

EDITORIAL

THE IMMUNOLOGY OF GLUTEN SENSITIVITY BEYOND THE INTESTINAL TRACT

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Celiac disease and gluten-sensitive enteropathy are terms that have been used to refer to a disease process affecting the small bowel. However, evidence has been accumulated in literature demonstrating that gluten sensitivity or celiac disease can exist even in the absence of enteropathy, but affecting many organs. Based on overwhelming evidence, immunological pathogenesis has been demonstrated in the joint, the heart, thyroid, bone, and, in particular, the brain cerebellum and neuronal synapsin I. When blood samples of patients with celiac disease are tested against gliadin and different tissue antigens, in addition to gliadin antibody, a significant percentage of them exhibit elevation in antibodies against transglutaminase, heat shock protein, collagen, thyroid, myosin, endothelial cell, bone antigen (transglutaminase), myelin basic protein, cerebellar and synapsin. This elevation of autoantibodies in patients with celiac disease may result in neuroimmune disorders. In fact, in comparison to the general population, the incidence of various autoimmune disorders, including gluten ataxia, is increased up to 30-fold in patients with celiac disease. Therefore, immune evaluation of patients with gluten sensitivity or celiac disease, in addition to gliadin and transglutaminase, should include antibody measurement against thyroglobulin, thyroid peroxidase, heat shock protein, bone transglutaminase, myelin basic protein, cerebellar peptide and synapsin. This novel laboratory approach to gluten sensitivity and autoimmunity may enable clinicians to detect markers of autoimmune diseases. Early identification of gluten sensitive and celiac disease patients and implementation of a gluten-free diet may result in significant improvement and control of associated diseases.

Gluten sensitivity, celiac disease (CD) and gluten-sensitive enteropathy are terms that have been used synonymously to refer to a disease process affecting the small bowel and characterized by gastrointestinal symptoms and malabsorption. However, since 1966 scientific evidence has been accumulated demonstrating that gluten sensitivity can exist even in the absence of enteropathy. For example, patients with dermatitis herpetiformis and presentation of blistering skin do not have any gastrointestinal symptoms but have elevated

gliadin antibody in the blood which improves on a gluten-free diet (1). Additionally, associations of CD with the involvement of other organs such as the central and peripheral nervous systems also go as far back as 1966 (2). However, until recently, this phenomenon of immune reaction against neural tissue, in particular the cerebellum, was attributed to vitamin deficiencies. During the past five years, based on overwhelming evidence of immune pathogenesis involving organs other than gut and skin, many scientists have begun to re-evaluate

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the notion that gluten sensitivity is solely a disease of the gut. Other organs suspected of involvement include: the joints (3-11), the heart (12-15), thyroid (16-18), bone (19-20), the brain cerebellum and the neuronal synapsins (14-28). Although it is believed that the prevalence of CD is one in one hundred, for every symptomatic patient with CD there are eight patients with CD with no GI symptom. In addition 10% of the healthy population have significant elevation in gliadin antibody but no obvious classic disease manifestations. In our laboratory, when the blood of these individuals is tested against different tissue antigens (joint, myosin, endothelial cell, bone antigens, myelin basic protein, cerebellar and synapsin peptides) more than 90% of them exhibit elevation in IgG, IgM and IgA antibodies against one or all these organ-specific antigens.

The gut-joint axis: cross-reactive food antibodies in rheumatoid arthritis

Patients with rheumatoid arthritis (RA) often feel there is an association between food intake and rheumatoid disease severity. In a recent study of this putative immunological link between gut immunity and RA, food IgG, IgA and IgM antibodies were measured in serum and perfusion fluid from the jejunum of 14 RA patients and 20 healthy controls to determine the systemic and mucosal immune response. The antigens originated from cow's milk (α -lactalbumin, β -lactoglobulin, casein), cereals, hen's egg (ovalbumin), cod fish and pork meat. In the intestinal fluid of many RA patients, all three immunoglobulin classes showed increased food specific activities, including gliadin antibodies (3).

It is well-known that some 80% of untreated RA patients have been shown to have reduced maximum gastric acid output leading to a marked reduction in dietary protein degradation, which contributes to enhanced food immunoreactivity (4-5).

