

Cutting-Edge Issues in Celiac Disease and in Gluten Intolerance

N. Bizzaro · R. Tozzoli · D. Villalta · M. Fabris · E. Tonutti

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Abstract Celiac disease (CD) is a gluten-dependent immune-mediated disease with a prevalence in the general population estimated between 0.3% and 1.2%. Large-scale epidemiological studies have shown that only 10–20% of cases of CD are identified on the basis of clinical findings and that laboratory tests are crucial to identify subjects with subtle or atypical symptoms. The correct choice and clinical use of these diagnostic tools may enable accurate diagnosis and early recognition of silent CD cases. In this review, we have considered some relevant aspects related to the laboratory diagnosis of CD and, more extensively, of gluten intolerance, such as the best combination of tests for early and accurate diagnosis, the diagnostic role of new tests for detecting antibodies against neoepitopes produced by the transglutaminase–gliadin complex, the forms of non-celiac

gluten intolerance (gluten sensitivity), and the use and significance of measuring cytokines in CD.

Keywords Celiac disease · Gluten sensitivity · Cytokines · Neoepitopes

Introduction

Celiac disease (CD) is an illness about which much is now known, including the cause (gluten), the genetic basis, and the pathogenic mechanism; we may rely on accurate diagnostic tests and effective therapy. The disease epidemiology is also known: in particular, that for each person who has been diagnosed there are at least another five or six in the population who have not yet been identified—the so-called celiac iceberg—most of whom are adults without gastrointestinal symptoms. All this knowledge taken together has brought significant attention by physicians to a disease which can have grave consequences in terms of morbidity if it remains unidentified and untreated.

In any case, notwithstanding the notable progress of recent years, there remain some points to clarify. In particular, the best diagnostic algorithm for identifying the submerged part of the “iceberg” remains to be defined. In addition, new evidence suggests the existence of a form of gluten intolerance that doesn’t meet the diagnostic criteria for CD and in which patients probably will never develop CD. This evidence points to an even deeper part of the iceberg made up of subjects with hypersensitivity to wheat protein components but who don’t experience damage to the intestinal mucosa and don’t have detectable antibodies.

This review undertakes to examine some of these diagnostic “hot spots” in subjects with gluten intolerance: the role of new tests for detecting antibodies against

N. Bizzaro
Laboratory of Clinical Pathology, S. Antonio Hospital,
Tolmezzo, Italy

R. Tozzoli
Laboratory of Clinical Pathology, Civic Hospital,
Latisana, Italy

D. Villalta
Allergology and Clinical Immunology,
S. Maria degli Angeli Hospital,
Pordenone, Italy

M. Fabris · E. Tonutti
Immunopathology and Allergology,
University Hospital S. Maria della Misericordia,
Udine, Italy

N. Bizzaro (✉)
Laboratory of Clinical Pathology,
Ospedale S. Antonio via Morgagni, 18,
33028, Tolmezzo, UD, Italy
e-mail: nicola.bizzaro@ass3.sanita.fvg.it

neopeptides produced by the transglutaminase–peptide complex, the best combination of tests for early and accurate diagnosis, the forms of non-celiac gluten intolerance (gluten sensitivity), the significance and the potential use of measuring cytokines in gluten intolerance.

Key Laboratory Issues Related to CD Diagnosis

While intestinal biopsy is still considered the gold standard for diagnosing CD, the presence of highly specific autoantibodies in patient serum has been used clinically for more than 20 years as a marker for screening candidates for duodenal biopsy [1, 2]. In addition, the importance of antibody assessment in predicting CD has increased along with the number of patients with minor or atypical symptoms. Indeed, the increase in CD diagnosis during recent decades is due in large part to the availability of immunoassays with excellent diagnostic accuracy, such as the anti-endomysium (EMA) and the anti-transglutaminase (tTG) assays [3–10]. Conversely, the classical anti-gliadin (AGA) antibody assay has lost diagnostic significance since the AGA autoantibodies can be detected in non-CD enteropathies as well as in healthy individuals. Therefore, international guidelines have recommended that the AGA test be completely abandoned and replaced by the anti-tTG test [11].

