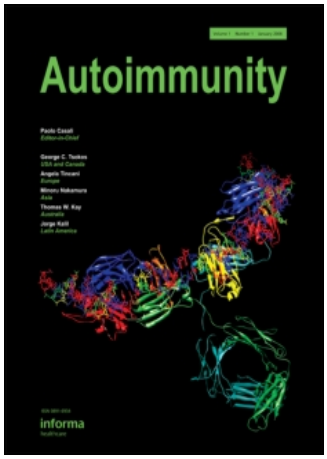

Autoimmunity
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713453864

Autoantibodies in celiac disease

Armin Alaedini [a]; Peter H. R. Green [b]
[a] Department of Neurology and Neuroscience, Cornell University, New York, NY, USA
[b] Celiac Disease Center, Columbia University College of Physicians and Surgeons, New York, NY, USA

Online Publication Date: 01 February 2008
To cite this Article: Alaedini, Armin and Green, Peter H. R. (2008) 'Autoantibodies in celiac disease', Autoimmunity, 41:1, 19 - 26
To link to this article: DOI: 10.1080/08916930701619219
URL: http://dx.doi.org/10.1080/08916930701619219

Autoimmunity, February 2008; 41(1): 19–26

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Autoantibodies in celiac disease

ARMIN ALAEDINI[1] & PETER H. R. GREEN[2]

[1]*Department of Neurology and Neuroscience, Cornell University, New York, NY, USA, and* [2]*Celiac Disease Center, Columbia University College of Physicians and Surgeons, New York, NY, USA*

(Submitted 22 February 2007; accepted 13 March 2007)

Abstract
Autoantibody production is an important feature of many autoimmune disorders, signifying a breakdown of immune tolerance to self-antigens. In celiac disease, an autoimmune enteropathy with multiple extra-intestinal manifestations, autoantibody reactivity to transglutaminase 2 (TG2) has been shown to closely correlate with the acute phase of the disease. It serves as a specific and sensitive marker of celiac disease, and is highly useful in aiding diagnosis and follow-up. Immune reactivity to other autoantigens, including transglutaminase 3, actin, ganglioside, collagen, calreticulin and zonulin, among others, has also been reported in celiac disease. The clinical significance of these antibodies is not known, although some may be associated with specific clinical presentations or extra-intestinal manifestations of celiac disease. This review examines the presence of anti-TG2 and other autoantibodies in celiac disease, discussing their diagnostic value, their potential role in disease pathogenesis and current hypotheses that explain how their release may be triggered.

Keywords: *autoimmunity, antibody, transglutaminase, enterpathy*

Introduction

Celiac disease is a common inflammatory disorder, characterized by an improper immune response to ingested wheat gluten (composed of gliadin and glutenin) and related cereal proteins in genetically predisposed individuals, resulting in villous atrophy, crypt hyperplasia and lymphocytic infiltration in the small intestine. Elimination of the immunogenic proteins from diet leads to clinical and histological improvement [1]. Although once thought of as a pediatric enteropathy, celiac disease is now recognized as a complex disorder that affects multiple organs and can arise at any age. The extra-intestinal manifestations of celiac disease include bone disease, dermatitis, malignancies, endocrine disorders and neurologic deficits [2]. Some of the associated complications of celiac disease are the direct product of the characteristic mucosal lesion or the subsequent malabsorption that leads to nutrient deficiency. Other extra-intestinal associations are probably due to common genetic background, most importantly linked to the HLA region of chromosome 6, and to immunologic factors, possibly involving immune reactivity to specific autoantigens. The presence of antibodies to the autoantigen transglutaminase 2 (TG2) is a hallmark of active celiac disease. The anti-TG2 antibody response is believed to be driven by the intestinal immune reaction to gluten, although the mechanism of its release and its role in the pathogenesis of celiac disease are not entirely clear yet. Other autoantibodies have also been reported in celiac disease, some in association with specific extra-intestinal complications. In this review, we discuss the autoantibodies of celiac disease and examine their diagnostic value, origin and potential for pathogenesis.

