

## Research article

# Serum cytokine elevations in celiac disease: Association with disease presentation

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## ARTICLE INFO

## Article history:

Received 11 April 2009

Received in revised form 19 August 2009

Accepted 1 September 2009

Available online 6 September 2009

## Keywords:

Cytokines

TTg IgA antibodies

Celiac disease

## ABSTRACT

Celiac disease (CD) is an autoimmune disorder that is triggered by an immune response to gluten in genetically predisposed individuals. Although considered a primary gastrointestinal disease, CD is now known to have widespread systemic manifestations. We attempted to define the nature and role of systemic cytokine levels in the pathophysiology of CD. Multiplex cytokine assays were performed on four different groups of adult patients; patients with active CD (ACD), patients on a gluten-free diet (GFD) with positive TTG IgA antibodies, patients on a GFD with negative antibodies, and those with refractory CD (RCD). The results were compared with values in healthy adult controls. Patients with active CD and those on GFD with positive antibodies had significantly higher levels of proinflammatory cytokines, such as interferon- $\gamma$ , interleukin (IL)-1 $\beta$ , tumor necrosis factor- $\alpha$ , IL-6 and IL-8, and also T<sub>H</sub>-2 cytokines such as IL-4 and IL-10, compared with normal controls and patients on GFD without antibodies. Interestingly patients on GFD for less than 1 year had significantly higher levels of both proinflammatory cytokines and T<sub>H</sub>2 cytokines compared with the patients on GFD for more than 1 year. In addition, a statistically significant correlation between levels of TTG IgA titers and serum levels of T<sub>H</sub>-2 cytokines IL-4 ( $p < 0.001$ ), IL-10 ( $p < 0.001$ ) and inflammatory cytokines such as IL-1 $\alpha$  ( $p < 0.001$ ), IL-1 $\beta$  ( $p < 0.005$ ), and IL-8 ( $p < 0.05$ ) was observed.

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## 1. Introduction

Celiac disease (CD) is an immune-mediated malabsorption syndrome that occurs in genetically susceptible individuals intolerant to dietary gluten. Although the small bowel mucosa is the primary target of damage, the incidence of extraintestinal autoimmune disorders is also increased in individuals with this disorder [1]. It is generally accepted that celiac disease is a T-cell-mediated disease in which gliadin-derived peptides are deaminated by tissue transglutaminase and presented by antigen presenting cells to lamina propria T helper (Th) lymphocytes. Upon activation, the latter as well as macrophages release pro-inflammatory cytokines that lead to the activation of intraepithelial lymphocytes and result in the characteristic histologic alterations of CD [2–4]. It has also been demonstrated that the levels of cytokine elevations correlate with disease activity [5,6]. In active CD (ACD), activated T cells in the small intestinal mucosa produce the T<sub>H</sub>1 cytokine interferon (IFN)- $\gamma$  and express T-bet, a T<sub>H</sub>1 transcription factor [7,8]. There is also increased production of interleukin-15 by the intestinal epithelial cells, which influences the intraepithelial lymphocytes

(IELs) to produce IFN- $\gamma$  [9,10]. A role for cytokines in mediating mucosal damage has also been proposed for interleukin (IL)-1 $\beta$  and tumor necrosis factor (TNF)- $\alpha$ , as these cytokines have been shown to increase the expression of certain metalloproteinases (MMP-1 and MMP-3) with consequent degradation of extracellular matrix (ECM) components [11–13]. The activation and synthesis of ECM-degrading enzymes immediately beneath the epithelium is associated with tissue remodeling and could contribute to the architectural abnormalities, *i.e.*, villous atrophy and crypt hyperplasia, in CD.

Most studies pertaining to cytokine elevations in CD have been performed using whole duodenal or jejunal biopsy samples with measurement of cytokine production at the local level in small bowel mucosa [14–19] using immunohistochemical or *in situ* hybridization methods [4,13,20]. At the systemic level, a limited number of studies have assessed serum cytokine levels by enzyme-linked immunosorbent assays (ELISAs). Such studies have shown increased levels of IL-2, the soluble form of its surface receptor (sIL-2R), IL-18, IFN- $\gamma$ , and TNF- $\alpha$  in individuals with active CD [21–23]. Lahat *et al.*, using reverse transcription polymerase chain reaction (RT-PCR) on peripheral blood mononuclear cells, also reported high expression of IFN- $\gamma$ , IL-2, IL-4, and IL-10 in patients with active

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CD [24]. Gliadin has also been shown to stimulate the production of IL-8 and TNF- $\alpha$  by peripheral blood monocytes from patients with active CD *in vitro* [25].

Cytokines are implicated in both enhancing and suppressing immune responses through their influence on T-cells and other immune effectors. IL-2, IL-12, INF- $\gamma$ , and TNF- $\alpha$  activate T<sub>H</sub>-1 lymphocytes, whereas IL-4, IL-5, and IL-10 lead to T<sub>H</sub>-2 cell activation [26]. In CD, both T<sub>H</sub>-1 and T<sub>H</sub>-2 cytokines have been shown to be elevated [27]. Because only limited published data are available, we decided to perform a comprehensive evaluation of serum cytokine levels in individuals with CD. The goals of our study were threefold: (1) to define serum cytokine profiles of different subsets of CD patients; (2) to evaluate the effect of a gluten-free diet (GFD) on serum cytokine levels; and (3) to correlate serum cytokine levels with anti-TTG IgA antibody levels, mode of clinical presentation, and grade of villous atrophy.