However, there are at least three good reasons to keep the anti-gliadin test in the panel of CD tests.

The first is that the peptide regions of gliadin recognized by the T lymphocytes of genetically predisposed individuals were recently identified [12, 13]. These peptides have a relatively constant aminoacid pattern consisting of the sequence QPEQPF, where the triplet PEQ is the epitopic core. The recognition of the epitopes containing the sequence QPEQPF led to the development of a new immunometric (ELISA) assay to detect deamidated gliadin peptide (DGP) antibodies and a relevant number of studies have shown that the new anti-DGP IgA ELISA tests have a sensitivity and a specificity comparable to those of anti-tTG and EMA and higher than AGA [14–18].

The second reason is that anti-gliadin antibodies, especially AGA-IgG class, can still be useful in pediatric patients with CD who test negative for anti-tTG [6, 19–21]. Simell et al. have described the development of CD-associated antibodies in the first years of age in patients genetically at risk for CD [22]. They demonstrated that EMA, anti-tTG, and IgA-class AGA may fluctuate during the first 2 years of age whereas IgG-class AGA usually remains positive. It is noteworthy that the same results were obtained using the DGP IgG test, confirming its usefulness in routine diagnostic practice in all cases in which anti-tTG tests are inconclusive [23–26].

The third point that still favors the use of new anti-DGP antibody test derives from the role that DGP IgG class antibodies play in detecting CD patients with IgA deficiency. In one study by our group [27], we found that the DGP IgG test was positive in 80% of cases of CD patients with IgA deficiency as compared to 40% for AGA IgG. Although in the same study, anti-tTG IgG performed equally well in patients with IgA deficiency, the use of DGP IgG has the advantage of being more sensitive in children who test negative for both IgA and IgG anti-tTG.

As described below, assays to detect antibodies against neopeptides of the tTG–DGP complex provide a new approach to screening and diagnosis of celiac patients. Results of initial studies show diagnostic sensitivity (95%) and specificity (97%) comparable to—if not greater than—that of the consolidated antibody assays [28].

Anti-actin IgA antibodies are another type of autoantibody that can be found in patients with CD. Although the anti-actin test has limited usefulness for the diagnosis of CD and cannot replace anti-tTG in the diagnostic algorithm of CD, it can be used in the evaluation of disease severity because the antibody level is related to the degree of intestinal damage [29–33].

Taken together, eight to ten different assays (considering both the IgA and the IgG isotype) are currently available to diagnose and monitor CD, each one of them with its own characteristics of sensitivity and specificity and each giving different results in adult and pediatric patients. There is ongoing debate as to which of these tests should be used for screening, which tests should constitute a confirmatory panel, and which isotypes of the various antibodies should be included in these panels.

The introduction of the anti-tTG antibody test, which is far more sensitive and specific than the AGA test, and has in its human recombinant antigen formulation a specificity almost equivalent to that of the EMA, has given rise to the question of whether the determination of the anti-tTG IgA antibodies was sufficiently accurate so as not to require the use of other laboratory tests, either in conjunction with or as confirmation of the results [2].

Our own experience [27, 34, 35] and recent studies by other groups [18, 21, 36] show that the anti-tTG IgA assay is not sensitive enough to be used alone and that the addition of the anti-DGP IgG test would greatly increase the accuracy for CD diagnosis especially in young children. Given also the high accuracy of the anti-DGP IgG assay in IgA-deficient CD patients, we believe that the association of anti-tTG IgA and anti-DGP IgG assays should be adopted as the best test combination to screen for CD. Furthermore, in monitoring of patients on a gluten-free diet, positivity with a low titer of anti-DGP antibodies suggests that the diet should be reassessed, even if the anti-tTG test is negative. Indeed, the introduction of small quantities of

gluten into the diet of celiac patients on a gluten-free diet has been shown to be associated with positivity for DGP but not for anti-tTG [37].

