Tissue transglutaminase—the key player in celiac disease: a review

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Abstract

Gluten-sensitive enteropathy, otherwise known as celiac sprue, is characterized by an abnormal proximal small intestinal mucosa arising as a result of an inappropriate inflammatory response to ingested gluten antigens present in wheat in genetically susceptible individuals. This immune response is directed to a 33-mer peptide of the \( \alpha \) gliadin component of gluten. The generation of an epitope for the recognition by CD4\textsuperscript{+} T cells requires deamination of the protein by tissue transglutaminase (tTG). Moreover, IgA anti tTG is highly sensitive and is specific serologic marker (95–99\%) of celiac disease. They can be easily determined quantitatively, by ELISA of an accurate and relatively inexpensive technique. Therefore, tTG can be used as the first line diagnostic test in the work-up of celiac disease, as well as for screening purposes. Finally, tTG may contribute to future strategies in treating celiac disease either by producing nontoxic wheat or by generating oral vaccination that can prevent the disease.

Keywords: Celiac disease; Tissue transglutaminase; Gluten

1. Introduction

Celiac disease (CD) is an enteropathy triggered by the ingestion of gluten-containing grains in genetically predisposed individuals. Elimination of gluten, the cornerstone of treatment for CD, may dramatically influence the course of the disease and prevent complications, such as osteopenia, malignancy and miscarriage [1]. With the recent introduction of newer and more precise serologic tests, such as antibodies against tissue transglutaminase (tTG), significant progress has been achieved, not only in exploring the genetic basis, clinical course and epidemiology of the disease, but also in understanding its pathogenesis. This greater knowledge may contribute to enhanced treatment, and possibly, to the prevention of CD [2]. This review will outline tTG as a diagnostic

\textsuperscript{Abbreviations:} tTG, tissue transglutaminase; CD, celiac disease; GFD, gluten-free diet.

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tool and describe its role in the immunologic basis of CD and subsequent therapeutic implications.

2. Role of serologic testing in CD

Celiac patients exposed to gluten express high levels of serum antibodies to gliadin, endomysium, reticulin, jejwnum and tTG [3,4]. Elimination of gluten from their diet results in a decrease of the titer of these antibodies and to their eventual disappearance. Anti-reticulin antibodies comprise a specific, but not a very sensitive test. Antibodies of the immunoglobulin IgA and IgG class to gliadin have also been used in the past to diagnose CD and monitor response to therapy. Sensitivities range from 76 to 88% for IgG and 52 to 91% for IgA antigliadin antibodies, while the respective specificities vary from 88 to 92% and from 45 to 94% [5–7]. The main advantage of using the IgG anti-gliadin is seen in patients with IgA deficiency, a condition that is more common among CD patients than in the general population [8,9].

Anti-endomysium antibodies (EMA) of the IgA class have a reportedly much better sensitivity and specificity (97 to 100% and 98 to 99%, respectively), and a combination of antigliadin and antiendomysium antibodies can give a negative and positive predictive value approaching 100% [10]. Because these specific antibodies were found to be useful and reliable diagnostic tests for CD, the diagnostic criteria for CD was revised by the European Society for Pediatric Gastroenterology, Hepatology and Nutrition. It has been argued that serologic tests should be used in screening and initial workups, but a small intestinal biopsy with characteristic histologic abnormalities should still be the main criterion for diagnosis [11]. An intestinal biopsy to show improvement of the villous structure under Gluten free diet (GFD) could be now replaced by the measurement of serologic markers [11].

3. Alteration in the incidence of CD due to the use of serologic tests

CD was believed to affect 1:1500 individuals throughout Europe, with a higher prevalence (1:150) in Ireland [12]. Recent serologic screening of normal populations, however, has yielded prevalence between 1:100 to 1:300 [13,14]. In the past, CD had been considered a disease of childhood that was usually diagnosed before the age of 2 years, and presented with symptoms of diarrhea, malabsorption and weight loss following the introduction of cereals into the infant diet. Adults and adolescents often present atypically, with vague and non-specific or problems that at first glance appear unconnected to an intestinal pathology. Such ‘atypical’ symptoms are listed in Table 1. Indeed, the variety of symptoms, signs, and severity of CD led to the concept of there being a ‘celiac iceberg’, with the patients who had complaints associated with the gastrointestinal tract being the ones ‘above the surface’, and many more as yet undiagnosed patients with minimal or no symptoms or with atypical ones making up a silent, unseen majority hidden ‘below the surface’ [15]. Testing for CD is, therefore, recommended even when the clinical symptoms are indicative—however, subtle—for the disease, or for individuals who are at increased risk either because of heredity,
extra-intestinal symptoms, or an associated disorder (e.g. IgA deficiency, Diabetes Mellitus [16]).

