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Transglutaminase 2 and Transglutaminase 2 Autoantibodies in Celiac Disease: a Review

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Abstract Celiac disease is a common inflammatory disorder with a prevalence of 1–2 % in which a distinct dietary wheat, rye, and barley component, gluten, induces small-bowel mucosal villous atrophy, crypt hyperplasia, and inflammation. The small-bowel mucosal damage can be reversed by a strict lifelong gluten-free diet, which is currently the only effective treatment for the condition. A key player in the pathogenetic process leading to the enteropathy is played by a protein called transglutaminase 2 (TG2), which is able to enzymatically modify gluten-derived gliadin peptides. The TG2-catalyzed deamidation of the gliadin peptides results in their increased binding affinity to the disease-predisposing human leukocyte antigen (HLA) DQ2 and DQ8 molecules, thus enabling a strong immune response to be launched. Blocking the enzymatic activity of TG2 has thus been suggested as a suitable novel pharmacological approach to treat celiac disease. By virtue of its transamidation capacity, TG2 is also able to cross-link gliadin peptides to itself, this resulting in the generation of TG2-gliadin peptide complexes whose presence might provide an explanation for the generation of the TG2 autoantibodies characteristic of celiac disease. Due to their excellent specificity for the disorder, the TG2-targeted autoantibodies are widely used in the diagnostics as a first-line test to select patients for gastrointestinal endoscopy. More recently, it has come to be appreciated that these autoantibodies and also the TG2-specific B cells might play an active role in the disease pathogenesis. In this review, we assess the role of TG2, TG2-specific B cells, and autoantibodies in celiac disease.

Keywords Celiac disease · Transglutaminase 2 · Transglutaminase 2 autoantibodies

Introduction

Transglutaminase 2 (TG2) is a ubiquitously expressed protein with multiple functions, including enzymatic, cell adhesion, cell signaling, and G-protein activities. Interestingly, TG2 has been implicated in a variety of human disorders including several neurodegenerative conditions, cancer, and celiac disease. Celiac disease is an inflammatory disorder characterized by a loss of tolerance against dietary gluten present in wheat, rye, and barley in a subset of genetically predisposed individuals. Typically, in celiac patients, the ingestion of gluten leads to small-bowel mucosal villous atrophy, crypt hyperplasia, and inflammation, which recovers with adhesion to a strict gluten-free diet (GFD). TG2 is an important player in the pathogenetic process leading to the enteropathy and has therefore been suggested as a suitable target for novel future treatment options in celiac disease.

A hallmark of celiac disease is the presence of circulating autoantibodies targeted against TG2, which have proved eminently useful in the diagnostic workup of celiac disease. It has even been suggested that in some cases, a firm celiac disease diagnosis could be established without the demonstration of small-bowel mucosal villous atrophy in biopsies taken upon endoscopy.

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In addition to being a valuable diagnostic tool, the TG2 antibodies have been demonstrated to exert a variety of effects in vitro and in vivo. Both the TG2-specific B cells and the autoantibodies might be active players in the disease pathogenesis. In this article, we review the role of TG2 in the development of celiac disease. In addition, we assess the usefulness of the TG2 autoantibodies in diagnostics and discuss the potential role of the TG2-specific B cells and the TG2 autoantibodies in the pathogenesis.

Transglutaminase 2

General Introduction to the Transglutaminase Protein Family

Transglutaminases (TGs) are a family of enzymes which catalyze Ca^{2+}-dependent post-translational modification of proteins [1]. They were first discovered in the 1950s, since when nine TG isoforms have been described in humans, eight of them being active enzymes [2, 3]. The TG family comprises TGs 1–7, blood coagulation factor XIII, and the enzymatically inactive erythrocyte band 4.2; all of which are encoded by a set of structurally related genes [2].

All TGs share the same basic four-domain tertiary structure, with minor variations specific for each isoform. Even though the isoforms have different expression patterns, substrate specificities, and mechanisms for activation and regulation, their catalytic mechanism is conserved, resembling that of the cysteine proteases [1]. The enzymatic reactions catalyzed by TGs generally occur through transamidation, esterification, and hydrolysis; all of which lead to post-translational modifications in the target proteins [1]. The best-established function of TGs is the ability to mediate selective protein cross-linking by forming covalent isopeptide linkages between two target proteins [4]. The resulting cross-linked products in many cases have high molecular masses and are unusually resistant to proteolytic degradation and mechanical strain. They are thus of functional significance in tissues and processes in which these properties are important, for example, extracellular matrix stabilization, apoptosis, blood clotting, and wound healing [1].

Characteristics of TG2

TG2, also known as tissue transglutaminase, is the most intensively studied member of the TG family and was the first to be discovered [3]. Human TG2 is a monomeric protein of 686 amino acids, encoded by the *TGM2* gene located on chromosome 20q11–12 [5]. The protein has a structure typical of all TGs, consisting of the following four distinct domains: an N-terminal β-sandwich domain containing fibronectin and integrin binding sites; a core domain containing the catalytic core and regulatory sites; and two C-terminal β-barrel domains, one of which contains a site for phospholipase C (PLC) binding [6, 7]. In addition, it has a unique guanine nucleotide binding and hydrolyzing site located in a cleft between the catalytic core and the first β-barrel, a feature distinguishing the structure of TG2 from that of other TGs [6]. Altogether, four alternatively spliced forms of the *TGM2* gene have been identified, each variant lacking part of the C-terminal region [8].

Unlike other members of the TG family, TG2 is ubiquitously present throughout the body, being constitutively expressed in many tissues and cell types such as endothelial cells, smooth muscle cells, and fibroblasts [9]. The expression is tightly regulated, mainly at transcriptional level, and influenced by several, often stress-related factors. The best-known upregulators are retinoids [10]. In addition, the expression of TG2 is induced in response to various inflammatory cytokines and growth factors such as interleukin (IL) 6, tumor necrosis factor (TNF)-α, and transforming growth factor (TGF)-β [9].