Anti-transglutaminase 2 antibodies

In the 1960s, it became clear that celiac disease is associated with autoantibodies against endomysial

Correspondence: A. Alaedini, Department of Neurology and Neuroscience, Weill Medical College of Cornell University, 1300 York Avenue, Room LC807, New York, NY 10021, USA. E-mail: ara2004@med.cornell.edu; Peter H. R. Green, Department of Medicine, Columbia University College of Physicians and Surgeons, 180 Fort Washington Avenue, Room 956, New York, NY 10032, USA. E-mail: pg11@columbia.edu

ISSN 0891-6934 print/ISSN 1607-842X online © 2008 Informa UK Ltd.
DOI: 10.1080/08916930701619219

tissue (loose connective tissue surrounding smooth muscle fibers) [3,4]. These antibodies became known as anti-endomysial and anti-reticulin antibodies and were generally detected by immunofluorescence assays. In 1997, TG2 was identified as the main autoantigen for the anti-endomysial antibody [5]. The so-called anti-reticulin antibodies were later shown to target the same antigen [6,7]. TG2 appears to be an important player in celiac disease, not only as a target autoantigen, but also as a deamidating enzyme that can enhance the immunostimulatory effect of gluten. The activity of TG2 includes catalysis of calcium dependent acyl transfer cross-linking reactions between glutamine residues and lysine residues (or other groups with primary amines), leading to formation of N^{ε}-(γ-glutamyl)-lysine isopeptide bonds [8–10]. This cross-linking activity of TG2 is thought to have a role in triggering the immune reaction against itself in celiac disease, as will be discussed in more detail later. TG2 can also convert glutamine residues to glutamic acid by deamidation when the amine is replaced by water. This deamidating activity has been shown to render gliadin peptides negatively charged, significantly increasing their affinity for the HLA-DQ2 and -DQ8 molecules that are involved in presenting them to T cells [11,12].

With establishment of TG2 as the main autoantigen for anti-endomysial antibodies, an enzyme-linked immunosorbent assay (ELISA) was developed, which has been rapidly replacing the more labor-intensive immunofluorescence assays. Use of purified or recombinant human TG2 in the ELISA has improved performance over earlier versions that used guinea pig transglutaminase, especially with regard to specificity [13–16].

The sensitivity and specificity of the anti-TG2 antibody for celiac disease vary in different studies, resulting from differences in the choice of gold standard, patient selection bias and test standardization. However, a systematic and rigorous review of the literature, using strict criteria to exclude studies with methodological flaws, indicates that the anti-TG2 antibody is currently the most sensitive and specific antibody marker for celiac disease [17]. IgA anti-TG2 antibodies have sensitivities over 90% and specificities that exceed 95%. IgG anti-TG2 antibodies have good specificities (above 95%), but they suffer from poor sensitivities (around 40%) [17]. Therefore, antibodies of the IgA isotype are recommended for initial screening, as they are much more sensitive than the IgG.

Currently, the anti-TG2 antibody test is an important tool in the diagnosis of celiac disease. Patient serum is tested for anti-TG2/endomysial antibodies upon suspicion of celiac disease, either due to presence of characteristic symptoms, or if the individual is in an at-risk group [2]. If the patient is positive for IgA anti-TG2/endomysial antibodies, or if IgA deficiency is noted and an IgG antibody is positive, or in less common cases of negative serology but high clinical suspicion, an intestinal biopsy is performed. Positive identification of the characteristic histologic abnormalities leads to a presumptive diagnosis of celiac disease and institution of gluten-free diet. Clinical improvement on the diet would yield a definitive diagnosis. It should be noted that while the anti-TG2/endomysial antibody test is a highly valuable tool in the screening of patients, the actual diagnosis of celiac disease solely on the basis of serologic markers is not yet accepted, and that identification of the characteristic mucosal abnormalities upon intestinal biopsy remains a requirement for diagnosis [18,19]. In addition, the sensitivity of the anti-TG2 antibody test drops significantly with milder histologic grades of celiac disease [17,20,21]. Therefore, cases of the disease with partial villous atrophy may go undetected if relying only on the serologic tests in their currently devised formats.

Other autoantibodies in celiac disease

Although TG2 is the main autoantigen of anti-endomysial antibodies, it is not the only one, as shown by absorption studes [22–24]. In addition, there are other autoantibodies in celiac disease that are not related to the endomysial staining. Most of these antibodies do not appear to have high sensitivity or specificity for celiac disease, but they may be associated with particular clinical manifestations of the disease, such as dermatologic, neurologic or endocrine complications. As such, they have the potential to become valuable diagnostic markers in the future after their utility has been demonstrated (Table I).