A germ-free state prevents the development of gut and joint inflammation in HLA-B27 transgenic rats, thereby giving strong support to a connection between mucosal immunity and arthritis. Also, reactive arthritis in humans appears to be caused by a combination of a mucosa-associated microbial impact and genetic predisposition. Interestingly, some 90% of patients with reactive arthritis or ankylosing spondylitis express HLA-B27, and these

disorders can be associated with Crohn's disease, ulcerative colitis, and jejuno-ileal bypass surgery – again emphasizing the putative gut-joint axis which is also supported by shared homing properties of activated intestinal immune cells (6-7).

Moreover, animal experiments have demonstrated a widespread tissue distribution of food antigens shortly after feeding, which could predispose to synovial immune complex formation and thereby autoimmune joint reactions (3). Additionally, it was reported that intestinal levels of IgM and IgA are increased in patients with ankylosing spondylitis related to disease activity. Antigens from the gut microbiota rather than food are apparently involved in that disease (5-6).

Gluten sensitivity and its association with autoimmune myocarditis

Myocarditis can be associated with systemic autoimmune disorders that if unrecognized and untreated can prevent the recovery of, or even worsen, myocardial function.

Several studies have demonstrated a close association between CD and autoimmune disorders, such as insulin-dependent diabetes mellitus, thyroid disorders, Addison's disease, and connective tissue disorders. An increased prevalence of CD (5.7%) has been recently recognized in patients with idiopathic dilated cardiomyopathy, and an immunologic associative mechanism has been suggested (8).

Although a reciprocal negative interaction between the heart and small intestine is known to occur whenever either organ is severely compromised, less recognized is the possibility of simultaneous damage of the two organs due to a common pathogenetic mechanism. A study five years ago showed the presence of an intestinal inflammatory disease in a large population of patients with myocarditis, with a prevalence that was 14 times higher than that in normal control subjects (8).

This amount appears to be rather reliable, because for both anti-endothelial cell antibodies (AEA) and tTG antibodies, high sensitivity (95% and 100%, respectively) and specificity (90% and 100%, respectively) have been found. Moreover, every single patient with anti-transglutaminase antibodies and symptoms of autoimmune

myocarditis was found to be positive for anti-heart antibody. These patients' clinical manifestations of myocarditis and heart failure markedly improved after a gluten-free diet or a combination of gluten-free diet and immunosuppressive therapy. Prior to administration of the gluten-free diet, these patients with chronic heart failure failed to respond to supportive treatment administered for more than six months (9). This responsiveness to a gluten-free diet and immunosuppressive therapy strongly suggest the existence of an autoimmune disorder directed toward antigenic components of both the myocardium and small bowel. Indeed, both myocarditis and gluten sensitivity are known to occur in association with systemic autoimmune disorders. The observation that these entities can be combined in the same patient is of clinical relevance (10-11).

In fact, gluten sensitivity is invariably associated with an increase of intestinal permeability, which could lead to the translocation of many intestinal luminal antigens (such as ingested food proteins, bacterial breakdown products, endotoxins, heat shock proteins and active enzymes) that can exacerbate myocardial inflammation. Indeed, some other extraintestinal findings of CD, such as chronic unexplained hypertransaminasemia, are attributed to the mechanism of antigenic overload (8).

Furthermore, active CD is accompanied by consistent production of IgA autoantibodies to reticulin, a common constituent of the extracellular matrix; serum IgA antibodies of patients with untreated CD have been reported to strongly react against human brain-blood vessel structures, and this mechanism has been hypothesized to be involved in the abnormal nervous system manifestations frequently described in association with CD. Recent studies have demonstrated that anti-gliadin autoantibodies react with common epitopes on gliadin, calreticulin, enterocytes, and with a nuclear autoantigen expressed in intestinal endothelial cells and in fibroblasts (10-12). On the other hand, tTG, recognized as the target antigen of CD-specific autoantibodies, is an extracellular enzyme that is distributed in the cells of all organs. This is a possible link between tTG and cardiac damage (8). An interesting finding in this study is the clear demonstration that the risk of autoimmune disorders is significantly more elevated in untreated CD and

that the prevalence of autoimmune disorders in CD patients is related to the duration of exposure to a gluten-containing diet; compared with healthy subjects, patients with early diagnosis of CD do not show an increased prevalence of autoimmune disorders. This observation suggests the need for early diagnosis, prompt implementation of a gluten-free diet, and strict compliance to gluten withdrawal.