## 2. Subjects and methods

### 2.1. Serum samples

Serum samples were obtained from patients with active CD (ACD;  $n = 18$ ), patients on a gluten free-diet (GFD;  $n = 26$ ) and patients with refractory CD type I (RCD;  $n = 6$ ; *i.e.*, patients with persistence of symptoms despite a dietitian-assessed gluten-free diet for more than 6 months and the absence of phenotypically aberrant IELs). All patients had biopsy-proven CD, were >18 years of age at diagnosis, and had tissue transglutaminase (TTG) IgA antibody serologic tests performed at the time of diagnosis. Serum was isolated from peripheral blood obtained from patients and controls by the gastroenterologist after informed consent and stored at  $-80^{\circ}\text{C}$  before testing. All samples were taken in accordance with the regulations and approval of the Institutional Review Board of Columbia University.

### 2.2. Small-bowel histology

Biopsy specimens were obtained from the second portion of the duodenum during gastroduodenoscopy. Histologic evaluation was performed by a pathologist blinded to the results of serologic findings according to the modified Marsh classification as described previously [28].

### 2.3. Assessment of gluten-free diet

Compliance and absence of contamination of the diet by gliadin was established by an expert dietician. This included review of a 3-day food log of patients, 24-hour food recall in the presence of the dietician, as well as assessment of specific food preparations, brands, and eating habits by the dietician. Normal controls included 16 healthy adults in whom CD and/or other inflammatory processes were excluded by a questionnaire and serum TTG analyses. We excluded patients who were originally identified solely on the basis of screening of asymptomatic first-degree relatives with antibody tests, if the patients had selective IgA deficiency (defined as a total serum IgA level <0.05 g/l) or if the patients were taking immunosuppressive medications at the time of diagnosis.

### 2.4. Measurement of TTG IgA antibody

Serum anti-TTG IgA antibody levels were tested for patients with ACD ( $n = 10$ ), patients on a GFD ( $n = 20$ ) and normal controls ( $n = 6$ ) with an assay that used recombinant human TTG as antigen (INNOVA Diagnostics, San Diego, CA). Patients with anti-TTG levels <20 Eu/ml were labeled as negative, those with values between 20 and 25 Eu/ml as borderline, and those with values >25 Eu/ml as positive, based on the manufacturer's protocol. The range for the patient groups evaluated was as follows: ACD patients (30–118 Eu/ml); GFD patients (10–104 Eu/ml), and normal sub-

jects (10–27 Eu/ml). The percentage above cut-off was calculated as follows:  $(\text{serum value Eu/ml} - 20 \text{ Eu/ml}) \times 100$ .

### 2.5. Measurement of serum cytokine levels

The Evidence Investigator™ (Randox Laboratories, Crumlin, UK) is a bench-top semi-automated instrument used for imaging, capture, and analysis of protein biochip array assays. The immunoassay procedure was performed manually, followed by an automated readout of the biochips by the instrument.

An Evidence Investigator human cytokine and growth factor kit (Randox Laboratories) was used for the simultaneous measurement of human cytokines IL-2, IL-4, IL-6, IL-8, IL-10, IFN- $\gamma$ , TNF- $\alpha$ , IL-1 $\alpha$ , and IL-1 $\beta$ . This kit contained ready-to-use, bar-coded reagents, including multi-analyte calibrators complete with parameter details, multi-analyte conjugates, assay reagents, signal reagent (luminol and peroxide), wash buffer, and the biochips. Multi-analyte controls complete with parameter details (Randox Laboratories) were used according to the manufacturer's protocol. The methodology of the assay was a sandwich immunoassay. Specific antibodies, which are bound to the biochip at separate X- and Y-coordinates, captured the cytokines present in the sample. A horseradish peroxidase enzyme-labeled multi-conjugate produced a chemiluminescent reaction with the signal reagent. The resulting chemiluminescent signal was directly proportional to the concentration of the cytokines in the serum sample. The assays were defined on discrete test regions as immobilized antibodies on the activated solid substrate ( $9 \times 9 \text{ mm}$ ) protein biochip. The protein biochips were supplied in individually labeled carriers in a  $3 \times 3$  format, totaling nine reaction wells with one biochip per well. One biochip contained 12 cytokine and growth factor assays. Single samples and reagents were added to each well to perform the assay. A single biochip carrier was placed into the processing compartment of the imaging module. Chemiluminescent reactions produced on the surface of the biochips at the different discrete test regions (DTRs) were recorded and detected, by a cooled charge coupled device (CCD) camera and the system software, respectively. The instrument-specific software carried out image processing, quantification, and validation. Images and numerical data were automatically stored. The system software had the added facility of checking the performance of manual steps (washing, absence of conjugate, absence of signal reagent) in its validation process.