As there is considerable sequence homology in the transglutaminase family of enzymes, it is not surprising if the anti-TG2 antibodies in celiac disease originating from the mucosal immune response in the small intestine are found to bind other transglutaminases in the body. In fact, such antibody cross-reactivity may

Table I. Reported autoantibody reactivities in celiac disease.

Autoantigen	Reference
Transglutaminase 2	[5] and many others
Transglutaminase 3	[26]
Factor XIII	[28]
Actin	[29–32]
Calreticulin	[81]
Gangliosides	[36,39,82]
Collagens	[55]
Synapsin I	[48]
Zonulin	[44]
Cardiolipin	[45]
ATP synthase β chain	[30]
Enolase α	[30]

be associated with specific extra-intestinal manifestations of celiac disease in some cases. In dermatitis herpetiformis, presence of antibodies against both TG2 and transglutaminase 3 (TG3), a cytosolic enzyme involved in cell envelope formation during keratinocyte differentiation [25], has been demonstrated [26,27]. TG3 is also found in complex with the IgA precipitates on the skin, and may therefore have a role in the pathogenesis of the skin manifestations of the disease [26,27]. In another study, antibodies to factor XIII, a plasma transglutaminase, have been detected in some patients with CD, although they are not found to be associated with any clinical manifestations [28].

Several studies have reported the presence of antibodies to cytoskeletal actin in patients with celiac disease [29–32]. These antibodies are not specific to celiac disease, as they have been found in chronic hepatitis in the past [33,34]. But in celiac disease, the presence of IgA anti-actin antibodies correlates with the degree of villous atrophy, appearing in more severe forms of the disease [31,32]. This may indicate that the antibodies follow mucosal injury and are a product of the release of actin from apoptotic cells, which triggers the autoimmune response. Furthermore, their appearance in celiac disease is dependent on gluten intake, indicating that they are related to the anti-gluten immune response in celiac disease [35].

Recent studies have described the presence of anti-ganglioside antibodies in conjunction with gluten sensitivity and idiopathic central or peripheral nervous system deficits in some patients [36–39]. Gangliosides are sialic acid-containing glycosphingolipids present in high concentrations in the nervous system, but they are also found in abundance on gut epithelial cells [40,41]. Antibodies to these glycolipids are generally associated with and serve as diagnostic markers for specific autoimmune peripheral neuropathies, such as Guillain-Barré syndrome [42,43]. In celiac disease, the presence of anti-ganglioside antibodies may be related to the gluten sensitivity, as it appears to depend on gluten intake in some patients [39].

Several other antibodies have been reported in conjunction with celiac disease, though in relatively small numbers of patients. One of these is the anti-zonulin antibody [44]. The zonulin protein is involved in the modulation of intestinal permeability by interaction with intercellular tight junctions. Its expression is upregulated in several autoimmune diseases, including celiac disease. Presence of IgA anti-zonulin antibody is found to correlate with the acute phase of celiac disease and to disappear during remission in some patients [44]. IgA autoantibodies to collagen types I, III, V and VI have also been found in celiac disease. No specific clinical manifestation is reported to be associated with these antibodies yet, but the prevalence of connective tissue diseases in patients with celiac disease may be related to an anti-collagen immune reactivity. Presence of antibodies to cardiolipin, single- and double-stranded DNA, ATP synthase β chain and enolase α in some celiac patients has also been reported [30,45]. The clinical relevance of these antibodies remains to be determined.

At least two potential autoantigens are reported to bind to anti-gliadin antibodies in celiac disease, presumably as a result of structural similarity. IgA antibodies to calreticulin, a multifunctional calcium-binding protein, have been described in celiac disease, as well as in some other autoimmune disorders [46,47]. However, the reported findings remain to be confirmed, as the presence of specific high affinity anti-calreticulin antibodies in autoimmune disease has been questioned [34]. Another protein that appears to bind to anti-gliadin antibodies is synapsin I. Both animal and human affinity purified anti-gliadin antibodies have been shown to cross-react with synapsin I [48]. However, anti-gliadin antibody levels do not necessarily correlate with anti-synapsin antibody reactivity and the cross-reactivity is observed only in a subset of celiac patients. As synapsin I is a protein that is relatively specific to the nervous system, the possibility that it might be associated with neurologic deficits is intriguing.