Gluten sensitivity and its association with osteoporosis

Osteopenia and osteoporosis are well-recognized complications of CD and constitute a major problem through their association with bone fractures, mainly in the peripheral skeleton. Many factors have been suggested to play a role in the development of osteopenia in patients with CD, including calcium and vitamin D malabsorption, malnutrition, and menopause. However, bone demineralization has been demonstrated in patients with no evidence of malabsorption. Therefore, the precise pathophysiological and molecular mechanisms inducing bone disease in gluten-sensitive patients are still only partially known. It is now well established that gluten sensitivity is caused by a T-cell-mediated hypersensitivity with the subsequent release of imbalanced quantities of pro-inflammatory and anti-inflammatory cytokines. Because bone loss is an effect observed in a tissue distant from the primary site of inflammation (small intestine), it was speculated that the effect could be attributable to systemic immunological factors. Another possibility that has not been explored until very recently is molecular mimicry between target antigens of gluten sensitivity such as tissue transglutaminase and bone structures (13-14).

Interestingly, tissue transglutaminase, which belongs to a very ubiquitous family of enzymes catalyzing a Ca^{2+} -dependent acyl-transfer reaction in which new γ -amide bonds are formed, has been shown to be relevant in bone calcification. Hence, it has been demonstrated that native bone tTG has a specific and key role in modulating maturation of bone/cartilage matrix and facilitating its stabilization and finally mineralization. This effect has been attributed to the tTG cross-linking activity, which generates deamidation and negative

charges on specific bases, in which calcium and hydroxyapatite will deposit. In a recent study (13), the presence of circulating anti-bone antibodies in sera from patients with CD was investigated by analyzing the immunoreactive profile of these autoantibodies using immunofluorescence staining and Western blot. Furthermore, it was determined that tissue transglutaminase was the main target of these autoantibodies. Finally, the study explored the relationship between anti-bone antibodies and the presence of bone impairment in these patients (13).

In fact, sera from 51.5% of gluten sensitive patients had antibodies that recognized antigenic structures in chondrocytes and the extracellular matrix along mature cartilage, bone interface, and perichordium of bone. Among controls, only two osteoporotic patients showed very low titers of anti-bone autoantibodies. The immunostaining was localized in areas where an active mineralization process occurred and was similar to the distribution of the native bone tissue transglutaminase. The frequency of patients with positive baseline titers of anti-bone antibodies diminished significantly after treatment with a gluten-free diet. This study provided original evidence that patients with celiac disease have IgA-type circulating autoantibodies against intra- and extracellular structures of tibia. Further, it was suggested that these antibodies recognize bone tissue transglutaminase as the autoantigen, and based on the localization of the immunoreactivity, it was speculated that they might have an active role in the pathophysiology of celiac disease-associated bone complications, including osteopenia and osteoporosis (13).

Association between gluten sensitivity and neuroautoimmunity

During the past two decades, gluten sensitivity and CD has been recognized as a multi-system autoimmune disorder. A growing body of distinct neurologic conditions such as cerebellar ataxia, epilepsy, myoclonic ataxia, chronic neuropathies, and dementia have been reported. However, recent studies suggest that the variability of neurologic disorders that occur in gluten sensitivity is broader than previously reported and includes “softer” and more common neurologic disorders, such as chronic headache, developmental delay (autism), hypotonia,

and learning disorders or ADHD (2, 15).

In relation to these diseases, a question has been raised: Could neural-cell degeneration, resulting in antibody formation as an epiphenomenon, be the trigger to an immune response to gluten at the lamina propria of the gut? After all, the reverse has been shown to be true: anti-gliadin antibodies cross-react with epitopes on Purkinje cells. If such a contention is true, the explanation might well come from the transglutaminase story discussed earlier in relation to gliadin and bone antibody (13-14).