Once expressed, TG2 is primarily localized in the cell cytoplasm [11]. However, it is also found in other cellular compartments. TG2 can be translocated into the nucleus and mitochondria [11]. Furthermore, despite lacking a secretory leader sequence, the enzyme can be transported into the cell surface and extracellular matrix by an as yet poorly known mechanism, possibly through an unconventional pathway involving recycling endosomes [12].

The catalytic activity of TG2 inside and outside cells must also be tightly regulated, and this occurs post-translationally. The most clearly established physiological regulators are Ca^{2+} ions, guanine nucleotides, and redox potential [6, 13, 14]. Under normal physiological conditions, intracellular TG2 is predominantly maintained in an inactive state [15]. However, in situations of stress such as physical or chemical injury, TG2 is transiently activated and released from the cells. The enzymatic activation is regulated by Ca^{2+} ions and guanine nucleotides and characterized by an unusually large conformational change [7]. In the absence of Ca^{2+} and in the presence of guanosine triphosphate (GTP) or guanosine diphosphate (GDP), the prevailing conditions inside the cell, TG2 has a closed, inactive conformation with the C-terminal β-barrels folding over the core domain. When Ca^{2+} homeostasis is altered and its level rises, the enzyme adopts an open, catalytically active conformation in which the four domains are aligned and the catalytic region is exposed. Similarly to intracellular TG2, extracellular TG2 has also been shown to be mostly inactive in spite of conditions favoring activation (high Ca^{2+} concentration, low GTP). This is due to the oxidizing extracellular environment, where TG2 is rapidly inactivated in its open conformation by the reversible formation of a disulfide bond between two vicinal cysteine residues in the enzyme [14]. Such transient activation of extracellular TG2 is thought

to occur in response to certain types of inflammatory stimuli or tissue injury [15, 16].

In addition to the above-mentioned regulators, nitric oxide-mediated nitrosylation [17] and phosphorylation [18–20] have been noted to modify or inhibit TG2 activity. Furthermore, protein-protein interactions such as those between TG2 and its extracellular substrate proteins can affect its activity.

The Functions of TG2

The function TG2 evinces depends on its cellular location, prevailing conditions, and the substrates and interacting proteins available [21, 22]. The principal and best-characterized function of TG2 is the Ca^{2+}-dependent covalent and irreversible cross-linking of proteins with a glutamine residue to a primary amine, often another protein with a lysine residue, in a two-step reaction, resulting in the formation of an isopeptide bond between the target proteins (Fig. 1) [4]. Alternatively, when local pH falls and no suitable amines are available, the glutamine substrate reacts with water in a reaction termed deamidation. As a result, the reactive glutamine residue in the substrate is converted into negatively charged glutamic acid [4, 23]. In addition, TG2 catalyzes the incorporation of small-molecule amines such as polyamines and histamine into proteins and, furthermore, is capable of cleaving isopeptide bonds [1].

Besides its ability to modify proteins in a manner similar to that of other TGs, TG2 also possesses additional, Ca^{2+}-independent enzymatic activities. It can bind and hydrolyze adenosine triphosphate (ATP) and GTP and act as a G-protein, thus participating in transmembrane signaling by binding and activating PLC upon stimulation of various cell surface G-protein-coupled receptors (Fig. 1) [13, 24, 25]. Moreover, TG2 has been reported to exhibit protein disulfide isomerase (PDI) [26] and protein kinase activities [18, 27].

In addition, TG2 can also function as an adaptor/scaffold protein independently of its enzymatic activity. This property is particularly important in the extracellular environment, where TG2 promotes a number of cellular functions through

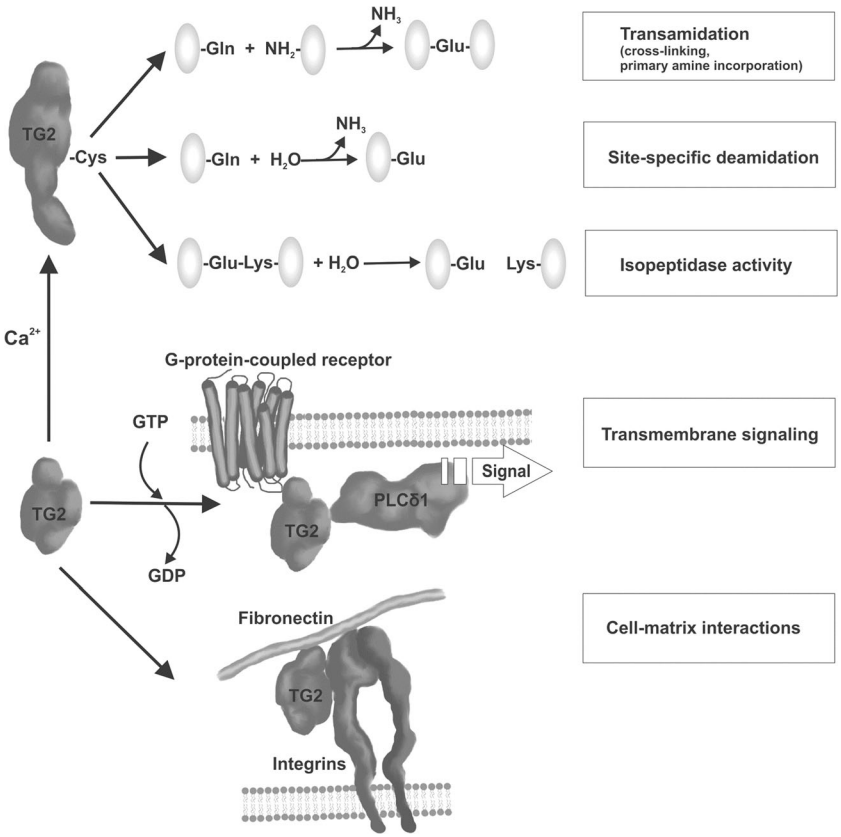

Fig. 1 Main biochemical functions of transglutaminase 2 (TG2). TG2 catalyzes Ca^{2+}-dependent post-translational modification of proteins by transamidation or deamidation of specific polypeptide-bound glutamines. Depending on the substrate, the transamidation can lead either to cross-linking of proteins through the generation of ε-(γ-glutamyl)lysine isopeptide bonds or incorporation of small-molecule amines such as polyamines and histamine into proteins. Alternatively, the glutamine substrate can react with water in a TG2-catalyzed deamidation reaction resulting in the conversion of the reactive glutamine residue into a negatively charged glutamic acid. TG2 is also capable of cleaving isopeptide bonds by virtue of its isopeptidase activity. In addition, TG2 possesses GTPase activity allowing it to participate in signal transduction and regulate the activity of phospholipase C δ1 (PLCδ1). Moreover, independent of its enzymatic activity in the extracellular environment, TG2 acts as an integrin-binding adhesion coreceptor for fibronectin and thus has a role in cell attachment and spreading. *GTP* guanosine triphosphate and *GDP* guanosine diphosphate

