfort	8 (5–8)	5 (3–8)	0 (0–2)	0	< .0001[a]
Nausea	7 (3–8)	3 (1–6)	0 (0–1)	0	< .0001[a]
Bloating	8 (5–9)	7 (4–8)	2 (0–4)	1 (0–2)	< .0001[a]
Diarrhea	8 (5–10)	6 (3–8)	0 (0–1)	0	< .0001[a]
Constipation	1 (0–3)	1 (0–4)	0 (0–1)	1 (0–1)	.03[a]

Symptom data are shown as median (interquartile range).

Abbreviations: NA, not applicable; NS, not significant; PI-FGID, postinfectious functional gastrointestinal disorder.

[a]Kruskal–Wallis test across 4 groups.

[b]Fisher's exact test.

328 [282–425]; $P = .001$). However, there were no differences in CD3 IEL levels between the 4 study groups.

Microscopic inflammation was seen in a high proportion of patients with CG (85.7%), some of the patients with PI-FGID (28.8%), and a few of the RCs, but not among HCs (Table 1). Twenty PI-FGID patients tested for *H. pylori* were all negative.

Abdominal Symptoms

There were significant differences in abdominal symptoms between the groups (Table 1). Abdominal pain and discomfort, diarrhea, and bloating were the most common symptoms in the patients with CG and PI-FGID. Only diarrhea found to have a significantly higher score among CG cases compared to PI-FGID ($P = .009$). There were few symptoms among recovered and healthy controls. Females scored significantly higher than males for nausea ($P < .0001$) and abdominal pain ($P = .005$) in all 3 *Giardia*-exposed groups.

Of 66 PI-FGID patients, 42 (64%) completed a ROME II form between October 2005 and April 2007, at median illness duration of 19 months (range, 12–34 months). Thirty-seven of 42 (56%) fulfilled criteria for IBS, 3 of 42 (5%) had functional dyspepsia (FD), 5 of 42 (8%) had both FD and IBS, and 2 patients (3%) had functional abdominal bloating. Subtyping of the 37 PI-IBS patients revealed 54% IBS-A, 35% IBS-D, and 11% IBS-C.

Correlation Between T and B Cells and Illness Duration

In the preliminary analysis of lymphocyte subsets, we saw gradual changes over time in the PI-FGID and RC groups. In the PI-FGID group, illness duration was positively correlated with Lpc CD4 cells ($P = .0005$, Spearman $r = 0.4$) and with Lpc CD8 cells ($P = .005$, Spearman $r = 0.3$). Illness duration in RC correlated positively with Lpv CD4 cells ($P = .005$, $r = 0.6$), and Lpc CD4 cells ($P = .0002$, $r = 0.8$). We therefore divided the PI-FGID and RC groups into subgroups of 4-month intervals with regard to onset of symptoms.

In patients with chronic giardiasis, we found a significant correlation between illness duration and a small gradual increase in Lpc CD8 cells ($P = .005$, Spearman $r = 0.5$). The CD3, CD4, or B-cell populations were not altered over time in CG.

Macroscopic and Microscopic Inflammation

Macroscopic duodenitis was not common in the participants (Table 1). Enough patients were available in the PI-FGID group with illness duration 3–10 months to assess duodenal inflammation grade and lymphocyte populations. Histologically normal duodenal biopsies in PI-FGID had lower CD20 cell counts in both villus (0 [0–5] vs 25 [5–43]; $P = .0001$) and crypt (16 [12–26] vs 37 [25–42]; $P = .0002$) than those with microscopic inflammation (Figure 2).

The same trend was seen in the PI-FGID patients with illness duration 11–19 months, in RCs, and in the CG group (Supplementary Table 2). No significant differences in CD4 and CD8 cell counts between histologically normal and inflamed biopsies were found.

Intraepithelial Lymphocytes

When compared to healthy controls, there were no significant differences in CD8 IELs between the 3 *Giardia*-exposed groups (Supplementary Table 1). However, CD4 IELs were significantly increased in chronic giardiasis compared to healthy controls, as shown in Figure 3. Somewhat surprisingly, there was a dip toward normal levels in the PI-FGID group at 7–10 months of illness duration, with a later increase. The same development was seen in RCs with significantly higher levels of CD4 IELs at 15–19 months compared to HCs.