### 2.6. Statistical analysis

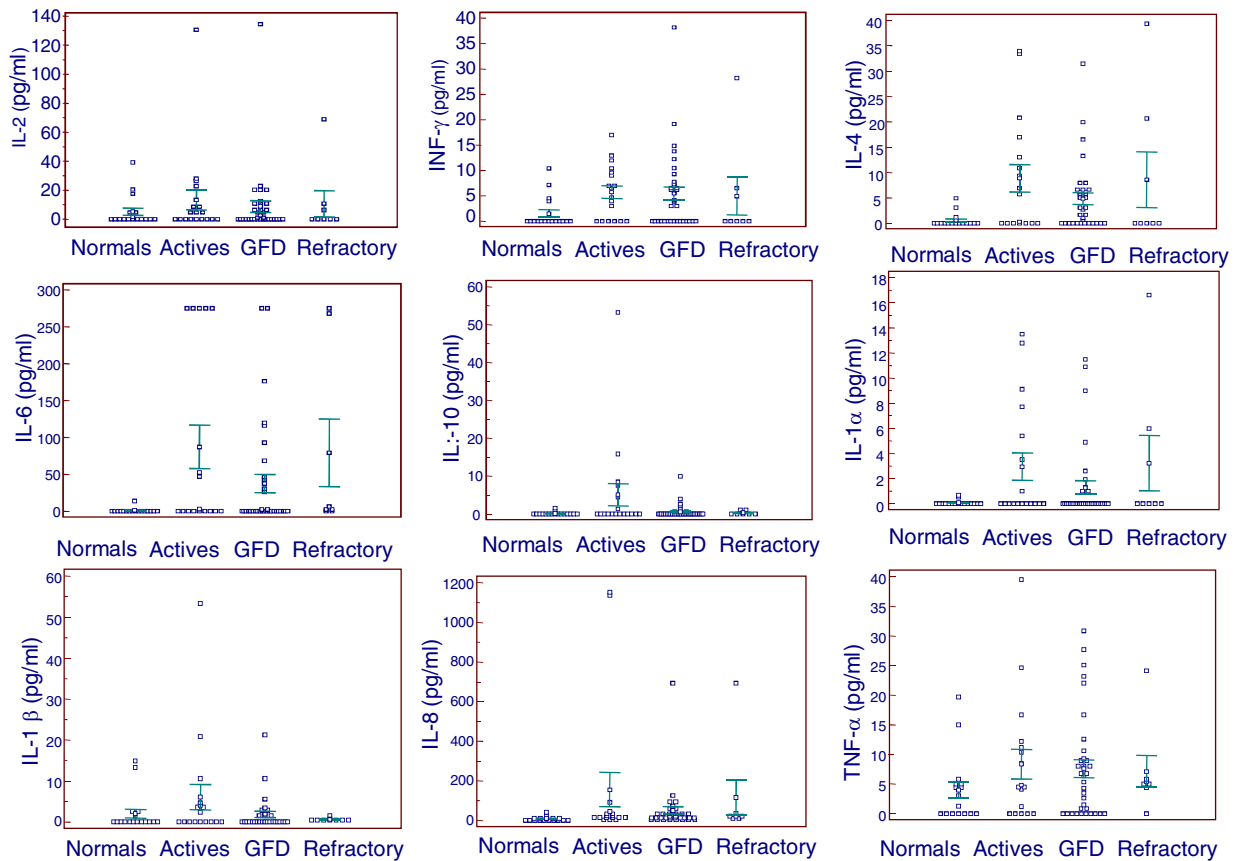
Statistical analysis was performed using a Mann-Whitney rank test or Student's *t* test. Variables were calculated as mean  $\pm$  SE. TTG-antibody levels were standardized to one single assay and plotted as the percentage above the cut-off. Correlation between cytokine levels and antibody levels, clinical presentation and degree of villous atrophy was determined by linear regression analysis. All statistical analyses were performed using Sigma Plot (SYSTAT software, Richmond, CA) and MedCalc software (Mariakerke, Belgium).

## 3. Results

### 3.1. Celiac disease patients versus controls

Serum cytokine levels were evaluated in all patient groups (ACD, GFD, and RCD) and compared with controls. The cytokines analyzed were divided into three categories based on their primary cell source; T<sub>H</sub>1 type cytokines included IL-2 and IFN- $\gamma$ ; T<sub>H</sub>2 type cytokines were represented by IL-4, IL-6, and IL-10 and APC-derived cytokines included IL-1 $\alpha$ , IL-1 $\beta$ , IL-8, and TNF- $\alpha$ .

We found that ACD patients had significantly higher mean serum levels of both T<sub>H</sub>1 cytokines tested (IL-2 and IFN- $\gamma$ ), and all three T<sub>H</sub>2 cytokines tested (IL-4, IL-6, and IL-10) compared with controls (Fig. 1, Tables 1 and 2). Moreover, regarding the APC-



**Fig. 1.** Multiple scatter plots of serum cytokine levels (pg/ml) in the three patient subsets and normal controls. Error bars indicate standard errors of the mean.

derived cytokines, mean levels of IL-8 and TNF- $\alpha$  were significantly higher in patients with ACD compared with controls, whereas the mean levels of IL-1 $\alpha$  and IL-1 $\beta$ , although higher, were not statistically significant (Tables 1 and 2). The mean serum levels of four cytokines (IFN- $\gamma$ , IL-4, IL-6, and IL-8) were significantly higher in the subset of patients on GFD compared with those in normal subjects. Interestingly, the mean serum levels of three of these cytokines (IFN- $\gamma$ , IL-4, and IL-6) were not significantly different when GFD patients were compared with ACD patients. Serum levels of IL-8 were significantly lower in GFD patients than in ACD patients. Of the remaining five cytokines tested, only IL-2 and IL-10 were significantly higher in ACD patients compared with GFD patients.