Finally, although it has been shown that the EMA probably recognizes the same antigen target of the anti-tTG antibodies, the anti-tTG test should be preferred because it is quantitative, automated, and not prone to subjective interpretation as the EMA immunofluorescence test. In addition and very importantly, the EMA IgA test has proved to be less sensitive than the anti-tTG test but completely specific [17, 38–40]. As a consequence, it is important that positive findings in the anti-tTG antibody assay are confirmed by the EMA test [41]. In the case of negative EMA results, it is only after clinical evaluation that it may be decided whether to proceed with further testing (HLA, intestinal biopsy) in order to confirm or exclude a diagnosis of CD.

The Transglutaminase–Gliadin Peptides Complex: Is It the Real Autoantigen? A New Way to Explore the Celiac Iceberg

The molecular mechanism of gluten intolerance is poorly understood, but some recent reports have partially explained the pathogenesis of the disease. Gliadin is a glutamine–proline-rich protein that is only partially digested in the gastrointestinal tract, producing digestion-resistant peptides which are able to cross the intestinal epithelial barrier due to increased intestinal permeability, the binding of the chemokine receptor CXCR3, and the release of zonulin, a protein that disassembles the intercellular tight junctions [42]. In the lamina propria, such digestion-resistant peptides can be modified by tTG in two ways: deamidation (25%) and transamidation (75%), in which a glutamine residue of the gliadin and a lysine residue of the acceptor protein are being cross-linked. The cross-linking increases the antigenicity of the gliadin peptides and occurs within and outside of the active site of tTG and results in permanently linked DGP–tTG complexes [43], which are found in small intestine biopsies of patients with CD [44]. Understanding remains poor regarding the mode of development of the well-known autoantibodies to DGP and tTG, but the formation of the tTG–DGP complex is known to be the cornerstone epitope spreading from gliadin to tTG [45].

This autoantigenic complex is able to drive the development of three different types of autoantibodies: one against deamidated gliadin peptides, one against tissue transglutaminase, and one against newly formed epitopes (neopeptides) derived from the cross-linking between the two molecules. Extracellular tTG in the lamina propria is predominantly structured in a closed conformation: once tTG is activated and cross-linked with gliadin peptides, the

enzyme exposes self-epitopes that are ordinarily inaccessible to the immune system. These neopeptides are able to trigger an autoantibody response via a hapten-carrier-like model [46].

It is possible that the autoantibody response against these neopeptides appears early during the course of CD and precedes the formation of anti-DGP and anti-tTG through a mechanism of epitopic spreading in a similar way as the mechanism of formation of anti-nucleosome, anti-dsDNA, and anti-histone antibodies that has been suggested for systemic lupus erythematosus.

Quantitative measurement of all three classes of autoantibody may therefore represent the best means of screening populations or groups of at-risk subjects for identifying patients with silent or subclinical celiac disease, which are 7–8 times more frequent than clinically manifesting subjects and are frequently undiagnosed, according to the epidemiological model of the celiac iceberg.

A new ELISA assay for the simultaneous detection of the three types of autoantibodies was recently proposed as a screening test [28]. This diagnostic method seems to offer superior analytic sensitivity in identifying patients with subclinical or silent CD, as shown in studies of general populations [47] or groups of at-risk subjects [48]. In our recent experience, we have been able to demonstrate the superior analytical sensitivity of the new method compared to consolidated tests:

1. In a sample of 471 at-risk subjects affected by systemic or organ-specific autoimmune diseases, the positivity frequency in anti-complex autoantibody research has been 2.8% (13/471), superior to that found in the general population where it is approximately 1%. Of the 13 positive subjects, three were affected by classic CD and three by silent CD, while another three were DQ2-DQ8-positive [49].
2. In 208 patients affected by non-celiac gastrointestinal diseases (infectious diseases, liver cirrhosis, diverticulitis, irritable colon, etc.), seven patients (3.4%) tested positive for anti-complex antibodies. Of these, three were affected by silent CD [49].
3. In a group of 165 elderly subjects aged greater than 65 years and coming from the general population, 16 (9.7%) tested positive only for anti-tTG–DGP complex. Of these, three were affected by silent CD (unpublished data).
4. In a group of 1,006 patients consecutively referred for diagnostic celiac testing, 26 were found to be affected by CD (new diagnosis). Test sensitivity was the same (96%) for anti-tTG IgA and anti-tTG–DGP complex IgA. Another 34 subjects who were anti-tTG-negative but anti-tTG–DGP complex-positive, were recalled after 6 months: 16 (47%) carried a haplotype compat-