non-covalent interactions with its abundant interaction partners, particularly fibronectin and specific β-subunits of the integrin family on the cell membrane (Fig. 1) [28, 29].

Through its enzymatic and non-enzymatic activities, TG2, similarly to the other members of the TG family, is involved in a variety of cellular processes, among them cell adhesion, proliferation, differentiation, apoptosis, and stabilization of the extracellular matrix [9]. Owing to the diversity of biological functions ascribed to TG2, defects in its function may also contribute to pathological conditions. Indeed, TG2 has been implicated in the pathogenesis of a number of human diseases such as degenerative disorders, certain types of cancer, and inflammatory and autoimmune conditions [30].

Clinical Aspects of Celiac Disease

Overview of Celiac Disease

One of the disorders in which TG2 has been shown to play a role is celiac disease, a systemic autoimmune disorder induced by ingestion of dietary wheat-, rye-, and barley-derived gluten. In celiac disease patients, dietary gluten induces small-bowel mucosal damage with characteristic villous atrophy and crypt hyperplasia, which are ameliorated when gluten is excluded from the diet. Further, an antibody response presenting with circulating antibodies against modified gliadin peptides (deamidated gliadin peptide antibodies, DGP antibodies) and autoantibodies such as TG2 and endomysial antibodies (EmA) is representative of celiac disease [31–33].

Even though celiac disease was formerly considered a rather rare condition affecting mainly children, it is nowadays recognized as one of the most common food-related lifelong disorders in Western countries, with a prevalence of 1–2 % [34]. It would also appear that celiac disease is a more widespread disorder in the USA than has previously been appreciated, since prevalence figures comparable to those described in Europe have been reported [35]. Thus, celiac disease is indeed a major health care problem in Western countries. Similarly to several other autoimmune disorders, the prevalence of celiac disease has been shown to increase over time [36], and further, parallel to many autoimmune diseases, a marked female predominance with a female to male ratio between 2:1 and 3:1 prevails in this condition [37]. Further, the prevalence of celiac disease has been shown to increase with age [36, 38, 39]. Even though celiac disease can be diagnosed at any age, there appears to be peak incidences in early childhood and in the fourth or fifth decades of life [40].

Environmental and Genetic Factors Facilitating Celiac Disease

Thus far, the only verified environmental factor underlying celiac disease is dietary gluten derived from wheat, rye, and barley, which activates a detrimental immune response in the intestinal mucosa [41]. The role of environmental causes other than gluten in the development of the disorder is far more obscure. Infant feeding habits as a risk factor for celiac disease have been scrutinized for decades. It has been suggested that prolonged breast feeding, breast feeding during the introduction of dietary gluten, and introducing gluten to infants between 4 and 6 months of age are associated with a reduced risk of the disease [42, 43], but according to recent evidence, no specific general recommendations concerning gluten introduction or breast feeding can be made with a view to lowering the risk [44, 45]. Also, early life infections [46] and gastrointestinal infections such as rotavirus infections [47] have been suggested to serve as additional triggers for celiac disease development. In contrast, there is a possibility that clinical or subclinical infections by Epstein-Barr virus, cytomegalovirus, and rubella might have a protective effect on the emergence of celiac disease [48], a conception well in keeping with the finding that the prevalence of celiac disease is lower in areas with inferior prosperity and standard hygiene [49].

In addition to the identified exogenous trigger of the disease, dietary gluten, the development of celiac disease requires a genetic component, namely, human leukocyte antigen (HLA) DQ2 or DQ8 [50]. HLA genes are polymorphic genes located in a gene cluster called the major histocompatibility complex on chromosome 6p21.3, and this region is known to contain hundreds of genes with immunological functions and to be responsible for the strongest association signals observed in most immune-mediated diseases [50]. Even though HLA DQ2 and DQ8 constitute the highest genetic risk by far for celiac disease, these molecules alone are not sufficient for disease development. It is known that approximately 40 % of individuals possess these haplotypes [51], while only some 1 % of the population acquires the disease during their lifetime. At least 57 non-HLA variants associated with celiac disease have so far been identified [52], but their exact role in the onset of the disease remains to be elucidated.

Clinical Presentations

Gastrointestinal symptoms such as diarrhea, abdominal pain, and malabsorption are considered the classical signs of celiac disease. These were the predominant symptoms until the mid-1970s, but with more in-depth studies and increased awareness of the multiplicity of celiac disease, it has since been shown that the clinical symptoms are highly variable. In children, the severity of symptoms related to celiac disease have become milder [53, 54], and interestingly, in adults nowadays, a majority of patients either suffer from some extraintestinal manifestation or have no symptoms at all [55].

Dermatitis herpetiformis is an itching, blistering rash manifesting predominantly on elbows, knees, and buttocks [56], and it is known to be a cutaneous and also the most prevalent

extraintestinal manifestation of celiac disease. Intriguingly, even though the majority of dermatitis herpetiformis patients evince TG2 antibodies in the serum and intestine similarly to celiac patients [57, 58], in dermatitis herpetiformis, there are pathognomonic IgA deposits in the skin proved to be directed against TG3, not TG2 [59]. Likewise, TG6 antibodies have been suggested to serve as a marker identifying celiac disease patients with neurological symptoms [60] such as ataxia, neuropathy, and encephalopathy [61].