We also looked at the serum cytokine levels of six patients who were on GFD but were nonresponsive to the diet and were considered to have RCD (all type I). In this subset of patients, serum levels of six cytokines (IL-1 $\alpha$ , IL-4, IL-6, IL-8, IFN- $\gamma$ , and TNF- $\alpha$ ) were significantly higher compared with those in healthy controls. The levels of the remaining three cytokines were higher compared with those in controls, although not statistically significant. Compared

with ACD and GFD patients, there was no significant difference in the levels of any of the serum cytokines in the RCD group.

These results suggest that patients with CD have high levels of circulating pro-inflammatory cytokines and anti-inflammatory cytokines (IL-10), especially during the active phase of the disease. Interestingly, most of these cytokines decrease when the patients commence a GFD; however a few cytokines that have been implicated in the pathogenesis of tissue damage in CD, such as IFN- $\gamma$ , persist in the circulation despite GFD.

### 3.2. Effect of duration of gluten free diet

We next determined whether the serum levels of the cytokines tested changed with the duration of GFD. For this, we evaluated 26 patients on GFD; 14 were on GFD for <1 year and 12 for >1 year. In the latter group, all the patients were on the diet for <5 years except one patient who was on GFD for >20 years. Patients on GFD for >1 year showed significantly lower levels of all T<sub>h</sub>-1 and T<sub>h</sub>-2 cytokines tested when compared with patients on the diet for <1 year (Table 3). With respect to the APC-derived cytokines, only the

**Table 1**  
Mean serum cytokine levels in the different subsets of celiac disease patients and controls

	Primarily T <sub>h</sub> -1 derived		Primarily T <sub>h</sub> -2 derived		Primarily APC derived				
	IL-2	IFN- $\gamma$	IL-4	IL-10	IL-1 $\alpha$	IL-1 $\beta$	IL-6	IL-8	TNF- $\alpha$
Normal [16]	4.4 $\pm$ 2.6	0.97 $\pm$ 0.5	0.3 $\pm$ 0.2	0.13 $\pm$ 0.1	0.07 $\pm$ 0.05	0.07 $\pm$ 0.05	1.07 $\pm$ 0.84	9.66 $\pm$ 2.4	3.01 $\pm$ 0.9
Active [18]	14.6 $\pm$ 5.2	5.41 $\pm$ 0.9	8.9 $\pm$ 2.2	4.7 $\pm$ 2.2	2.6 $\pm$ 1.8	2.65 $\pm$ 0.88	84.2 $\pm$ 24.4	113.3 $\pm$ 61.	9.26 $\pm$ 2.0
GFD [26]	6.59 $\pm$ 1.3	6.18 $\pm$ 1.3	8.03 $\pm$ 2.2	1.89 $\pm$ 1.1	1.91 $\pm$ 0.80	1.91 $\pm$ 0.81	55.53 $\pm$ 15.0	40.6 $\pm$ 16.0	8.05 $\pm$ 1.4
Refractory [6]	35.0 $\pm$ 22.7	9.63 $\pm$ 4.7	11.58 $\pm$ 6.4	0.41 $\pm$ 0.2	5.01 $\pm$ 2.5	0.63 $\pm$ 0.3	121.2 $\pm$ 50.7	34.8 $\pm$ 7.6	9.11 $\pm$ 3.0

Values are mean (pg/ml)  $\pm$  SE.

**Table 2**

Comparison of mean serum cytokine levels between normal controls and the different subsets of celiac disease patients

	IL-2	IFN- $\gamma$	IL-4	IL-6	IL-10	IL-1 $\alpha$	IL-1 $\beta$	IL-8	TNF- $\alpha$
Normal vs ACD	<b>&lt;0.04</b>	<b>&lt;0.005</b>	<b>&lt;0.004</b>	<b>0.008</b>	<b>&lt;0.01</b>	0.13	0.38	<b>0.0007</b>	<b>0.03</b>
Normal vs RFD	0.28	<b>0.01</b>	<b>&lt;0.04</b>	<b>&lt;0.03</b>	0.21	<b>0.03</b>	0.76	<b>0.003</b>	<b>0.004</b>
Normal vs GFD	0.09	<b>&lt;0.01</b>	<b>0.0006</b>	<b>&lt;0.01</b>	0.13	0.42	0.87	<b>&lt;0.003</b>	0.09
Normal vs GFD <1 year	<b>0.05</b>	<b>0.005</b>	<b>0.001</b>	<b>0.004</b>	0.13	<b>0.05</b>	0.16	0.21	<b>0.008</b>
Normal vs GFD >1 year	0.96	0.77	0.09	0.37	<b>0.02</b>	0.86	0.90	0.35	0.76

*p* values shown were determined using Mann-Whitney rank test. Significant *p* values are in boldface type.

mean levels of IL-1 $\beta$  showed a significant decrease in patients on GFD >1 year compared with patients on GFD for <1 year. The mean levels of IL-1 $\alpha$  and TNF- $\alpha$  were lower in patients on GFD >1 year compared with patients on GFD for <1 year but were not statistically significant. Interestingly, the mean serum levels of IL-8 were similar in both patient groups.

### 3.3. Serum cytokine levels versus CD diagnostic criteria

Since the identification of tissue transglutaminase (TTG) as the autoantigen of CD [29], detection of anti-TTG IgA antibodies in the serum of CD patients has become an essential tool for the diagnosis of this disorder.