ible with CD and two of these also had become positive for anti-tTG in the follow-up [50].

Our studies confirm the data of Reeves [51], which have compared various methods for measuring autoantibodies related to CD, showing a very high sensitivity of the analytical method for measuring anti-complex antibodies. Detection of anti-tTG–DGP complex antibodies might therefore be able to integrate in one single diagnostic assay the information provided by either tTGAb or DGPAbs and could be used as a reliable test for screening in the general population or in at-risk groups.

The meaning of the positive results found in patients who tested negative in consolidated single tests is not clear. One can hypothesize that these antibodies are present early in the natural course of CD and, therefore, have a predictive value for clinical or subclinical disease, or that they might be present even in non-celiac subjects, either healthy ones or those affected by gluten sensitivity (see below) or other autoimmune or non-immune diseases.

Non-celiac Gluten Sensitivity

Until only a few years ago, almost the whole of the scientific world maintained that gluten would provoke negative effects only in CD and in cases linked to IgE-mediated allergies, both of which are rare in adults and more frequent in the pediatric population. For this reason, patients who complained of abdominal symptoms (abdominal discomfort or pain, bloating or abdominal distension, diarrhea) associated with wheat ingestion but who presented normal values of anti-tTG autoantibodies and absence of villous atrophy, or normal values of IgE against gluten or wheat, were counseled to continue to consume gluten because gluten was not considered as the cause of the symptoms. More recently, however, progress has been made toward the hypothesis that a form of gluten intolerance exists that's different from celiac disease, defined as non-celiac gluten intolerance or gluten sensitivity (GS), and it is estimated that for every person who has CD, there are at least six or seven people who have GS. Therefore, wheat allergy, CD, and GS combined together may affect about 10% of the general population [52, 53]. But what is the evidence for the existence of GS?

1. *There is clinical evidence* from patients who report suffering from gastrointestinal symptoms after consumption of cereals, that is, wheat, rye, and barley and who do not meet the criteria for classification as carriers of overt or latent CD. A few years ago, Kaukinen and coworkers [54] demonstrated that of 94 consecutive adults reporting abdominal symptoms after consumptions of cereals, only eight (9%) had CD and seven (8%) showed evidence of latent celiac disease.

Of the other patients, 19 (20%) were suffering from cereal allergy, but 59 (63%) were not classifiable as CD or as allergic. Of these last, 10 presented an increment of CD3 $\gamma\delta$ +T cell receptor bearing intraepithelial lymphocytes (IELs), but were negative for HLA DQ2 and DQ8, excluding CD latency. In about 40% of patients not affected by CD, there were anti-gliadin antibodies of the anti-IgG or IgA classes.