Finally, as celiac disease is associated with several autoimmune endocrine disorders, including type 1 diabetes and autoimmune thyroid disease, autoantibodies that are specifically affiliated with them can be found in the serum of patients with celiac disease [49]. The link between these disorders and celiac disease is mainly a result of common genetic background, associated with the HLA region of chromosome 6 [49–52]. Nevertheless, the development of these organ-specific autoantibodies may be directly related to gluten intake in some cases, as thyroid disease- and diabetes-related antibodies in children with celiac disease have been reported to disappear in response to gluten-free diet [53].

Mechanisms for triggering autoantibody production in celiac disease

Autoantibodies are the result of a breakdown of tolerance to self-antigens, which results in the activation of autoreactive B cells (and usually T cells) that may have escaped negative selection. The initial release of antibodies by plasma cells may be followed by affinity maturation toward the target and isotype switching. But what is the trigger for this, and how is tolerance to an autoantigen, like TG2, compromised in celiac disease? One way of triggering and maintaining the autoimmune response in celiac disease may be through epitope spreading, where the initial immune response to gliadin or an autoantigen is diversified to include other epitopes on that antigen (intramolecular help) or other antigens (intermolecular help). Because covalent complexes can easily form between TG2 and

gliadin proteins, the TG2 antibody reactivity in celiac disease is believed to be a result of epitope spreading via intermolecular help. In this proposed mechanism that relies on complex formation between TG2 and gliadin, gliadin acts as a carrier protein for TG2. As a result, gliadin-specific T cells provide cognate help to TG2-specific B cells [11], which leads to their activation and the eventural release of anti-TG2 antibodies (Figure 1). This presumably gliadin-dependent T cell-driven mechanism of intermolecular help can result in an anti-TG2 antibody response in the absence of TG2-specific T lymphocytes. The strict dependence of anti-TG2 antibodies on gliadin intake and the apparent absence of TG2-reactive T cells in patients seems to support this mechanism [11,54].

Intermolecular help may also be proposed to explain the presence of antibody reactivity to other autoantigens that have been shown to bind gliadin. Collagen, for example, is a highly abundant protein of the extracellular matrix in the lamina propria, and an excellent substrate for TG2. The cross-linking of gliadin peptides with collagen has been shown to be catalyzed by TG2 [55]. The complex formation between gliadin and intestinal autoantigens may also take place in a TG2-independent manner. Gliadin is found to bind to GM1 ganglioside and to the GM1-rich intestinal brush border membrane in the absence of TG2 activity [56]. The haptenization of GM1 by gliadin may be responsible for driving the anti-ganglioside antibody response in some patients with gluten sensitivity, granted that GM1-gliadin complexes occur *in vivo*.

Another way by which autoreactive antibodies may be generated in celiac disease is by a form of molecular mimicry. A specific immune response targeting gliadin, TG2, or another molecule, is proposed to cross-react with other antigens due to structural similarity. The observed antibody reactivities to TG3 and factor XIII are likely to be the result of cross-reactive anti-TG2 antibody binding. Similarly, anti-gliadin antibodies have been shown to cross-react with calreticulin and synapsin I. Although the initial reactivity is due to structural homology between the antigens, additional immune reactivity to unique epitopes of the secondary antigen may eventually develop as a result of epitope spreading via intramolecular help, in a manner similar to that described in other autoimmune disorders [57,58].