We determined whether there was a correlation between serum anti-TTG IgA levels and the serum levels of the cytokines tested. We found statistically significant positive correlations between the levels of anti-TTG IgA and the levels of all the cytokines except IFN- $\gamma$  and TNF- $\alpha$  (Table 4). The strongest correlations were observed with IL-1 $\alpha$  ( $p < 0.001$ ,  $r^2 = 0.5$ ) and IL-4 ( $p < 0.001$ ,  $r^2 = 0.35$ ; Fig. 2).

A characteristic diagnostic feature of CD is the finding of villous atrophy and crypt hyperplasia on histologic examination of small-bowel biopsy samples [30]. In our study, 44 patients had intestinal biopsies at presentation, of which 12 had histologic evidence of varying degrees of villous atrophy. Using linear regression analysis we found that serum levels of IL-4, IL-6 and IL-8 correlated with the degree of villous atrophy ( $p < 0.003$ ;  $p < 0.002$  and  $p < 0.05$ , respectively; Fig. 3).

We also compared serum cytokine levels with the mode of clinical presentation of the patients. For this we divided the patients into two groups: classic presentation (*i.e.*, diarrhea/malabsorption syndrome;  $n = 12$ ) and atypical presentation (*i.e.* nondiarrheal;  $n = 10$ ). No statistically significant correlations were observed between the levels of serum cytokines and mode of clinical presentation.

## 4. Discussion

Our findings suggest that higher levels of T<sub>H</sub>-1, T<sub>H</sub>-2 and primarily APC derived cytokines in patients with CD characterize the inflammatory response of this disease, with certain serum cytokine elevations being similar to those observed in the small intestinal mucosa and others being different, the latter possibly deriving from

**Table 3**

Comparison of mean serum cytokine levels between celiac disease patients on GFD for less than 1 year and those on GFD for more than 1 year

	Cytokine	<1 Year mean (pg/ml) $\pm$ SE	>1 Year mean (pg/ml) $\pm$ SE	<i>p</i> -value
Primarily	IL-2	8.40 $\pm$ 2.69	2.72 $\pm$ 1.37	$p < 0.05$
TH1 derived	IFN-g	9.10 $\pm$ 3.02	1.83 $\pm$ 0.83	$p < 0.02$
Primarily	IL-4	11.66 $\pm$ 5.28	3.48 $\pm$ 1.54	$p < 0.05$
TH2 derived	IL-10	3.61 $\pm$ 2.91	0.83 $\pm$ 0.75	$p < 0.05$
Primarily	IL-1a	2.75 $\pm$ 1.76	0.91 $\pm$ 0.83	$p = 0.16$
APC derived	IL-1b	3.07 $\pm$ 1.34	0.56 $\pm$ 0.31	$p < 0.02$
	IL-6	68.28 $\pm$ 26.35	35.11 $\pm$ 24.09	$p < 0.05$
	IL-8	16.89 $\pm$ 4.03	22.00 $\pm$ 4.0	$p = 0.62$
	TNF- $\alpha$	9.86 $\pm$ 2.34	16.89 $\pm$ 4.03	$p < 0.02$

nonintestinal sources. Many *in vitro* studies have demonstrated that gluten (gliadin) induces sustained T<sub>H</sub> type 1 pro-inflammatory cytokine production [6,16,24,31–33]. The T<sub>H</sub> type 1 response to dietary gluten in the small intestinal mucosa most likely drives the lymphocytic and monocytic infiltration of the lamina propria. Although overlapping in their function, T<sub>H</sub>-1- and T<sub>H</sub>-2-derived cytokines mediate different functions. The T<sub>H</sub>-1 response augments cell-mediated immunity and pro-inflammatory responses, whereas the T<sub>H</sub>-2 cytokines predominantly affect the humoral immune response and play a role in downregulating inflammatory processes. Both responses have been observed in CD [24]. Of note, immune effectors other than T cells can also secrete cytokines, which contribute to the polarized T<sub>H</sub>-1 or T<sub>H</sub>-2 responses. We measured a wide spectrum of T<sub>H</sub>-1-, T<sub>H</sub>-2-, and APC-derived cytokines. The elevated serum levels of cytokines that are known to be high at the level of the intestinal mucosa as well as in PBMC suggests that systemic activation and secretion of cytokines occurs in patients with CD. Both IFN- $\gamma$  and IL-2 are important mediators of the T<sub>H</sub>-1 immune response, and the roles of these cytokines in CD are well documented. Gluten-responsive, mucosal, and peripheral blood major histocompatibility complex class II-restricted CD4<sup>+</sup> T cell clones express high levels of IFN- $\gamma$  [24]. Moreover, *in vivo* gliadin challenge of CD patients on GFD leads to increased production of IFN- $\gamma$  in the lamina propria coincident with histologic changes of the small intestinal mucosa [16]. In our study, IFN- $\gamma$  was persistently elevated in patients with active and refractory disease as well as in patients on GFD. There are data documenting high serum levels of this cytokine in autoimmune diseases, some of which are associated with CD [34]. IFN- $\gamma$  as well as IL-15 are pro-inflammatory cytokines that are known to be involved in the pathogenesis of CD [35,36]. We did not assess IL-15 levels, but prior studies have documented an important role of this cytokine in the pathogenesis of epithelial lesions and the activation of cytotoxic intra-epithelial lymphocytes [9,10]. Evaluation of IL-15 levels in the different clinical phases of CD remains an important subject for further research. IL-2 levels demonstrated a weak but still significant elevation in patients with ACD. However, no significant differences in IL-2 levels were observed in patients with RCD type I and those on a GFD. The exact reasons for the latter finding are unclear, but a possible explanation might be that serum levels of IL-2 could be influenced by binding of this cytokine to its soluble receptor sIL-2R, which is released into the blood during T-cell activation [22]. T<sub>H</sub>-2 cytokines (IL-4, IL-6, and IL-10) were also elevated in patients with ACD, RCD, and those on GFD. The role of these cytokines in CD pathogenesis is not well understood, but immune regulatory functions at the mucosal level have been suggested [18]. The role of IL-4 remains unclear and prior studies have indicated that it may not be involved in the pathogenesis of CD [37]. IL-4 is responsible for B-cell activation and, specifically, induction of IgE [38]. This cytokine also suppresses T<sub>H</sub>-1 responses by downregulating the effect of IFN- $\gamma$  on macrophages [26]. Our findings support a role of IL-4 in the inflammatory response associated with CD, given the significantly higher levels of this cytokine observed in all groups of patients except those on GFD for more than 1 year. Moreover, IL-4 levels showed a positive correlation with the levels of anti-tTG antibodies. In addi-