2. *There is laboratory evidence.* Patients with GS are always anti-tTG-negative and EMA-negative. In 40–50% of cases, IgG-type AGA or IgA-type AGA may present, and only 50% of patients carry the HLA-DQ2 or DQ8 haplotype. That which characterizes such patients, however, is that they do not show histological alterations in the small intestine upon biopsy, but at the most there is a positive increment of intrahepatic CD3 lymphocytes, albeit in a smaller amount than in CD (Marsh 0–1).
3. *There is experimental evidence.* Some of this is indirect and is tied to the “toxicity” demonstrated in vitro of gliadin on intestinal cells, which causes a reduction of the F-actin component, an inhibition of cellular growth, the induction of apoptosis, a rearrangement of the cytoskeleton, and an activation of zonulin signaling pathway [55]. Moreover, the gluten proteins present a huge content of proline and glutamine, which prevent a complete proteolysis by the intestinal enzymes. As a consequence, long toxic gluten oligopeptides are abundantly present at the level of the small intestine. Among subjects in whom the intestinal defense systems, including the innate immune response, are not able to counterbalance the toxic effect, the toxic oligopeptides may give way to an intestinal inflammatory response which in any case has characteristics that are clearly different from those of celiac subjects. This has been well documented by the study of Sapone and coworkers [56]. They showed that GS patients had a number of CD3+ IELs intermediate between CD patients and controls in the context of conserved villous architecture. The most interesting aspect, however, is that IL-17A gene expression in biopsy specimens was significantly elevated in active CD patients compared to GS and controls. The absence of an increment of IL-17 in GS, a cytokine that is thought to be involved in inflammatory and autoimmune processes, combined with the other observed clinical and histological differences, supports the idea that CD and GS are distinct entities and that the immune system deals with gluten in different ways, possibly depending on the genetic makeup of the subject [57, 58]. Such a concept is reinforced the experience of one of the authors, which showed that the levels of B-lymphocyte simulator (BlyS), a molecule significantly overexpressed in several autoimmune and lymphoproliferative diseases,

were increased in CD [59, 60], while in patients with GS, they were similar to those found in healthy blood donors (HBDs; GS=674±58 pg/mL; CD=1,433±468 pg/mL; HBDs=660±240 pg/mL; unpublished data). Finally, Sapone et al. [61], in a recent study, reported that GS patients, in comparison with CD patients, showed normal intestinal permeability and activation of the innate but not adaptive immune response. These changes cause only minimal gut inflammation, suggesting that in GS, lack of adaptive immune response involvement prevents the autoimmune gut insult typical of CD.

Inasmuch as reported above, it appears evident that GS exists distinctly from CD although it lacks definite criteria for a diagnosis. Until now, in the absence of one or more specific markers, once CD has been excluded, the best available test for diagnosing GS is the “gold standard” of food sensitivity testing, e.g., gluten elimination diet for 2–3 months. If, with the gluten-free diet, remission of symptoms is obtained, a definitive demonstration of GS can be counted if the symptoms reappear upon open challenge (gluten reintroduction).

Analogously to what has already been demonstrated for CD, extraintestinal symptoms also have been described in patients with GS. Among these, reports include muscular disturbances [62], neurological disorders, attention deficit, hyperactivity disorder, migraine, headache, sleep disturbance, lethargy, tiredness, and cerebellar ataxia [63], bone or joint pain [56], and also psychiatric disorders such as autism [64] or schizophrenia [65].

Although GS continues to gain medical credibility, only now are the first steps happening toward a recognition of this form of gluten intolerance. At this time, the pathogenic mechanism is not yet known; we don't know if affected persons can tolerate some minimum amount of gluten; if GS be a reversible condition and, therefore, if patients with GS after a more or less long period of gluten-free diet might be able to again consume gluten without manifesting symptoms; and, finally, we don't know if there will be long-term complications of not following a gluten-free diet. Future studies must seek answers to all of these questions, in addition to looking for highly sensitive and specific serological markers which might be used as first-level diagnostic tests analogous to those already in use today with anti-tTG for CD. All this can be hoped for to aid in preventing that GS remain a “no-man's land” [66] as it has been up to now and continues to be, at least in part.

Cytokines and Celiac Disease

The immunological mechanisms which cause damage to the intestinal mucosa are complex, constituting a reaction in

which a large number of immunocompetent cells and immunological mediators participate. The innate immune response, which is normally directed against microbial antigens, becomes in celiac patients directed also against components of some grain cereals [67, 68] and seems to favor the development of the adaptive response to gluten in patients who carry the haplotype HLA-DQ2 or HLA-DQ8 [69]. It has been demonstrated that the p31–43 peptide of α 2-gliadin is able to stimulate the innate immunity in the intestinal epithelium [69, 70]; dendritic and macrophagic epithelial cells secrete IL-15 which causes the expression of the NKG2D receptors on the intraepithelial lymphocytes (IELs) and of their ligands [71]. Therefore, IL-15 bridges innate immunity and adaptive immunity. In addition, it has been demonstrated that gliadin peptides can directly activate the innate immune response by linking themselves to the TLR-4 of the macrophages and of the dendritic cells with a synthesis of inflammatory cytokines such as IL-1 β , IL-8, and TNF α . Besides IL-15, even IL-21, produced by T CD4+ cells, can activate the innate immune mechanisms [72].