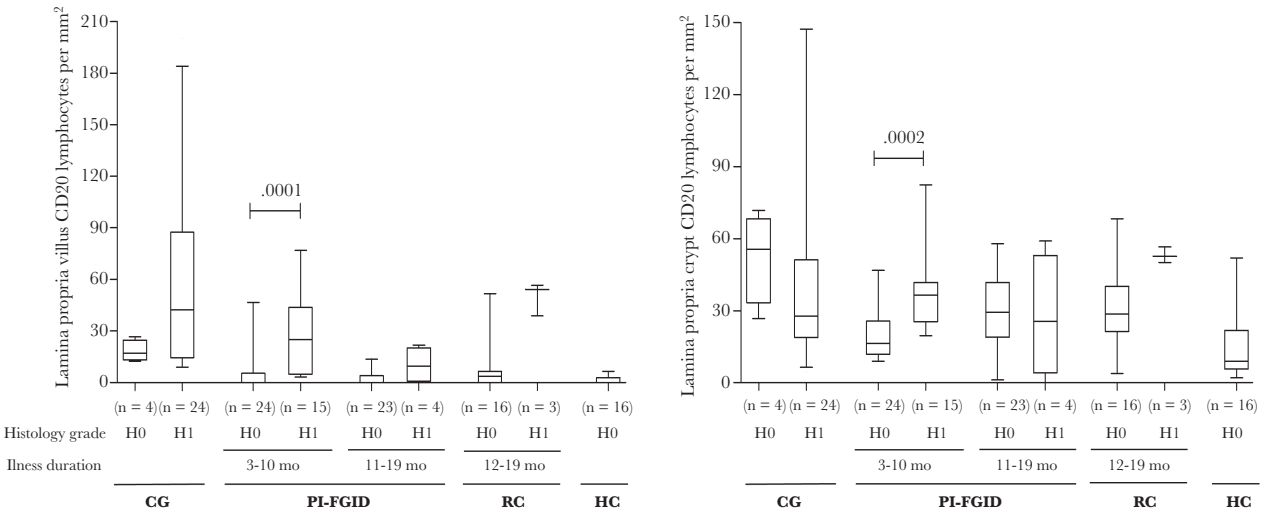

Figure 2. Duodenal lamina propria villus and lamina propria crypt CD20 cells in biopsies with normal histology (H0) and histological inflammation (H1) in patients with chronic giardiasis, those with PI-FGID, recovered controls, and healthy controls. Values above horizontal whiskers are *P* values for comparisons between groups.