**Table 4**  
Correlations between serum levels of anti-TTG IgA and levels of other serum cytokines in celiac disease

	IL-2	INF- $\gamma$	IL-4	IL-6	IL-10	IL-1 $\alpha$	IL-1 $\beta$	IL-8	TNF- $\alpha$
p Value	<0.01	0.60	<0.001	<0.001	<0.001	<0.001	<0.005	<0.05	0.123
r <sup>2</sup>	0.35	0.006	0.35	0.30	0.32	0.50	0.20	0.09	0.05

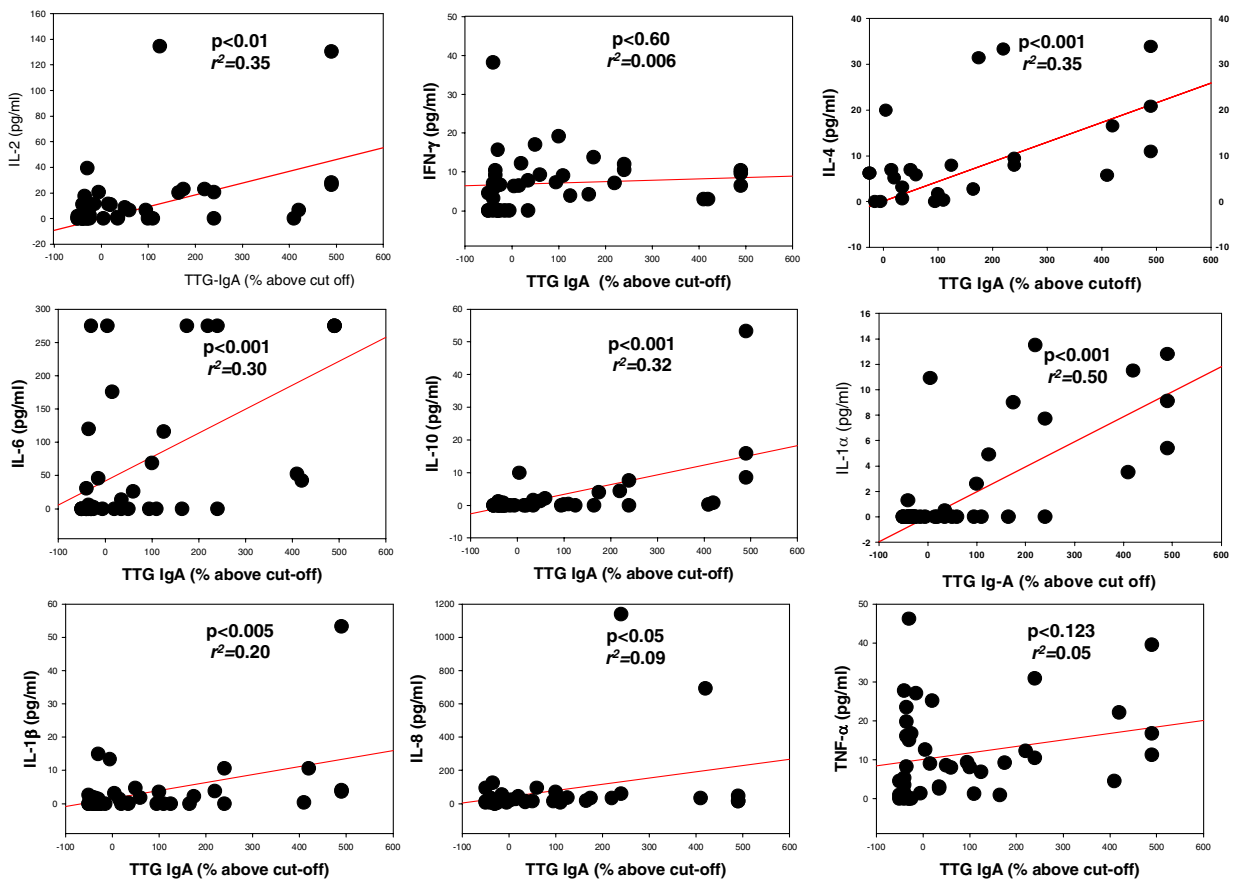
Coefficient of correlation ( $r^2$ ) between cytokine levels and antibody levels was determined by linear regression analysis.