Finally, the presence of autoantibodies in celiac disease may be directly attributed to the characteristic tissue remodeling and intestinal cell apoptosis that result in the release of new epitopes into the extracellular environment. These intracellular antigens, which are generally hidden from the body's immune system, may be seen as foreign in the pro-inflammatory enviroment of the mucosal lesion by specific B or T cells that have escaped negative selection. In addition, the subsequent breakdown of the released antigens—into peptides, for example—often unmasks many cryptic epitopes that have

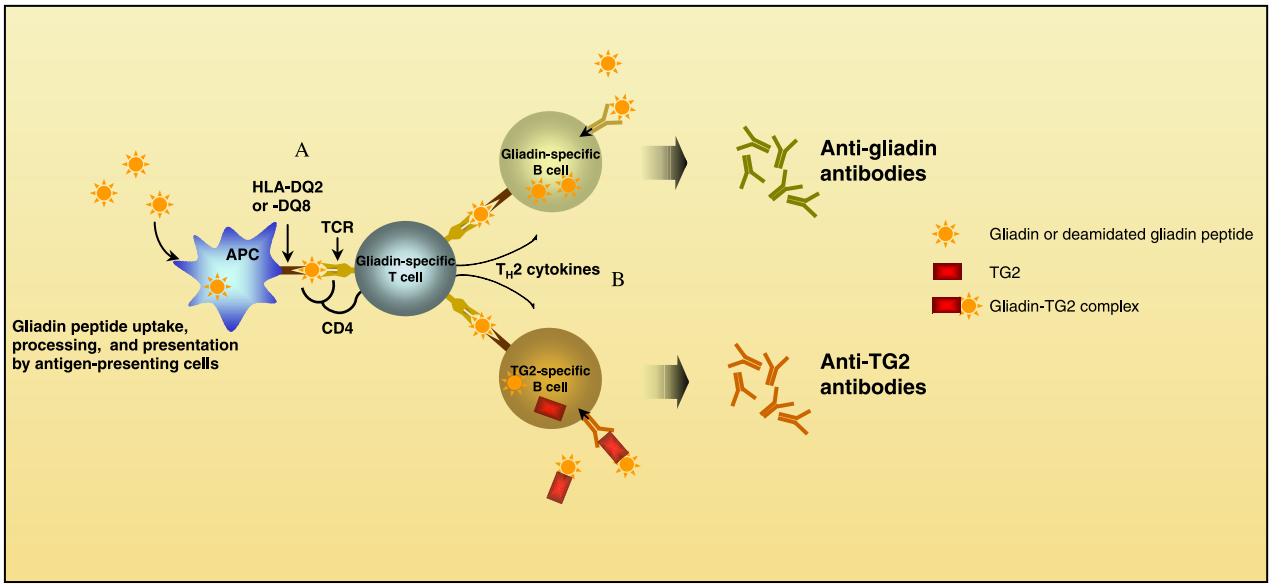

Figure 1. Simplified schematic depicting the proposed process of intermolecular help in celiac disease. (A) Gliadin peptides are presented in complex with HLA-DQ2 or -DQ8 molecules of antigen presenting cells, such as dendritic cells, macrophages, or B cells, to $CD4^+$T cells. (B) Gliadin-specific B cells receive help from the activated gliadin-specific T cells, leading to B cell clonal expansion and release of antibodies to gliadin. TG2-specific B cells can also receive help from the gliadin-specific T cells if they take up and process gliadin-TG2 complexes and specifically present gliadin peptides to the activated T cells. This would result in the release of anti-TG2 antibodies in the absence of TG2-specific T cells. A similar process may be proposed to explain the presence of antibodies to other autoantigens that bind gliadin, such as collagen and ganglioside.

the potential to activate the otherwise silenced self-reactive lymphocytes and drive the autoantibody response. New epitopes can also be generated by the heightened enzymatic activity of TG2 released during apoptosis in the mucosal lesion. Many proteins are good substrates for TG2, which can deamidate them, or cross-link them to itself and other molecules, thus creating novel epitopes that the immune system would regard as non-self and mount a response to. Other post-translational modifications common to apoptosis, such as phosphorylation and oxidative fragmentation, may also trigger autoantibody responses. The presence of antibodies to antigens like actin, DNA, ATP synthase β chain and enolase α may be at least partly a result of these neoepitope release phenomena in celiac disease.