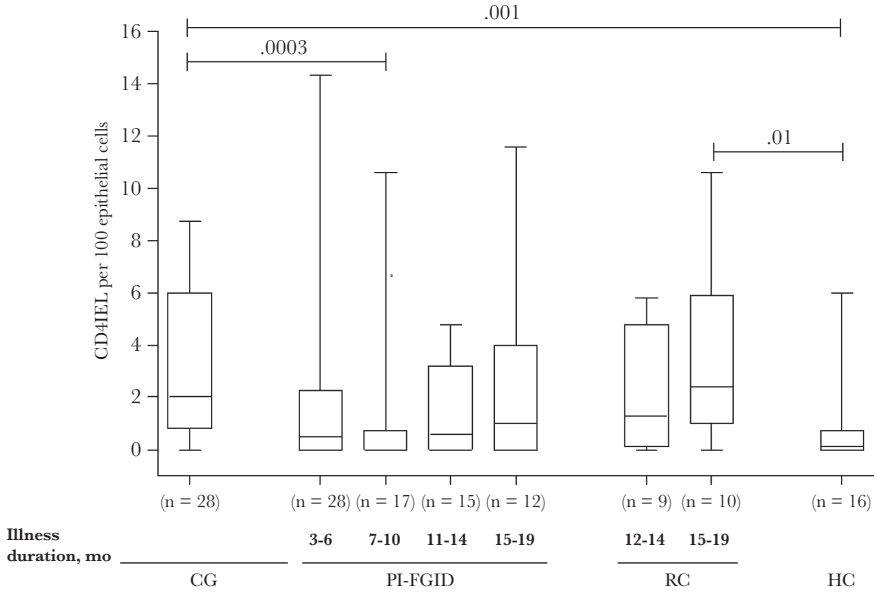

Figure 3. Duodenal CD4 intraepithelial lymphocytes (IELs) in patients with chronic giardiasis (CG), those with postinfectious functional gastrointestinal disorder (PI-FGID), recovered controls (RC), and healthy controls (HC). Values above horizontal whiskers are *P* values for comparisons between groups.

Using 30 IELs per 100 epithelial cells as an upper limit of normal, CD3 IELs were found to be elevated in only 1 patient with chronic giardiasis, 2 with PI-FGID, and 2 HC subjects.

Lamina Propria T Cells

In patients with chronic giardiasis, the number of Lpc CD4 T cells was significantly lower (181 [144–226]) compared to HCs (323 [250–376]) ($P < .0001$) (Figure 4 and Supplementary Table 2). PI-FGID patients with recent *Giardia* infection had low levels of Lpc CD4 cells, similar to CG, but a gradual increase was observed in patients as illness lasted ≥11 months. At 15–19 months it reached the same level as HCs and was significantly increased compared to previous months.

The same pattern was observed in RCs where Lpc CD4 cells at 12–14 months of illness duration were significantly lower compared to HCs ($P = .0004$), but rose to normal levels from 12–14 months to 15–19 months (190 [138–216] vs 321 [254–405]; $P = .0009$).

Lpv CD4 T cells showed were very variable in CG, but decreased after eradication of the parasite, and then gradually increased in both PI-FGID and RC groups (Supplementary Table 1).

We did not observe any significant differences between the 4 study groups regarding CD8 cells in crypts or villus.

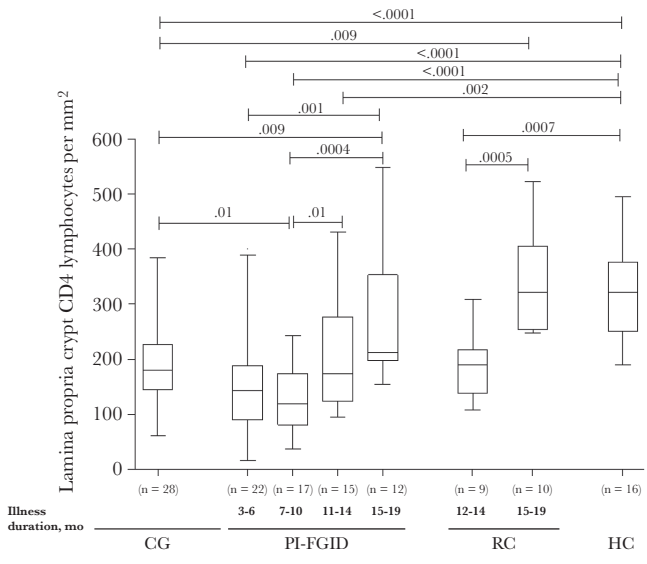

Figure 4. Duodenal lamina propria crypt lymphocytes in patients with chronic giardiasis (CG), those with postinfectious functional gastrointestinal disorder (PI-FGID), recovered controls (RC), and healthy controls (HC). Values above horizontal whiskers are *P* values for comparisons between groups.

Duodenal Lamina Propria B Cells

All 3 *Giardia*-exposed groups had significantly higher number of CD20 B cells in Lpv and Lpc lymphocytes compared with healthy controls (Figure 5).

The CD20 cell counts in the PI-FGID group was lower than in CG, decreased somewhat over time, and became more similar to HC levels. Recovered controls had significantly higher levels of levels of CD20 B cells in both villus and crypt lymphocytes compared to HCs.