The adaptive immune response begins when the antigen-presenting cells (APC) recognize the primary antigen (gliadin peptides) deamidated by the transglutaminase enzyme, process this antigen, and present it to the T CD4+ lymphocytes in association with class II major histocompatibility complex (MHC) molecules. CD4 Th2 lymphocytes direct their humoral immune response secreting interleukin 4 (IL-4), interleukin 5 (IL-5), and interleukin 10 (IL-10) which induce the proliferation and the differentiation of B lymphocytes [73, 74]. The importance of both Th1 and Th2 responses has been demonstrated in that they determine damage to the CD intestinal mucosa itself, while it is not yet clear which of the two CD4 functional components gets stimulated first [74]. It is notable that the cell-mediated immune response by CD4+ Th1 plays a central role in the genesis of tissue lesions at the level of the intestinal mucosa even though the principal cytokine involved in the Th1 polarization, interleukin 12 (IL-12), is not found in the intestinal mucosa of celiac subjects [75]. Analogous to that which is indicated by IL-12, no traces can be found in lesion sites of IL-23, which has been demonstrated to be a potent inducer of the Th1 response. It seems that this function in inducing Th1 polarization is carried out by other cytokines such as IFN α and IL-18 [76].

The humoral response initiates when B lymphocytes, present in the lamina propria, come into contact with the antigen. The T cells implicated in the development of humoral-type immune response are Th2 cells. Contact between B lymphocytes and T lymphocytes appears following re-expression of the antigen processed in association with class II MHC molecules on the part of B cells

and is mediated by molecules of adhesion (LFA1 and LFA-3) and by costimulation (B7/BB1). Following this contact, the T lymphocyte releases various cytokines (IL-4, IL-5, and IL-10) which favor the eventual development of the B cells and influence the “isotype switch” of immunoglobulins [77]. In celiac patients, an abnormal expression of IL-10 and TGF- β has been found; these two cooperate in increasing the secretion of isotype IgA antibodies on the part of activated B cells.

Cytokines, therefore, play a key role in the activation of the immune system and in inducing the classical histological alterations typical of a case of CD; it has been demonstrated that even modifications of the whole of the duodenal mucosa are mediated by TNF- α , TGF- β , IFN- γ and by IL-1 and IL-2 through the suppression of the transcription mechanism of the E-cadherin [78].

As previously stated, interferon-gamma (INF- γ) is of primary importance in the pathogenesis of the disease: elevated levels of INF- γ are in fact demonstrable in duodenal biopsies of celiac subjects in the acute phase of the disease [79] as for IL-18, cytokines which mediate the synthesis of INF- γ [80]. IL-15, the cytokine that bridges innate immunity and adaptive immunity, is found in high concentrations only in the first phases of the disease or after in vitro challenge with gliadin, while it is absent in patients with long-standing untreated CD [81] (Table 1). Another cytokine which is shown to be increased in lesion sites is IL-21, neutralization of which reduces the production of IFN- γ and Th1 activation [82].

A side discourse is merited for B cell-activating factor of the TNF family (BAFF), also known as BLyS. BAFF is a

member of the TNF superfamily which plays an important role in immune response [83]. BAFF is a survival factor of the self-reactive B cells as seen in BAFF transgenic mice [84]; high levels of BAFF have been described in several autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus, and systemic sclerosis [85–88]. BAFF is released by myeloid cells which exert their activity on mature B lymphocytes, promoting survival, proliferation, antibody production, and class switching [89, 90]. Significant increase in serum BAFF of celiac patients was recently demonstrated.