Anemia is also well documented in untreated celiac disease, and the most commonly recognized cause of anemia in celiac patients is iron deficiency, followed by folate and B12 vitamin deficiencies [62]. Other frequently encountered abnormalities in laboratory parameters are selective IgA deficiency [62] and chronic elevation in liver enzymes [63]. Hypertransaminasemia is generally asymptomatic, but celiac disease has also been reported in the context of severe liver disease [64].

Reduced bone mineral density is prevalent in celiac disease [65]; this is increasing the risk of bone fractures [66]. Further, reproductive difficulties and a higher risk of obstetric complications are associated with the condition [67]. It is moreover crucial to bear in mind that in addition to diverse clinical symptoms, also clinically mute celiac disease is increasingly detected not only among relatives of celiac patients but also among subjects with other autoimmune diseases. Patients particularly at risk of celiac disease are those with type I diabetes, autoimmune thyroid disorder, Sjögren's syndrome, and Addison's disease [68, 69]. In addition, the possibility of celiac disease should be explored in patients with Down's or Turner's syndrome in view of a fivefold to sixfold increased risk [70, 71].

Diagnosing Celiac Disease

Interpretation of small-bowel biopsies has been essential in celiac disease diagnostics since the 1950s. Small-bowel biopsies are obtained during esophago-gastroscopy, and according to current diagnostic criteria, the detection of small-bowel mucosal villous atrophy and crypt hyperplasia is mandatory for the diagnosis [72]. Unfortunately, there are pitfalls in this approach [73, 74]; the presence of villous atrophy is not pathognomonic for celiac disease alone [75], and further, celiac disease causes a continuum of intestinal alterations starting from increased densities of intraepithelial lymphocytes and only eventually leading to flat mucosa [76], and celiac disease symptoms and even complications can be present even before the development of marked villous atrophy [77, 78]. Intraepithelial lymphocytosis, albeit an early marker of celiac development, is also known to be unspecific for the disease [79]; an increased density of γδ intraepithelial cells detected in frozen small-bowel samples is indicative of celiac disease but not entirely restricted to celiac inflammation [80].

The development of celiac antibody detection has greatly improved celiac disease diagnostics, even though serology is considered to have a supportive role in the diagnostic procedure. The gliadin antibodies (AGA) targeting gliadin part in gluten were introduced in the 1980s but were later shown to be unspecific for celiac disease [81]. In contrast, a method subsequently developed for antibody detection against DGP has proved to be highly accurate in celiac disease diagnosis [82], and especially, IgG-DGP is a valuable diagnostic tool in IgA-deficient patients [83, 84].

Anti-reticulin antibodies (ARA) were introduced even before AGA tests [85], and these antibodies reacted against the reticular fibers in the endomysium. An EmA test was developed in 1984 by Chorzelski and coworkers [86], EmAs being detected by immunofluorescence (IF) using monkey esophagus or human umbilical cord as substrate [87]. The autoantigen of both ARA and EmA, TG2, was discovered in 1997 [88], and an enzyme-linked immunosorbent assay (ELISA)-based method for detecting TG2 antibodies was developed. TG2 antibodies would appear to be slightly more sensitive for celiac disease compared to EmA but less specific; the superiority of EmA accuracy is supported by a high concordance between EmA positivity and the presence of the celiac-type HLA DQ2 or DQ8, a connection not always seen with TG2 positivity [77, 89]. Further, the performance of commercially available ELISA TG2 antibody assays may vary. On the other hand, the IF method required for EmA measurements is relatively laborious and also subjective, and consequently, ELISA-based TG2 antibody tests remain currently widely used in the diagnostic workup of celiac disease. Further, TG2 antibody titers have shown to correlate with the degree of intestinal damage [90]. In recent guidelines from ESPGHAN, it was even suggested that in celiac children, small-bowel biopsies are no longer mandatory for the diagnosis; the decision can be made if serum TG2 antibody levels exceed 10 times the upper limit of normal and EmA and HLA DQ2 or DQ8 as confirmatory tests are positive [91]. It would thus appear that the significance of celiac antibodies in the diagnostic workup of celiac disease is gaining strength due to their accuracy and non-invasive nature. Further, in addition to the antibody tests measured in specialized laboratories, rapid on-site testing for celiac disease is also currently available, the benefits of these tests being presumably cost reduction, testing availability, and also shorter diagnostic delays. Whole-blood sample tests measuring for example IgA TG2, IgA and IgG TG2, and IgA and IgG anti-DGP are of interest, and their accuracy has been shown to be trustworthy [92–94].

In addition to serum, the celiac autoantibody response can also be detected in the small-bowel mucosa, which is known to be the origin of these antibodies [95, 96]. It was shown decades ago that untreated celiac disease patients have deposited IgA in their small-bowel mucosa [97, 98], and in 2004, Korponay-Szabo and associates demonstrated that this IgA

deposition was directed against TG2 [99]. This TG2-targeted IgA deposition can be detected using direct IF, and intestinal antibody deposits have been shown to precede villous atrophy [100], and are also present in seronegative celiac disease [101], being thus of diagnostic value in problematic cases. Interestingly, this intestinal TG2-targeted antibody response is also evident in dermatitis herpetiformis [58], where TG3 is considered to be the primary autoantigen [59]. The dermatitis herpetiformis diagnosis, however, is based on the demonstration of granular IgA deposits in the papillary dermis of the skin by direct IF [102], and it is known that IgA exists together with TG3 as an immunocomplex deposition of high avidity. Intriguingly, even though circulating TG2 antibodies are highly representative of celiac disease, as noted above, TG3 antibodies can also be detected in the serum of not only dermatitis herpetiformis patients but also celiac disease patients [59, 103, 104]. However, the frequency, titers, and also the avidity of TG3 antibodies have been shown to be higher in dermatitis herpetiformis patients compared to celiac disease patients [103, 105, 106]. Currently, TG3 antibodies have no role in the celiac disease diagnosis, and the precise implication in celiac disease in general remains to be unraveled in future studies. It has however been hypothesized that the TG3 antibody response develops as a result of epitope spreading from an antibody response initially directed against TG2, and this epitope spreading might develop as a result of prolonged gluten exposure. Results showing that serum TG3 antibody positivity is rare among celiac children support this hypothesis [105, 107].