Repeated Duodenal Biopsies

In paired samples from the same patient from 2 time points, we found a significant increase in Lpc CD4 cells (from 136

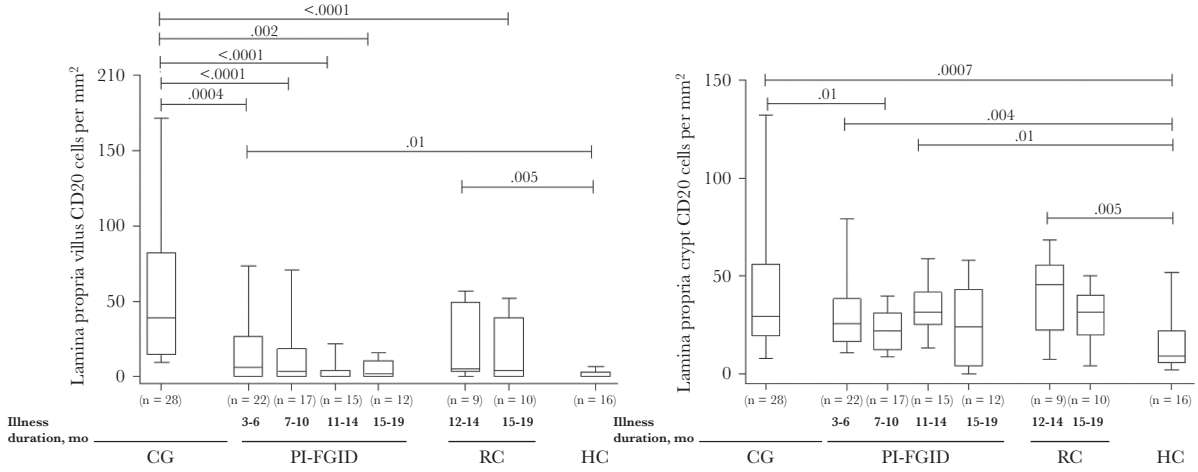

Figure 5. Duodenal lamina propria villus and lamina propria crypt CD20 lymphocytes in patients with chronic giardiasis (CG), those with postinfectious functional gastrointestinal disorder (PI-FGID), recovered controls (RC), and healthy controls (HC). Values above horizontal whiskers are *P* values for comparisons between groups.

[118–193] to 227 [199–371]; $P = .01$) and CD8 cells (from 219 [118–271] to 389 [275–434]; $P = .01$) (Figure 6).

Otherwise, there were no significant differences in duodenal intraepithelial or lamina propria cell counts for CD3, CD4, CD8, or CD20 lymphocytes in these 11 patients.

Correlation Between Duodenal EC Cells and T Cells

A previous study showed that EC cells were reduced in the PI-FGID group (n = 19) compared with the RC group (n = 19). When correlating lymphocyte data with available EC cell data (n = 38), we found EC cells to positively correlate with CD4 IELs ($P = .01$, Spearman $r = 0.4$) and a tendency for correlation with Lpc CD4 cells ($P = .02$, Spearman $r = 0.4$). Looking at the PI-FGID group only (n = 19), there was a positive correlation between EC cell numbers and both Lpv CD4 cells ($P = .01$, Pearson $r = 0.6$) and Lpc CD4 cells ($P = .008$, Pearson $r = 0.6$).

DISCUSSION

In the present study, we identified alterations in duodenal mucosal lymphocyte subsets during symptomatic chronic giardiasis, as well as prolonged differences in these subsets after successful treatment. Importantly, we did not observe important differences in these major lymphocyte populations between patients who did and those who did not develop long-term PI-FGID symptoms. We found increased frequency of CD4 IELs in chronic giardiasis that was sustained over time, especially in recovered asymptomatic patients, but also in patients with PI-FGID. In the lamina propria, CD4 T-cell numbers were high in the villus, but low in the crypt in chronic giardiasis. In both the PI-FGID and RC groups, lamina propria crypt CD4 cells and CD8 cells gradually increased to normal levels >1 year after giardiasis symptoms started. Compared with healthy