4. tTG as a serologic marker for CD

After it has been shown that tTG is the substrate recognized by Antiendomysium antibodies (EMA) [17], an Enzyme-linked immunosorbent assay (ELISA) was developed and validated as an alternative screening test for CD [18]. The currently used method employs a commercially available guinea pig tTG as a substrate for the ELISA. The recent cloning of human tTG [19], however, led to a human tTG base assay, which has higher specificity and sensitivity [20]. Furthermore, in certain circumstances, such as the evaluation of a possible CD among patients with elevated liver transaminases and obscure chronic liver disease, there was a high frequency of false positive anti tTG tested on guinea pig as the antigen due to the presence of hepatic proteins in the commercial tTG obtained from guinea pig liver, which disappeared when human tTG was used as the Ag in the ELISA system [21]. Initial series quoted a sensitivity of 95–98% and a specificity of 94–95% for the IgA anti-tTG assay, respectively, in untreated celiac patients [18,22,23]. The results of ELISA anti-tTG tests correlated well with the traditional IgA-EMA tests and the titer of tTG antibodies fluctuated with the dietary gluten exposure in a manner consistent with previous observation of EMA antibody monitoring [24]. Furthermore, EMA assays are based on indirect immunofluorescence using monkey esophagus or umbilical cord, which is expensive, labor intensive and subjective. In contrast to EMA, tTG autoantibodies can easily be measured in immunoassays with diagnostic sensitivity and specificity that are comparable to those of EMA [18,23]. Therefore, tTG emerged as the recommended standard initial diagnostic study [19–22]. The presence of a positive anti-tTG test should prompt a small intestinal biopsy: if the patient is IgA deficient—a condition with a 10-fold risk of developing CD—IgG antigliadin should be used or a direct duodenal biopsy should be performed [25]. In their recent study, Agardh et al. [24] suggested that since IgA–tTG is a more specific and sensitive test than IgG1–tTG and IgM–tTG, it should, therefore, be implemented as screening test for CD. Nevertheless, IgG1–tTG is more commonly used for children diagnosed at a young age (<2 years) and may be used as sufficiently sensitive substitute for IgA–tTG in IgA-deficient children; nevertheless, it has not been practically implemented.

Why do CD patients produce tTG antibodies? The most plausible explanation was put forward by Sollid et al. [26] where they reasoned that tTG can crosslink itself to gluten and this gluten–tTG complex will be taken up by B-cells that express tTG-specific immunoglobulin on their membrane. As a result of this uptake, the gluten–tTG complex will be degraded intracellularly and gluten peptides will bind to HLA-DQ and be expressed on the cell surface. In CD patients, gluten-specific T cells will recognize this HLA-DQ peptide complex and this results in T cell ‘help’ for the production of tTG-specific antibodies by the B cells. The fact that only CD patients have measurable numbers of gluten-specific T cells explains why only CD patients make tTG-specific antibodies and also, importantly, why the antibody titer drops after gluten withdrawal [27,28].

5. tTG: the master regulator of CD

In 1997, Dieterich et al. [17] described tTG as being the target of the endomysium-specific antibodies that are characteristic for CD [29]. As a result of that observation, a series of studies have revealed that screening for the presence of tTG-specific antibodies is a very specific indicator for CD [18–24]. tTG is expressed in many different tissue and organs, and is found intracellularly as well as extracellularly. In the extracellular environment, tTG was shown to play a role in extracellular matrix assembly, cell adhesion and wound healing [30,31]. The calcium-dependent tTG catalyzes selective crosslinking or deamination of protein-bound glutamine residues [32]. Binding of calcium ions induces conformational alterations in tTG that result in the acquisition of transglutaminase activity [33].

Recent evidence has indicated that tTG is not only a diagnostic marker of CD, but that it is also directly involved in the pathogenesis of the dis-
ease. Antibodies against tTG in CD patients interfere with fibroblast-induced differentiation of epithelial cells, possibly by inhibiting the crosslinking activity of tTG. In addition, tTG is necessary for activation of transforming growth factor β (TGF-β) [34], which is involved in the differentiation of intestinal epithelium [35,36]. Reduced formation of TGF-β could also lead to increased activation of mucosal Th1 cells that produce inflammatory cytokines, because TGF-β is a suppressor factor for T cells [37,38]. Moreover, there is now compelling evidence that tTG is linked to the humoral response that is also involved in generating gluten peptides, which stimulates the T cells in the small intestine of CD patients [39].