However, the evidence for gluten ataxia as a disease entity is now overwhelming. The disease is characterized by ataxia, the presence of anti-gliadin antibodies, the HLA haplotype (DQ2, DQ8) associated with gluten sensitivity, the presence of anti-Purkinje cell antibodies, the presence of high levels of the interferon- γ -inducible chemokine CXCL10 and often oligoclonal bands in the cerebrospinal fluid (CSF) and the presence of inflammatory pathology of the cerebellum at postmortem (2, 15-18). Perhaps even more compelling is the evidence of a clinical response in the form of improvement of ataxia after a gluten-free diet, even in the absence of an enteropathy. This was demonstrated in the largest control study ever to be published indicating the relationship between gluten, cerebellar antibody and the presence of ataxia (19-20).

What is the pathogenesis of neurological dysfunction in gluten sensitivity?

Experimental evidence suggests that there is antibody cross-reactivity between antigenic epitopes on Purkinje cells and gluten peptides. Thus, sera from patients with CD but no neurological symptoms demonstrate cross-reactivity with epitopes on Purkinje cells. The reactivity can be abolished after absorption of the anti-gliadin antibodies with crude gliadin. In the sera of patients with gluten ataxia, however, there is evidence of additional antibodies targeting Purkinje-cell epitopes (19).

To investigate the epitope responsible for cross-reaction between gliadin peptides and cerebellar peptides, in a study conducted in our laboratory we assessed the reactivity of sera from 50 autism patients and 50 healthy controls to specific peptides from gliadin and the cerebellum (21). A significant percentage of autism patients showed elevations in

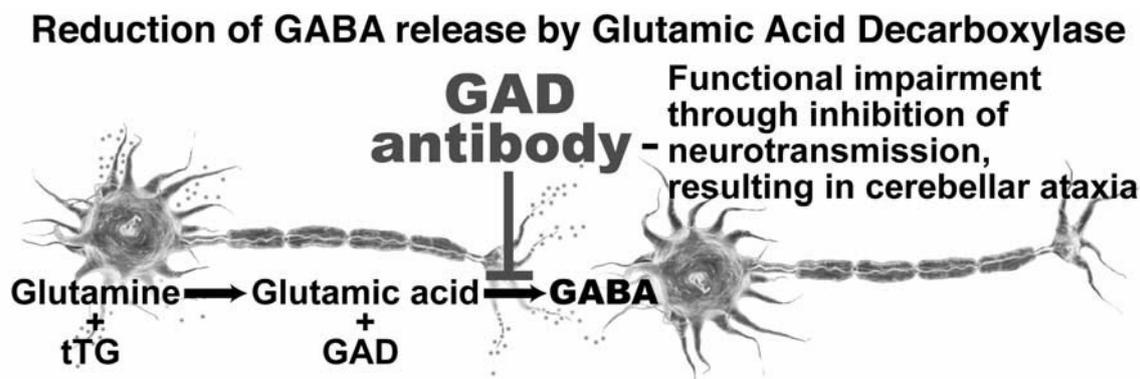


Fig. 1. Presynaptic impairment of cerebellar Purkinje cells by an antibody to glutamic acid decarboxylase (GAD).

antibodies against gliadin and cerebellar peptides simultaneously. For examining cross-reaction between dietary proteins and cerebellar antigens, antibodies were prepared in rabbits, and binding of rabbit anti-gliadin, anti-cerebellar peptides, anti-MBP, anti-milk, anti-egg, anti-soy and anti-corn to either gliadin- or cerebellar-antigen-coated wells was measured. In comparison to anti-gliadin peptide binding to gliadin peptide at 100%, the reaction of anti-cerebellar peptide to gliadin peptide was 22%, whereas the binding of anti-myelin basic protein (MBP), anti-milk, anti-egg and anti-soy to gliadin was less than 10%.

Further examination of rabbit anti-gliadin 8 amino acid peptide (EQVPLVQQ) and anti-cerebellar 8 amino acid peptide (EDVPLLED) with human serum albumin and an unrelated peptide showed no binding, but the reaction of these antibodies with both the cerebellar and gliadin peptides was greater than 60%. This cross-reaction was further confirmed by dot-immunoblot and inhibition studies. We concluded that a subgroup of patients with autism produce antibodies against Purkinje cells and gliadin peptides, which may be responsible for some of the neurological symptoms in autism (21).