Pathogenic role of autoantibodies in celiac disease

The gluten-driven mucosal lesion in celiac disease is believed to be mainly T cell mediated and to involve multiple effector mechanisms. The infiltration by $CD4^+$T cells into the lamina propria and of mainly $CD8^+$ and $CD4^-CD8^-$ T cells into the epithelium is an important feature of active celiac disease. In genetically predisposed individuals with the HLA-DQ2/DQ8 molecules, the intruding glutamine- and proline-rich gluten peptides are processed and presented to gluten-specific $CD4^+$T cells by antigen presenting cells within the lamina propria. Recognition of HLA-bound gluten peptides by T cells leads to their activation and release of various cytokines. Some of these cytokines drive the activation and clonal expansion of B cells that produce antibodies, while others promote various inflammatory mechanisms that contribute to the destruction of the mucosal matrix [59,60]. The intestinal immune response to gluten also occurs within the epithelium by mechanisms that appear to be different from those of the lamina propria [61]. Intraepithelial T cells interact with stress proteins expressed by epithelial cells and exhibit cytolytic activity that leads to destruction of the epithelium [62–64]. Unlike the antigen-specific activation of $CD4^+$T cells that involves the adaptive immune response, activation of intraepithelial lymphocytes appears to be additionally mediated by the innate immune system [64–67].

What part do the autoantibodies, especially those directed at TG2, play in the pathogenic process in celiac disease? The role of autoantibodies in disease pathogenesis is complex and varies from one disease to another. Autoantibodies can specifically interfere with the biologic activities of a specific antigen. But they can also form immune complexes that activate the complement system to cause tissue injury. While there is no consensus on the exact action of anti-TG2 and other autoantibodies in celiac disease, the available evidence suggests that they have the potential to have a pathogenic contribution. It has been shown that the anti-TG2 antibodies in celiac disease recognize the enzymatic core of the protein and interfere with TG2 bioactvity [68,69]. TG2, which is required for the activation of transforming growth factor β (TGF-β) [70], is therefore involved in differentiation of epithelial cells [71,72]. Considering this, the local production of these antibodies might have a deleterious effect on cell differentiation, contributing to the characterstic mucosal transformation in celiac disease. A reduction in eptithelial cell differentiation in response to anti-TG2 antibodies has been demonstrated [73]. Anti-TG2 antibodies have also been shown to increase epithelial cell permeability in an intestinal cell line and to induce monocyte activation upon binding to Toll-like receptor 4 [74]. Considering that transglutaminase is present in many different tissues, anti-transglutaminase antibodies may have a pathogenic effect not only in intestinal lesion, but also in some of the extra-intestinal manifestations of gluten sensitivity, including those affecting the nervous system and skin. For example, deposits of anti-TG2 antibody have been observed in the cerebellum and brainstem of a patient with gluten sensitivity and cerebellar ataxia, presenting the possibility that they may be involved in the associated ataxia [75]. Similarly, in dermatitis herpetiformis, antibody deposits in the papillary dermis of patients have been found to contain TG3 [26]. The reported presence of anti-TG2 antibody in other disorders and its association with increased patient mortality may be further indication for a role in disease pathogenesis [76].

Evidence for direct involvement of other celiac disease-associated autoantibodies in pathogenesis is scarce. In the case of anti-ganglioside antibodies, many studies support a pathogenic role in autoimmune neuropathies. Immunization with gangliosides and passive transfer of anti-ganglioside antibodies have been shown to induce neuropathy in experimental animals [77,78]. More specifically, binding to the neuromuscular junction and blockade of neuromuscular transmission by anti-ganglioside antibodies have been demonstrated in the presence, as well as in the absence, of complement [79,80]. Whether the antibodies can exert a similar pathogenic role in the celiac disease-associated peripheral neuropathy remains to be determined. These and other antibodies that target nervous system antigens may have a role in the neurologic complications of celiac disease, but only if they can cross the blood–brain or blood–nerve barrier and reach their targets.

Conclusions

The discovery and characterization of anti-TG2 autoantibody reactivity in celiac disease has dramatically altered our understanding of the disease and its

mechanism of pathogenesis. Being a highly specific and sensitive marker of the active phase of the disease, it has also significantly contributed to more efficient diagnosis and follow-up of patients. However, as in other multi-system autoimmune disorders, the spectrum of autoantibody reactivity in celiac disease is wide, involving a number of other antigens. Careful scrutiny of these antibodies with regard to their association with specific complications of celiac disease, their mechanism of development, and potential for pathogenesis will provide us with additional insights into this important and prevalent disease, which might help to identify new markers for diagnosis of individual extra-intestinal complications of the disease and offer new avenues for therapy.

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