tion, we also found low levels on IL-4 in patients without villous atrophy. Another cytokine, whose role in celiac disease pathogenesis has been studied, is IL-10 [17,39–43]. Several studies have shown that IL-10, concomitantly with IFN- $\gamma$ , is produced in significantly higher amounts by IELs from patients with active CD than IELs from treated CD patients or controls [39,41]. Interestingly, other studies have shown increased IL-10 production by IELs from patients on GFD, patients with “silent” CD, and controls compared with active CD patients [42,43]. We observed elevated serum levels of IL-10 in patients with ACD but not in those on GFD. Similar findings have only previously been described in patients with underlying IgA deficiency, a condition that none of our subjects had [5]. In addition, we found a positive correlation of IL-10 levels with anti-TTG antibody titers. Further studies are required to determine if certain circulating lymphocyte subsets secrete IL-10 in individuals with CD, which may help to elucidate the consequences of serum IL-10 (pro-inflammatory vs regulatory) elevations in this disease.

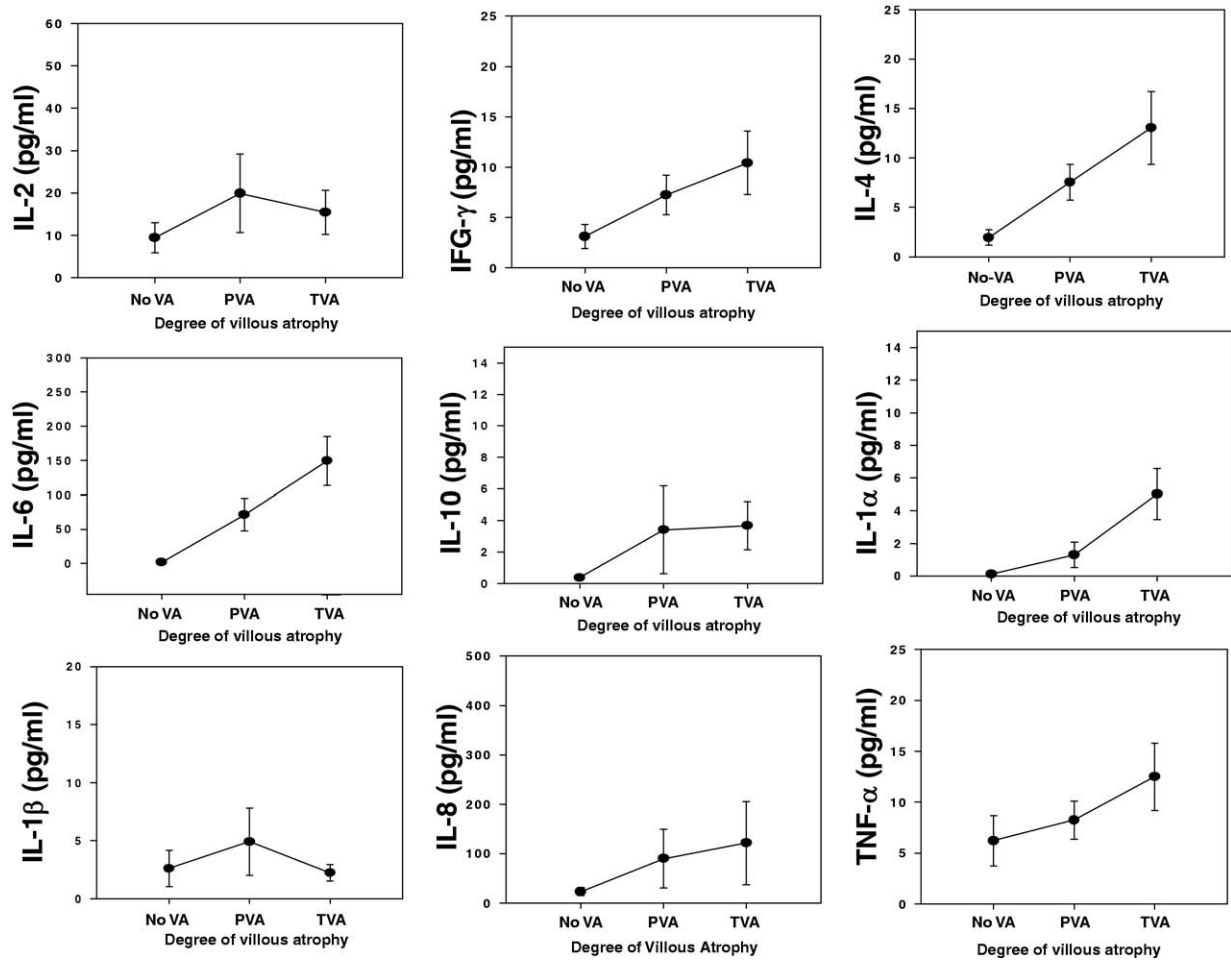
The APC-derived cytokines showed variable results. The levels of IL-1 $\alpha$  and IL-1 $\beta$  did not show significant elevations with the exception of the latter in patients with RCD type I. Although similar in function, these cytokines differ in structure, origin, enzymes that convert them to active peptides, and circulating receptor antago-

nists, all of which lead to divergent roles of these two homologous cytokines [44]. It is possible that differences in the levels of these cytokines seen in our study might be due to the aforementioned factors. Other APC-derived cytokines, such as TNF- $\alpha$ , did show consistent elevations in active CD patients compared with patients on GFD. Gliadin peptides have been shown, *in vitro*, to induce the production of higher levels of TNF- $\alpha$  and IL-8 by peripheral blood monocytes from patients with ACD compared with monocytes from GFD patients or healthy controls [25]. In one study, stimulation with gliadin of monocyte-derived DCs from healthy donors led to enhanced secretion of IL-6, IL-8, IL-10, and TNF- $\alpha$ , in contrast to other tested food proteins [45]. TNF- $\alpha$  produced locally in the small intestinal mucosa is known to be of pathogenic significance in the development of mucosal damage in CD [15].