Binding of anti-gliadin antibody to neuronal synapsin

Synapsin is a neuronal phosphoprotein involved in the regulation of neurotransmitter release. Celiac disease is also characterized by systemic manifestations that contribute to a complex clinical presentation. Neurologic deficits, including axonal neuropathy and cerebellar ataxia, are among the

most common extraintestinal symptoms associated with celiac disease which were discussed earlier. Based on these neurological manifestations of gluten sensitivity, a different study looked into the cross-reactivity of anti-gliadin humoral immune response with neural tissue (21-23). It was shown that both human and animal anti-gliadin antibodies can cross-react with synapsin I, a cytosolic phosphoprotein found in most neurons of the central and peripheral nervous systems. The anti-gliadin antibodies bound to both isomers of synapsin I, A and B, which have very similar amino acid sequences (22).

In the human serum samples, antibody to synapsin I was detected in several patients with gluten sensitivity, while control specimens without anti-gliadin antibody did not exhibit significant anti-synapsin antibody reactivity. The patient data also clearly demonstrated that anti-gliadin antibody levels do not necessarily correlate with anti-synapsin antibody reactivity and that only certain subsets of anti-gliadin antibodies cross-react with synapsin I. Because of the large number and heterogeneous nature of gliadins, as well as the high diversity of wheat phenotypes, the anti-gliadin immune response is likely to involve a sizeable repertoire of antigenic determinants. Therefore, varying degrees of cross-reactivity to synapsin I can be expected in different patients with gluten sensitivity (23). Such differences in the anti-gliadin antibody cross-reactivity in different patients may reveal clues about the potential pathogenic role of the antibody and its association with specific extra-intestinal complications.

Although pathogenic antibodies typically

target antigens in the extracellular matrix or on the cell surface, there is evidence that antibodies to intracellular antigens can also cause disease. For example, antibodies to glutamic acid decarboxylase, which catalyzes the production of the neurotransmitter γ -aminobutyric acid, have been shown to selectively suppress γ -aminobutyric acid-mediated synaptic transmission. As synapsin I is associated with synaptic vesicles, it might be similarly targeted by antibodies taken up from the extracellular compartment (24-26). Therefore, it is conceivable that, in some patients with gluten sensitivity, the anti-gliadin antibody response would affect synapsin I activity, thus interfering with neurotransmitter release and resulting in neurologic dysfunction (25-27).

Immune cross-reactivity may also lead to tissue damage through T cell-mediated mechanisms. Among the celiac patients in this recent study, anti-synapsin antibodies were present in subjects with neurologic disease, as well as those without (22). This implies that, like other autoimmune disorders, antibody reactivity is only one piece of the puzzle in the pathogenic mechanism of the neurologic complications of celiac disease. Therefore, the potential pathogenic role of anti-synapsin immune cross-reactivity in the neuropathy or CNS manifestations is likely to depend on a number of additional factors, including the type and fine specificity of the immune response, local integrity of the blood-nerve or blood-brain barrier, and presence of pro-inflammatory factors.

Gluten sensitivity and antibodies against glutamic acid decarboxylase, an enzyme responsible for the production of GABA

In addition to tissue transglutaminase, glutamic acid decarboxylase (GAD) is another target enzyme involved in gluten sensitivity and neuroautoimmune disorders. GAD is the enzyme responsible for the production of γ -aminobutyric acid (GABA), the most abundant inhibitory neurotransmitter in the central nervous system (26). Antibodies against GAD have been described in stiff person syndrome, insulin-dependent diabetes mellitus (IDDM), and autoimmune polyendocrine syndromes, as well as in some immune mediated ataxias (28). These antibodies are present in at least 60% of both

patients with gluten ataxia and patients with CD and no neurological manifestations. Furthermore, the levels and positivity of these anti-GAD antibodies can be significantly reduced by the introduction of a gluten-free diet in both of these patient groups. In the patients with neurological manifestations, who also have an enteropathy, the prevalence of these antibodies is 96%. These observations imply that the presence of these antibodies in the context of the enteropathy might predispose individuals to the development of neurological disease (28). However, this cannot explain the entire story within the whole spectrum of gluten sensitivity because the antibodies are still present in some patients with gluten-related neurological dysfunction and no enteropathy. The prevalence of GAD within the nervous system correlated with the clinical presentation of ataxia and/or peripheral neuropathy being the commonest neurological manifestations of gluten sensitivity. The presence of GAD in the enteric plexus could hold the key to the generation of anti-GAD antibodies in patients with CD (28). The mechanism by which GAD antibody induces presynaptic impairment of cerebellar Purkinje cells through inhibition of GABA is shown in Fig. 1.