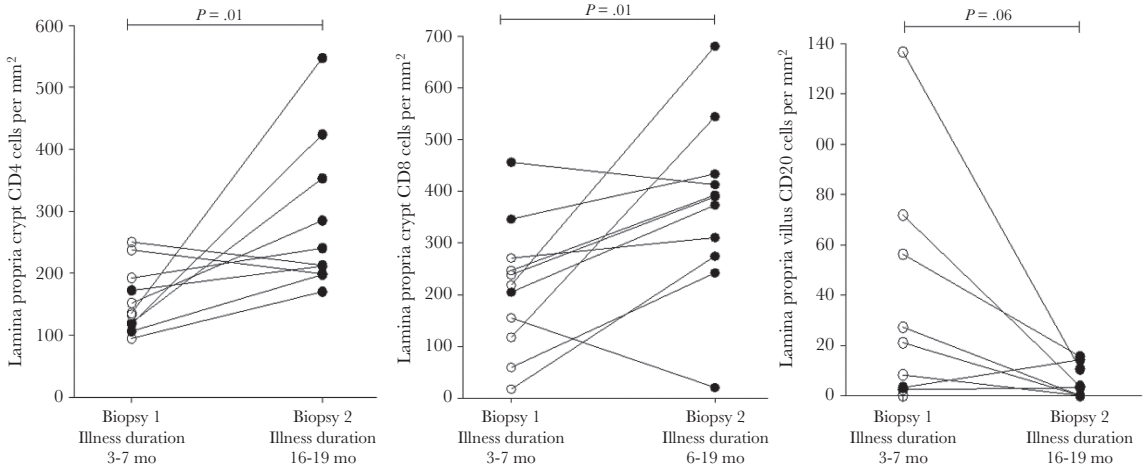

Figure 6. Duodenal lamina propria crypt CD4 cells, lamina propria crypt CD8 cells, and lamina propria villus CD20 cells in biopsy 1 (illness duration between 3 and 7 months) and biopsy 2 (illness duration between 16 and 19 months) in 11 *Giardia*-positive (○) and *Giardia*-negative (●) patients.

controls, lamina propria CD20 cells were elevated during the whole follow-up period in all 3 *Giardia*-exposed groups.

To our knowledge, this is the first study describing duodenal mucosal intraepithelial and Lpv and Lpc lymphocyte kinetics over 1.5 years in patients with and without persistent abdominal symptoms after gastroenteritis with a confirmed intestinal pathogen. A rigorous interpretation of the data was possible due to inclusion of groups with prolonged illness as well as recovered controls exposed to the same pathogen.

Inflammation in Chronic Giardiasis

Previous studies of giardiasis have concluded that there were no specific histological changes in patients with symptomatic giardiasis except slightly increased CD3 IELs [22, 23]. Only rarely there are signs of inflammation and villous flattening [24].

In our study, only a few subjects had increased CD3 IEL counts >30. However, many of the *Giardia* positive cases (by stool examination) presented in this study had duodenal inflammation, but *Giardia* trophozoites could only be identified above the duodenal mucosa in 2 of 28 cases. It is known that some patients may present with a more profound inflammatory reaction to the *Giardia* infection such as this. Recently a similar set of 10 giardiasis cases was reported with inflamed, but parasite-free, duodenal mucosa, but uninflamed ileal mucosa with discernible trophozoites above it [25].

Intestinal T-Cell Alterations

Our results show that duodenal CD4 T cells can be decreased for many months after giardiasis. Most studies of intestinal lymphocytes after gastroenteritis have investigated colonic or rectal mucosa [9]. In patients with persistent abdominal symptoms after *Campylobacter* or *Shigella* infection, an increased count of EC cells and lamina propria T cells have been found in the large intestine when compared with HCs [26–28]. The only study of duodenal lymphocytes in PI-FGIDs patients is a small study of 12 patients with PI-FD of unknown microbial etiology and 12 patients with unspecified FD, and no HCs [29]. A reduced number of intravillar CD4 cells in PI-FD was found. Their conclusion was that PI-FD patients showed an impaired ability of the immune system to terminate the inflammatory response after the acute insult. The long duration of recruitment into our study, and inclusion of a recovered control group exposed to the same infection, allows a better interpretation of the findings. As lower CD4 cell frequencies are present, and gradually increasing, in both the PI-FGID and RC groups, this finding seems to be a prolonged effect of the inflammatory response to the gastroenteritis, rather than associated with the presence of PI-FGID symptoms.