IL-8 is a chemokine that is expressed in tissue with neutrophilic infiltrates and plays an important role in inflammatory responses mediated by neutrophils [46]. In our study serum levels of IL-8, in contrast to other APC-derived cytokines, were increased in all groups of CD patients compared with controls. The levels of this cytokine remained elevated despite a GFD and remained so even after a year of gluten exclusion. This was in contrast to what has been described in patients with dermatitis herpetiformis, in



**Fig. 2.** Regression analysis plots showing correlations between serum cytokine levels measured and the percentage of TTG IgA serum levels above the cut-off value of the kit used in patients with celiac disease. The percentage above cut-off was calculated as follows: (serum value Eu/ml – 20 Eu/ml)  $\times$  100.



**Fig. 3.** Plots showing correlations between mean serum cytokine levels (pg/ml) and the degree of villous atrophy (VA). Abbreviations: No VA, no villous atrophy; PVA, partial villous atrophy; TVA, total villous atrophy.

whom the serum levels of IL-8 returned to normal levels within 2 years on a GFD [47]. The reason for this difference is not known, but could reflect prolonged activation of the inflammatory response in patients with intestinal predominant CD compared with those with dermatitis herpetiformis.

IL-6 is a pleiotropic cytokine produced by a variety of cells, including T cells, B cells, fibroblasts, endothelial cells, monocytes, keratinocytes, mesangial cells, and some tumor cells. IL-6 plays a crucial role in inflammation, immune regulation, hematopoiesis, and oncogenesis [48]. Similar to our findings, serum levels of IL-6 have been found to be significantly increased in patients with ACD compared with controls, and the serum levels of IL-6 decreased only after a year on the diet [31,49].

Several studies have shown that in CD, elevations of serum immunoglobulins against specific autoantigens, e.g., endomysial antibodies (EMA), and TTG-antibodies are predominantly of IgA isotypes [50–53]. At the mucosal level, cytokines favor the generation of IgA-producing plasma cells [54,55]. Although the degree of inflammation or systemic involvement cannot be estimated by assays performed at a given time point, our findings suggest roles for certain cytokines in eliciting or sustaining the humoral response in CD, with the exception of IFN- $\gamma$  and TNF- $\alpha$ . Considering that anti-TTG IgA antibodies are products of a humoral response, it is not surprising that the latter two cytokines did not show any correlation with serum antibody titers. Serum antibodies are more sensitive for diagnosing patients with severe degrees of villous atrophy than individuals with less advanced histologic changes [56–58].

Data regarding associations between local intestinal immune responses and systemic inflammation in CD are limited. Our findings of higher levels of serum cytokines in patients with severe grades of villous atrophy suggest a link between local mucosal alterations and systemic immune activation in this disease. Cytokine elevations have also been reported in other diseases or disorders frequently associated with CD, including autoimmune thyroiditis, diabetes, hepatitis, osteopenia, as well as psychiatric manifestations, especially depression [31,59,60]. It is likely that elevated levels of certain serum cytokines might underlie extraintestinal manifestations of CD. The current study did not address this possibility. Future studies exploring this issue might broaden our understanding of the roles of specific cytokines in the development of extraintestinal inflammatory and autoimmune disorders in CD patients. The clinical consequences of persistent elevations of the described cytokines in patients with RCD also need to be evaluated in larger studies to determine similarities or differences between patients with RCD types 1 and 2.

Our findings demonstrate a consistent presence of high serum levels of T<sub>H</sub>-1- and T<sub>H</sub>-2- as well as APC-derived cytokines in CD patients. Serum cytokine measurements have known limitations, such as increased clearance and presence of receptors leading to falsely low levels. Coexisting inflammatory processes may also elevate serum levels of certain cytokines. However, the consistent pattern of cytokine elevations in different patient subgroups leads us to believe that such considerations do not significantly affect the serum levels of the cytokines analyzed. Measuring serum cytokine

levels may have diagnostic and prognostic utility given the limitations of markers of disease activity as well as reliable indicators of response to gluten exclusion. We conclude that serum cytokines in CD patients show dual pro-inflammatory and anti-inflammatory profiles and differ from those of otherwise healthy individuals. The cytokine profile also varies with dietary gluten ingestion and severity of intestinal inflammation but, of interest, does not seem to mirror the cytokine profile of the inflamed small intestinal mucosa of ACD patients. The reasons for this discrepancy are likely multifactorial and probably relate to the different cellular sources of these cytokines and inflammatory mediators, as well as the possible existence of microenvironment related differences in regulatory programs or intercellular crosstalk controlling their synthesis, secretion, and degradation. A better understanding of cytokine abnormalities, both local and systemic, might not only provide insights into the pathogenesis, diversity of symptoms, and clinical presentations of CD but potentially also lead to the development of better tools to monitor disease activity and to identify new therapeutic as well as prognostic modalities for patients with CD.

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