Most studies have been performed using whole duodenal or jejunal biopsy samples with measurement of cytokine production at the local level in small bowel mucosa using immunohistochemical or in situ hybridization methods [91, 92]. A limited number of systemic studies have assessed serum cytokine levels by ELISA assays. Such studies have shown increased levels of IL-2, IL-18, INF- γ , and TNF- α in individuals with active CD [93, 94]. In vitro, gliadin has also been shown to stimulate the production of IL-8 and TNF- α by peripheral blood monocytes from patients with active CD [95].

Recently, Manavalan [96] has published a study in which he followed measurements of IL-2 and IFN- γ (Th-1-derived), IL-4 and IL-10 (Th-2-derived), and IL-1 α , IL-1 β , IL-6, IL-8 e TNF- α (APC-derived) in patients with active disease, in patients on gluten-free diet (GFD), and in patients with refractory celiac disease not responsive to GFD. Manavalan has shown that patients with CD have high levels of circulating pro-inflammatory cytokines and anti-inflammatory cytokines (IL-10) especially during the

Table 1 Distribution and levels of cytokines in serum and in duodenal mucosa in patients with active celiac disease (Active CD), in celiac patients on gluten-free-diet (GFD-CD), and in intestinal biopsies stimulated in vitro with gliadin (GS in vitro)

Cytokine	Source	Serum		Duodenal biopsy		
		Active CD	GFD-CD	Active CD	GFD-CD	GS in vitro
IL-1 α	APC	N	N			
IL-1 β	APC	N	N			
TNF- α	APC	H	N	N	N	H
IL-6	APC	H	N			
IL-8	APC	H	N			
IL-2	TH1	H	N			
IFN- γ	TH1	H	N	H		H
IL-4	TH2	H	N			
IL-10	TH2	H	H			
IL-12	B cell, APC			N		N
IL-15	Epithelial cells			N		H
IL-18	APC			H		H
IL-21	TH1			H		H
IL-23	Dendritic cells			N		N
IL-27	Dendritic cells			H		H
BAFF	Myeloid cells	H	H			

APC antigen-presenting cell, N normal level, H high level

active phase of the disease. 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- γ , persist in the circulation despite GFD. INF- γ , IL-4, IL-6, IL-1 α , and TNF- α correlate with histological damage showing higher mean values in patients with total villous atrophy than in those with partial or no atrophy. In addition, the concentrations of cytokines correlate to the titer of IgA anti-tTG antibodies.

That which appears from studies on serum cytokine testing in CD patients is that the mean concentration of each cytokine is higher than that in normal subjects due to the presence of a few subjects with very high values and many subjects with undetectable levels of the specific marker, thus, making clinical diagnostic use of serum testing of cytokines very problematic [96]. On the other hand, the serum levels of BAFF are higher in more than 80% of patients with active CD, with a significant difference relative to the 30–50% increases shown in systemic autoimmune pathologies [59]. Serum BAFF correlates with anti-tTG IgA antibody levels suggesting, as in other autoimmune diseases, the role of BAFF in sustaining B-cell proliferation, at least in a subgroup of patients. Changes in BAFF levels may be valuable for the follow-up of CD in GFD. Fabris [59] showed that, in a group of patients on GFD, BAFF continued to be above the normal range in most cases, even in patients whose anti-tTG antibodies had normalized. This observation points to the difficulty in eliminating the CD-related inflammatory process even after a strict GFD regimen [37]. BAFF is significantly more elevated in IgA-deficient subjects; this immunological condition is often associated with autoimmune disorders especially with CD [60]. Increased BAFF levels in IgA-deficient patients could represent a risk factor for developing autoimmune disease and eventually lymphoproliferative disorders.

Serum testing of cytokines in CD patients offers opportunity for speculation, since some cytokines seem to have a more specific and significant role than others in this clinical condition. Additional studies are needed to confirm the data presented in the literature and to verify the utility of these measurements in the clinical diagnostic arena.

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