Gluten-Free Diet Is Currently the Only Treatment

The only currently available treatment for celiac disease is avoidance of grains containing gluten, and it is considered indisputable that the exclusion of wheat, barley, and rye should be as strict as possible. The role of oats as a part of GFD has been controversial, but consumption of pure oats is nonetheless considered safe and well tolerated [108, 109]. The need for dietary treatment is currently considered to be lifelong, even though there are a few reports of celiac and dermatitis herpetiformis patients developing gluten tolerance and being able to reintroduce gluten to their diet [110–112].

The GFD has obvious benefits; clinical symptoms are alleviated and small-bowel mucosal damage heals. Moreover, the possibility of lymphoma, which is a well-known risk of celiac disease [113], decreases [114], and bone mineral density improves [115]. However, a strict GFD is not always easy to maintain; it is more expensive, there is limited availability of gluten-free products, it can be socially restrictive [116], and it further can have a negative effect on the quality of life.

In a minority of patients, a GFD is ineffective; in these subjects, even the strictest diet does not lead to symptom alleviation or healing of the small-bowel mucosa. Refractory celiac disease type I usually runs a benign course, but type II often progresses to overt intestinal lymphoma and premature death [117]. Such refractory cases are fortunately few in number; in a Finnish high-prevalence area, the prevalence of refractory celiac disease was 0.31 % among all diagnosed celiac disease patients [118]. The cause for incomplete clinical or histological response to a GFD is far more often connected to inadequate dietary compliance. Adherence rates have been highly variable in studies made; percentages of celiac patients on a self-reported strict GFD have ranged from 40 [119] to 96 [120] in adult celiac disease series.

Due to the challenges involved in keeping to a strict GFD, efforts have been made to develop new treatment options for celiac patients. New treatment modalities under investigation have been based on currently available knowledge of the pathogenetic mechanisms underlying the condition. Novel therapeutic approaches being developed include enzymatic degradation of gluten into harmless products, inhibition of intestinal permeability, antagonization of proinflammatory cytokines, and development of a gluten peptide vaccine or tolerance induction [121]. In addition, blockage of the enzymatic activity of TG2 has been suggested. However, even though TG2 inhibitor compounds have already been introduced and tested in vitro, clinical studies have yet to be instituted [122]. None of the new treatment modalities, including inhibition of TG2, is as yet part of clinical practice, and the GFD remains the sole treatment.

Biology Underlying Celiac Disease

Adaptive and Innate Immune Activation in Celiac Disease

The exogenous trigger of celiac disease, gliadin, is a particular dietary protein; in that, by virtue of its high proline content, the human gastrointestinal enzymes are not able to digest it to completion even in healthy individuals [123]. This imperfect digestion enables fairly long gliadin peptides to reach the small-intestinal lumen. Along with other luminal antigens, gliadin peptides are taken up by the various antigen-presenting cells in the small-intestinal mucosa and are presented to CD4-positive T cells (Fig. 2). Normally, in the case of dietary antigens, this results in the induction of oral tolerance. However, in celiac patients, such tolerance against gliadin either does not develop at all or is lost.

The presentation of gliadin peptides to CD4-positive cells occurs in the context of HLA DQ2 or DQ8 molecules on the surface of the antigen-presenting cells, but it is known that native gliadin peptides bind only poorly to these celiac-type HLA molecules [124]. Interestingly, in addition to being rich in proline, gliadin peptides are also characterized by their high glutamine content, which makes them suitable substrates for TG2. TG2 targets glutamine residues, preferentially followed