The currently accepted theory is that susceptible people, e.g. HLA type DQA*0501 and DQB1*0201, exhibit an aberrant response to dietary gluten, and that the resulting small intestinal damage is caused by locally activated CD4+ T-lymphocytes. Gluten-sensitive T lymphocytes recognize gluten-derived peptides epitopes when presented in association with DQ2. Activation of these normally silent CD4+ T-lymphocytes triggers a T-helper type 1 pattern of cytokine production, including the release of IFN-γ and leading to mucosal damage [3]. Glutamine is the most abundant amino acid in gliadin, making up 35% of its composition, and it may be central to the toxic effect of these proteins in celiac patients. The tTG enzyme selectively deamidates gluten protein glutamine to glutamic acid. This introduction of negative charges results in peptides that bind with relatively high affinity to the disease-associated HLA-DQ2 or HLA-DQ8 molecules [28,40]. In addition to this process, tTG increases their stimulatory effect on gluten-sensitive intestinal T lymphocytes and exposes neoepitopes in wheat proteins. One of several toxic epitopes may reside in the N-terminal region of α gliadin, a constituent of gluten, corresponding to amino acid residues 31–49. This peptide causes the histologic characteristic of CD in both organ culture and in-vivo challenge studies. In addition, it can bind DQ2 molecules, and when presented in association with DQ2, it can stimulate peripheral blood T-lymphocytes. In an attempt to find the precise structure of the toxic element of gluten, a single epitope of α gliadin was recently recognized as the dominant epitope recognized by T-lymphocytes in patients with CD [41]. This 33-mer peptide is stable in-vivo and in-vitro to breakdown by all gastric, pancreatic and intestinal brush-border membrane peptidase, and it reacts with tTG by deamination of specific glutamine residues within this sequence, which is essential for HLA-DQ2 binding and subsequent T lymphocyte stimulation [28,40].

The importance of the modification of gluten by tTG has been underscored by the observation that the enzyme specificity correlates with the toxicity of cereals for CD patients [42]. Moreover, tTG specificity can be combined with the requirements for peptide binding to HLA-DQ2, a finding that resulted in a search that identified T-cell stimulatory peptides in wheat, barely and rye but not in oats, the latter being considered safe for CD patients.

6. tTG as a main therapeutic target in treating CD

tTG can be considered a master regulator of CD. It is involved in both the humoral and cellular response and is tightly linked to cereal toxicity [43]. The enzyme tTG is considered the ideal assay for screening CD patients in making the initial diagnosis. An intestinal biopsy is then carried out for final confirmation and tTG level for monitoring clinical response to gluten-free diets [44]. Finally, the discovery of the immune regulation and the specific epitope in gluten-sensitive enteropathy opens the way for several potential interventional strategies for treating CD:

1. The discovery of the specific epitope of gluten—which is rich with proline—is a crucial factor in the resistance of the 33-mer gliadin peptide to gastrointestinal breakdown. This will enable the use of a bacterial prolyl endopeptidase that catalyzes this peptid, and diminishes its toxic effect.
2. The induction of tolerogenic T cells or promotion of T cells anergy.
3. tTGase-mediated endocytosis might be an effective mechanism for oral vaccination with immunogenic peptide epitopes as long as they are
resistant to the action of gastric and intestinal digestive enzymes. In addition, by blocking tTG activity, it would prevent the generation of the majority of T cells stimulatory gluten peptide.

Further knowledge of the substrate specificity of tTG will facilitate the design of specific blockers, bearing in mind, however, that long-term blocking of tTG might have undesirable consequences.

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Take-home messages

- Celiac Disease is an enteropathy caused by inflammatory response to ingestion of gluten in genetically susceptible individuals.
- The immune response is directed to a 33-mer peptide of the α gliadin component of gluten.
- Deamination of glutamin to glutamic acid by tissue transglutaminase (tTG) generate an epitope required for recognition by CD4+ T cells.
- TTG is the target of the endomysium specific antibodies that are characterized in Celiac disease.
- IgA anti tTG is highly sensitive and specific marker of celiac disease, except in IgA deficient patients.
- IgA against tTG can be easily and non expensively determined quantitatively by ELISA, and is now considered as the first line in the diagnosis of Celiac disease.
- Finally, tTG may be used in the future as a therapeutic target of celiac disease.

References