Interestingly, a recent mouse study found increased levels of anti-inflammatory cytokine interleukin 10 (IL-10) to be important in controlling *Giardia*-induced T-cell responses both in the small and large intestine [30]; thus, the prolonged lower T-cell count observed in the present study could be induced via an IL-10–dependent pathway. That mouse study also revealed the possibility that individuals with *Giardia*-induced duodenal inflammation could also have some degree of concomitant colitis. Further research is needed to examine whether this may occur, and if it could trigger a longer-term dysfunction and development of FGID.

Duodenal Lamina Propria B Cells

Antibody-producing B cells have an important role in adaptive immune response and are important in clearing *Giardia* infection [11, 13]. All *Giardia*-exposed participants had elevated CD20 B-cell counts in both Lpv and Lpc compared to healthy controls. CD20 B cells in the lamina propria mainly represent resident local memory B cells, and not plasma cells as these are CD20 negative [31]. These cells are likely to be part of the acquired immune response against *Giardia* observed in epidemiological studies [32].

There was a clear trend of gradual reduction over time of CD20 cells in the villi. However, in crypts, this trend was absent, and the increase was still present 15–19 months after initial infection. Separate counting of the 2 compartments avoided masking these differences.

Mucosal jejunal CD20 B cells have been found moderately elevated in a Spanish study of patients with IBS-D compared to healthy controls [10]. This cohort may have included PI-IBS patients, as IBS-D is a subtype commonly seen after gastroenteritis.

Surprisingly, there was a tendency for the RC group's Lpv B cells not to normalize as well as in the PI-FGID group at 12–19 months. An explanation for this might be that 6 of these 19 RCs had markedly elevated villus B cells compared to the rest of the group, indicating potential intercurrent recent enteric infection.

Duodenal EC Cells

EC cells are dispersed throughout the gut and are the main source of serotonin (5-HT). It has been shown previously that secretory products from CD4 T cells interact with EC cells to enhance the production of 5-HT in the gut via Th2-based mechanisms [33]. Our previous finding of lower number of EC cells that is now found to be associated with a prolonged dip in CD4 cells in the PI-FGID group could indicate a temporarily lower 5-HT production after *Giardia* infection.

Limitations and Strengths

The main strength of this study is the relatively large number of patients in the CG and PI-FGID groups and the long inclusion time, allowing a description of the kinetics of mucosal lymphocyte populations. A similar development in the 11 cases with repeated biopsies supported the finding in individual cases over time. The inclusion of *Giardia*-exposed RCs as well as HCs allowed results to be interpreted with more certainty.

There were some limitations of this study. We were not able to determine exactly when patients became *Giardia* negative after treatment, as many were referred after several courses of

metronidazole due to their prolonged symptoms. We also were not able to collect symptoms scores at the time of biopsy or ROME II follow-up forms from all patients in the CG and PI-FGID groups. Analysis of differences in lymphocyte counts according to histologic inflammation suffered from a low number of cases except in the PI-FGID group with symptom duration of 3–10 months.

Conclusions

Chronic symptomatic *Giardia* infection is associated with elevated CD4 IELs and with elevated B cells and decreased CD4 T cells in the duodenal lamina propria. In patients with PI-FGID symptoms after giardiasis and in RCs the same pattern was seen for more than a year before CD4 T cells were normalizing to levels seen in HCs. The findings implicate a cautionary approach to studies of PI-FGID not including a group of RCs. Further investigations into subsets and activation status of the prolonged alterations of mucosal lymphocyte subsets are warranted and may reveal clues for elucidating the pathogenesis of PI-FGID.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Potential conflicts of interest. All authors: No potential conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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