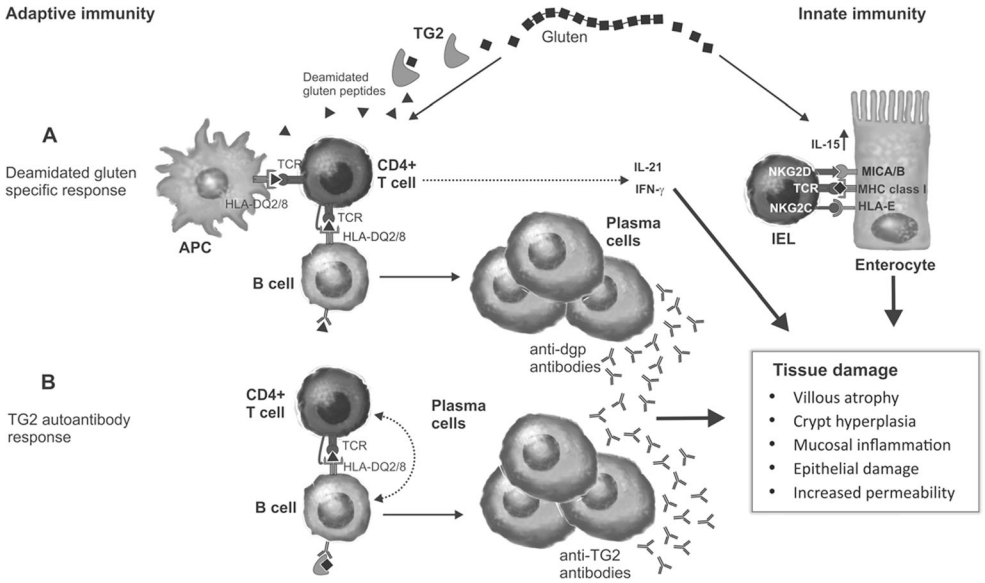

Fig. 2 The pathogenesis of celiac disease involves components of both adaptive and innate immunities. *left* TG2 is capable of deamidating gluten-derived gliadin peptides, which increases their affinity to celiac disease-specific human leukocyte antigen (HLA) DQ2 and DQ8 molecules expressed on various types of antigen-presenting cells (APCs). In the context of the HLA molecules, APCs present the peptides to gluten-specific CD4-positive T cells, which preferentially recognize deamidated gliadin peptides (DGPs) by their surface T cell receptor (TCR). Upon activation, the CD4-positive cells become pro-inflammatory and commence production of cytokines such as interferon γ (IFN-γ) and interleukin (IL) 21. The gluten-specific CD4-positive cells can also provide cognate help to deamidated gluten-specific B cells, which leads the B cells to differentiate into deamidated gliadin peptide antibody-producing plasma cells. The TG2-autoantibody response is thought to arise by a mechanism involving the cross-linking activity of TG2. The resulting TG2-gluten complexes are thought to be internalized by TG2-reactive B cells, whereafter the deamidated gliadin peptides can be released and presented to T cells when bound to DQ2 or DQ8. Thus, the gluten-reactive CD4-positive T cells provide help to the TG2-reactive B cells, enabling them to differentiate into TG2-autoantibody-secreting plasma cells

by C-terminal proline at position +2 [23, 125], and gliadin indeed contains an abundance of such sequence motifs. For augmented DQ2 or DQ8 binding, the conversion of the substrate glutamine residues to glutamic acid in a TG2-catalyzed deamidation reaction is crucial. Upon such modification, the peptides gain a negative charge in key anchor positions, thereby increasing their affinity to the HLA molecules [126].

CD4-positive T cells recognize the deamidated gliadin peptides bound to the HLA DQ2 or DQ8 molecules by their T cell receptors (TCR; Fig. 2). Interestingly, in celiac disease, the TCRs evince a biased use of distinct α- and β-chains and a strong structural convergence even between individual patients [127–130]. It would appear thus that high-affinity gluten-specific TCR repertoire has been selected for in celiac disease, and this population is likely to drive the detrimental disease-specific T cell response hallmarked by the secretion of pro-inflammatory cytokines such as IFN-γ and IL-21.

The gluten-reactive CD4-positive cells are also able to provide help to gluten-specific B cells, allowing them to differentiate into plasma cells which secrete anti-DGP antibodies (Fig. 2). However, celiac disease patients do not possess TG2-autoreactive CD4-positive cells which could assist TG2-specific B cells, thus raising the question how the TG2-targeting autoantibodies arise. In addition to deamidating gliadin peptides, TG2 is also capable of cross-linking peptides to itself [131], and the presence of such complexes is thought to provide an explanation for the celiac disease-specific TG2 autoantibodies. It has been suggested that TG2-specific B cells could take up TG2-gliadin peptide complexes and present the gliadin peptide after being released from TG2 to a gluten-specific CD4-positive cell in the context of an HLA DQ2 or DQ8 molecule [132]. In this way, the gluten-specific CD4-positive cells would also provide help to the TG2-specific B cells (Fig. 2). Indeed, B cells engineered to express HLA DQ2 and a TG2-specific B cell receptor have been shown to present TG2-gliadin complexes, resulting in enhanced celiac patient gluten-specific T cell responses [133]. The cooperation of the T and B cells is thought to result in the activation also of the B cells, leading them to differentiate into TG2 autoantibody-secreting plasma cells.

Taken together, the importance of TG2 enzymatic activity in the celiac disease pathogenesis is indisputable, but currently, the precise site of this crucial action remains unknown. One obvious site would be the small intestine, where TG2 has been detected in the epithelial and endothelial cells and also abundantly in the basement membrane [134]. However, under normal physiological conditions, the small-intestinal TG2 is enzymatically inactive [15]. Nonetheless, intestinal TG2 can be activated for instance by activation of the toll-like receptor 3 (TLR3) [15], thereby making it possible for deamidation and cross-linking of gliadin peptides to occur in the small-bowel

mucosa. The pH of the environment and the characteristics of the available substrates have an effect on the type of enzymatic activity TG2 evinces. At pH 7.3, transamidation is favored over deamidation [23], and such a pH is likely to prevail in the small-intestinal basement membrane, suggesting that cross-linking of gliadin peptides, but very likely not considerable deamidation, can occur in this compartment. In contrast, the pH in the intestinal lumen and thus probably also at the intestinal brush border is 6.6 [135]; at this pH, TG2-catalyzed deamidation occurs efficiently [23]. It has therefore been suggested that the brush border could be the site where the deamidation of the gliadin peptides occurs [23]. Interestingly, Rauhavirta and colleagues have shown that a strictly deamidation-dependent celiac patient T cell clone can be activated with intestinal epithelial cell media supplemented with non-deamidated immunogenic gliadin peptides [136]. This would imply that the deamidation of gliadin peptides could occur during their contact with the intestinal epithelial cells in permissive conditions. Alternatively, the crucial enzymatic reactions of TG2 could also occur outside the small bowel, for instance in lymphoid tissue, as suggested [132], where TG2 is also abundantly expressed [137].

Although the adaptive immune response is required for the development of small-bowel mucosal villous atrophy and crypt hyperplasia, it is not by itself sufficient and other factors must be envisaged. In celiac patients, gliadin peptides also launch an innate immune response hallmarked by increased expression of interleukin 15 (IL-15) by the intestinal epithelial cells [138] (Fig. 2). This upregulation has been attributed to a specific subset of toxic gliadin peptides which includes for instance the peptide p31–43 [139]. IL-15 induces a simultaneous upregulation of MICA expression in epithelial cells [140] and the activating NKG2D receptor in intraepithelial lymphocytes [141]. The engagement of the NKG2D receptor by an epithelial MICA ligand licenses the intraepithelial lymphocytes (IELs) to kill the epithelial cells in a T cell receptor-independent manner [140] (Fig. 2). However, according to one recent article, it appears that the upregulation of NKG2D is not solely sufficient for the development of small-bowel mucosal damage, as such upregulation has been described in TG2 antibody-negative celiac patient family members with normal small-bowel morphology [142]. Celiac patients also present with increased expression of another activating NKG2-receptor, NKG2C, and decreased expression of inhibitory receptor NKG2A [143], which may also be required for the development of villous atrophy. Thus, as suggested by Setty and coworkers, the cooperative action of both adaptive and innate immunity might be required for the development of the small-bowel mucosal damage in celiac disease [142].