Gluten sensitivity and autoimmune endocrine disorders

Polyglandular autoimmunity. Autoimmune polyglandular syndrome is a rare endocrine disorder comprising a combination of at least two of the following autoimmune endocrine disorders: Addison's disease, autoimmune thyroid diseases, hypoparathyroidism, type I diabetes, or primary gonadal failure (29-30). In addition, nonendocrine diseases that have been described in autoimmune polyglandular syndrome include mucocutaneous candidiasis, vitiligo, alopecia, pernicious anemia, autoimmune hepatitis, primary biliary cirrhosis, and intestinal malabsorption. There have been several studies on a link between autoimmune polyglandular syndrome (including type I diabetes), thyroiditis, and gluten sensitivity (10, 29-34). Therefore, gluten sensitivity may coexist with several extraintestinal diseases.

Many studies indicate an association between type I diabetes and celiac disease (34). This prevalence of CD in type I diabetes patients is 10 to

30 times that of the normal population. Since many type I diabetes patients have asymptomatic or silent CD or gluten sensitivity without enteropathy, this association is recognized in such a highly susceptible population only after administration of a gluten-free diet and an improvement in a diabetic condition, for example, decreased frequency of insulin reactions. The disappearance of diabetic instability after the introduction of a gluten-free diet in such patients emphasizes the importance of the early recognition and identification of CD (35- 36).

Thyroid autoimmunity is another example of an autoimmune disease that is associated with celiac disease. It is common and is due to an apparent immune reaction directed against self antigens of the thyroid. Three thyroid diseases are considered to have autoimmune etiology: Hashimoto's thyroiditis, idiopathic myxedema, and Graves' disease. The antigens against which the autoimmune reactions are directed to produce thyroid autoimmune disease include thyroglobulin (Tg), thyroid peroxidase (TPO), and the TSH receptor (37-38). It seems that these autoantibodies cause direct thyroid dysfunction, as in Graves' disease caused by antibodies to the TSH receptor, or a destructive process, as in Hashimoto's thyroiditis and idiopathic myxedema.

CD and autoimmune thyroid disorders share a common genetic predisposition, namely, the DQ2 allele. This common predisposing genetic background would explain the higher incidence of thyroid autoimmune disorders in CD than in the general population. For example, in one of many studies, the investigator found that the overall prevalence of autoimmune thyroid diseases was significantly higher in celiac patients than in controls (21% versus 11%). The prevalence of both hypo- and hyperthyroidism was not different from that of controls, while the prevalence of autoimmune thyroid disease with euthyroidism was 13% in patients and 4.7% in controls (32). In a different study, investigators performed thyroid antibody tests and thyroid echography in 47 patients with CD and 91 healthy controls and found that CD patients had a three- to fourfold increase in the incidence of thyroid autoimmunity (30). Moreover, other studies have reported an association of idiopathic hypoparathyroidism with CD. CD has been reported to be associated with both hypo- and

hyper- (both primary and secondary) thyroidism. CD has been associated with significant bone loss and hypocalcemia. Most CD patients with bone loss and hypocalcemia have secondary hypoparathyroidism. A gluten-free diet in patients with CD with secondary hypoparathyroidism has been reported to result in normal bone mineral density (39-40). Therefore, the presence of hypocalcemia or normocalcemic hypoparathyroidism should prompt an examination for CD. Treatment with a gluten-free diet should result in clinical improvement and restoration of normal calcium levels (39-40).

It is thus recommended that patients with autoimmune endocrine disorders (including patients with autoimmune thyroiditis, type I diabetes, Addison's disease) and patients with idiopathic hypo- and hyperparathyroidism be routinely investigated for gluten sensitivity, with or without enteropathy. If simultaneously antibodies against glutamic acid decarboxylase, transglutaminase, thyroglobulin, thyroid peroxidase, heat shock protein and gliadin, gliadin peptide and gluteomorphins are detected, then the patient should be monitored closely after the institution of a gluten-free diet.

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