Characteristics of Anti-TG2 Autoantibodies

In celiac disease, the anti-TG2 antibodies are produced by plasma cells located in the *lamina propria* of the small-bowel mucosa [95]. Interestingly, it has been reported that in patients with active celiac disease, on average as many as 10 % of antibody-secreting plasma cells in the duodenal mucosa produce TG2-specific antibodies [133]. Surprisingly, these antibodies show high affinity to TG2 and a single-cell analysis has revealed that the antibodies exhibit biased and limited heavy chain variable region (VH) gene-segment usage and only a few somatic mutations [133]. Recently, antibodies cloned from IgA-positive plasma cells, specific for deamidated gluten, have been shown to have similar characteristics [144]. Due to the scant presence of somatic mutations, the B cell response to deamidated gluten and TG2 may thus have shared origins [144].

Characterization of celiac patient anti-TG2 antibodies has revealed that they recognize certain conformation-dependent core domain regions of TG2 [145, 146]. The importance of TG2 conformation in celiac patient TG2 antibody binding has been further demonstrated in a study by Simon-Vecsei and colleagues [147]. They showed that antibodies from different celiac patients target the same conformational TG2 epitope involving residues Arg19, Glu153, and Met65 located in three different domains [147]. Subsequently, Iversen and colleagues further characterized the TG2 epitopes using human monoclonal antibodies generated by expression cloning of Ig genes from single intestinal plasma cells derived from the celiac disease lesion [148]. They found that the antibodies recognize at least four different conformational epitopes, including that earlier identified. In addition, they reported that anti-TG2 antibodies bind to an open conformation of TG2 and two of the autoantigenic epitopes are clustered in the N-terminal part of the enzyme [148]. Interestingly, these two epitopes were found to overlap with the fibronectin-binding site and none of the epitopes was accessible when TG2 was in cell surface-bound form [148].

In general, anti-TG2 IgA-class antibodies are highly specific for celiac disease, but both IgA and IgG-class antibodies against TG2 are also found in non-celiac individuals suffering from other disorders such as inflammatory bowel disease [149, 150], viral infection including HIV [151], or end-stage heart failure [152]. In addition, TG2 positivity has been detected in conjunction with autoimmune disorders other than celiac disease independently of gluten sensitivity [153]. However, it appears that at least in these cases, the TG2 autoantibodies target an epitope distinct from that recognized by celiac patient TG2 autoantibodies [147] which might provide clues as to the autoimmune mechanisms governing in celiac disease.

Biological Effects of Anti-TG2 Antibodies

The role of anti-TG2 antibodies in the pathogenesis of celiac disease remains controversial, although a number of studies have addressed this aspect. Firstly, since the disease-specific

anti-TG2 antibodies bind to TG2, it is logical to ask whether these antibodies can influence its enzymatic activity. This question has been investigated in several studies, but thus far, results have been contradictory. Celiac patient-derived antibodies have been shown to either enhance [154, 155], inhibit [156–158], or have no effect on TG2 activity [133]. Although methodological differences may explain these controversial findings, for the time-being, this issue remains to be solved.

However, both cell-based and in vivo studies show celiac disease patient antibodies to exert a number of biological effects on various cell types (Table 1). The epithelium is one cell type where their effects have been extensively studied. Both monoclonal anti-TG2 antibodies and celiac patient serum IgA have been shown to inhibit the differentiation of T84 intestinal crypt epithelial cells [159]. In addition, TG2 antibodies derived from celiac patients have been demonstrated to induce proliferation of intestinal epithelial cells [160]. Further, celiac patient sera or purified anti-TG2 antibodies are able to reduce the attachment of epithelial cells to the TG2-fibronectin matrix [161]. Moreover, treatment of intestinal epithelial cells with sera from celiac patients has been reported to increase the transepithelial permeability of these cells [162]. Particularly interesting are findings suggesting that celiac patient IgA is a factor which enables translocation of gluten-derived gliadin peptides across the epithelial barrier [136, 163–165]. However, the precise population of celiac antibodies responsible for these permeability-modulating effects remains to be identified, although at least the gliadin antibodies have been suggested to contribute [165]. Taken together, the celiac antibodies could play a role in the development of the epithelial alterations and increased permeability characteristic of celiac disease

In addition to affecting epithelial cell biology, the celiac patient antibodies also modulate the function of endothelial cells. Endothelial cells treated with celiac patient IgA evince only a weak attachment and a high susceptibility to detach when plated on fibronectin [166]. In addition, celiac patient TG2-specific antibodies are able to alter endothelial cell mobility and dynamics [167], which together with the weakened attachment might lead to the observed inhibition of in vitro, ex vivo, and in vivo angiogenesis [167, 168]. Interestingly, vascular permeability seems also to be increased in the presence of celiac patient IgA- or TG2-specific autoantibodies [155, 167]. Whether the effects exerted by the celiac patient antibodies on vascular biology are of significance in the development of small-bowel mucosal damage remains to be established, but at least they could play a role in the development of the vascular abnormalities observed in celiac disease [169].

The above-mentioned studies were largely conducted using cell culture and focused on the effects of the antibodies on a sole cell type. The results cannot therefore necessarily be translated to the level of the entire small intestine. Surprisingly, there are few studies where this aspect has been addressed. In order to clarify this issue, Freitag and coworkers [170] immunized mice with TG2 in order to study the specific effects of TG2 autoantibodies. Although the animals developed an anti-TG2 antibody response, none evinced any morphological changes in the small intestine. Parallel results were obtained in another study where celiac patient-derived single-chain TG2-specific antibody fragments were expressed in mice using adeno-associated virus vectors [171]. Upon vector injection into the skeletal muscles, high and persistent systemic levels of anti-TG2 antibodies were obtained, but no histological abnormalities in the small intestine were observed. In

Table 1 Biological effects of celiac disease antibodies

Biological effect described	Experimental system	References
Inhibition of differentation	Intestinal T84 epithelial cells	[159]
Induction of proliferation	NIH 3T3 fibroblasts and celiac small-intestinal epithelium	[160]
Reduction of cell attachment	Intestinal Caco-2 epithelial cells and human umbilical vein endothelial cells	[161, 166]
Increase in permeability	Intestinal T84 epithelial cells	[162]
Modulation of gliadin peptide permeability	Intestinal Caco-2 epithelial cells and celiac small-intestinal biopsy	[136, 163, 164, 165]
Inhibition of angiogenesis	Human umbilical vein endothelial cells, mouse aorta rings, and mouse matrigel plugs	[168, 167]
Increase in vascular permeability	Human umbilical vein endothelial cells and mouse matrigel plugs	[155, 167]
Inhibition of migration and induction of apoptosis	Swan-71 trophoblasts	[178]
Anti-idiotypic response	C57Bl/6J mice	[171]
Mild enteropathy and intestinal inflammation	Nude mice	[172]
Ataxia-like symptoms	C57Bl/6 mice	[174]

contrast to these two studies focusing specifically on TG2 autoantibodies, Kalliokoski and coworkers used an approach in which they injected either celiac patient serum or total immunoglobulin fractions into mice [172]. Interestingly, the mice had shorter villi than the controls along with positive TG2-specific serum autoantibodies [172]. However, the small-bowel mucosal alterations were not as pronounced as in overt celiac disease, and it thus appears that other factors, related possibly with epithelial cell stress as presented above, are needed for the development of small-bowel villous atrophy and crypt hyperplasia.

The Role of Celiac Patient Antibodies in the Induction of Extraintestinal Manifestations

In the study by Kalliokoski and colleagues, it was noted that mice injected with celiac patient serum or immunoglobulins evinced TG2-targeted antibody deposits also in several other tissues besides the small intestine [172]. This finding parallels that reported by Korponay-Szabo and coworkers [99], who observed that celiac patient IgA is found deposited on extracellular TG2 in the liver, lymph nodes, and muscles of patients with untreated celiac disease. Since these local extraintestinal TG2-targeted tissue antibody deposits often coincide with extraintestinal manifestations of celiac disease, it has been suggested that the autoantibodies might in fact play an active role in the development of the extraintestinal symptoms [99].

One interesting example of extraintestinal tissue deposits and extraintestinal symptoms is the presence of IgA-class anti-TG2 antibodies around blood vessels in the brain of a patient with gluten ataxia, a neurological manifestation of celiac disease [173]. By virtue of their capability to induce ataxia-like symptoms when injected into the mouse brain [174], the TG2 autoantibodies might well take part in the development of the neurologic impairment occurring in association with celiac disease.

Undiagnosed and untreated celiac disease can be associated with miscarriages and infertility [175, 176], and interestingly, TG2-bound celiac patient IgA has been detected in the wall of decidual blood vessels and on the surface of the chorionic villous structures [147]. It has been proposed that these antibodies might alter TG2 activity [177] and impair nutrient import [147]. Although IgA-class antibodies are not able to pass from the maternal to the fetal site of the placenta, maternal IgG anti-TG2 antibodies from an untreated celiac mother have been detected in the umbilical cord and serum of the newborn. Moreover, TG2 antibodies have been found on the surface of endothelial cells isolated from the umbilical cord exhibiting abnormal behavior [147]. Further, the interaction of circulating celiac antibodies with surface TG2 expressed on trophoblast cells has been thought to contribute to damage of the placenta and disrupt the phagocytosis of apoptotic bodies, which could in turn promote inflammation in the placenta [178]. Taken together, these findings make it plausible that celiac autoantibodies may play a role in the development of the reproductive problems associated with celiac disease.

In addition to TG2 autoantibodies, also antibodies targeting TG3 and TG6, which occur in the context of dermatitis herpetiformis and gluten ataxia, respectively, have been considered as potential contributors in the pathogenesis of the extraintestinal manifestations [56, 174]. Collectively, although experimental evidence supports the role of the antibodies in the development of the extraintestinal manifestations associated with celiac disease, further research on the subject is warranted.

Concluding Remarks

TG2 is indisputably a major player in the development of celiac disease by virtue of its enzymatic activity. TG2-mediated deamidation of gluten-derived gliadin peptides enables a strong immune response to occur, whereas TG2-mediated cross-linking of gliadin peptides is thought to account for the production of celiac disease-specific TG2-targeted autoantibodies. Due to its central role, TG2 has been regarded as a suitable target for future treatment options. Preliminary preclinical studies suggest that enzymatic inhibition of TG2 would be a feasible approach, but to date, clinical trials are eagerly awaited. Mounting evidence suggests that also the TG2-targeted B cells as well as the autoantibodies might play a role in the disease pathogenesis. The B cells might have an important role in the celiac pathomechanism by acting as antigen-presenting cells, thereby also contributing to the detrimental T cell activation. On the other hand, the antibodies targeting TG2, along with the other antibody populations, exert various biological effects on several cell types, thus rendering them potential players in the disease process. Although more experimental evidence is needed as to their contribution to the development of intestinal damage and extraintestinal manifestations, their value in diagnostics is unquestionable. We foresee that their importance will only increase in the future, as celiac disease diagnostics moves toward more non-invasive methods. In conclusion, it may be affirmed that both TG2 and the TG2-specific B cells and autoantibodies are important players in celiac disease.

In addition, gliadin also launches an innate immune response hallmarked by increased expression of IL-15 by the intestinal epithelial cells (right). IL-15 induces the upregulation of MICA expression in epithelial cells and the activating NKG2D receptor in intraepithelial lymphocytes. The engagement of the NKG2D receptor by the epithelial MICA ligand licenses the IELs to kill the epithelial cells. In celiac disease, the expression of activating NKG2C is also upregulated, whereas that of the inhibitory receptor NKG2A is downregulated. According to current view, the development of small-bowel mucosal damage requires all of these different components.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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