

Transglutaminase 2 and Anti Transglutaminase 2 Autoantibodies in Celiac Disease and Beyond: Anti- Transglutaminase 2 Autoantibodies: Friends or Enemies

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Abstract

Tissue transglutaminase is a multifunctional enzyme, exerting intra and extracellular, enzymatic and non-enzymatic, Ca²⁺ dependent and independent functions. Its specific autoantibody, the anti transglutaminase2 autoantibody is multifunctional, affecting many of the enzyme activities. Most of them are due to loss of function, the minority being gain of function of the enzyme. No beneficial protective effects, but only pathogenic ones, were assigned to those celiac disease associated anti TG2 autoantibodies. Taken together, celiac antibodies could collectively promote small bowel intestinal or extraintestinal damage. Yet, most of the transglutaminase2 autoantibody activities were explored *in vitro* and *ex-vivo*, very few in animal model but none *in vivo*, in human. The celiac disease serum contains numerous antibodies, IgA-transglutaminase2 is only one of them and up till now, its differential role in celiac disease induction and maintenance is far from being unraveled. Unraveling them might open some new therapeutic strategies for celiac disease.

Keywords: Tissue transglutaminase; TG2; Antibody; Autoantibody; Function; Celiac disease

Abbreviations

TG: Transglutaminase; CD: Celiac Disease; AB: Antibody; tTg: Tissue Transglutaminase; ESPGAHN: European Society for Pediatric Gastroenterology, Hepatology and Nutrition; GFD: Gluten Free Diet; GCD: Gluten Containing Diet

Introduction

Celiac disease (CD, OMIN number 212 750) is hallmarked by strong antibody (AB) response against dietary- derived gliadin peptides called anti gliadin AB [1], but the autoantibody production against self-protein is much more specific and important for the disease establishment and progression. Several specific autoantibodies were described in CD: Anti endomysial [2], anti deamidated gliadin [3], anti transglutaminase (TG2 OMIN number 190 196) [4,5] and anti neo-epitope tissue transglutaminase (tTg) [6-9]. All of them are used clinically for the diagnosis of symptomatic or high risk patients and for screening of populations [10,11]. The present review will concentrate on the IgA-TG2 autoantibodies, which are most frequently used and recommended by ESPGAHN [12] and some other gastrointestinal societies interested in CD. The anti TG2 autoantibodies are highly sensitive and specific for CD diagnosis, allowing to consider avoidance of intestinal biopsies in some settings. They reflect the adaptive immune response, but not directly measure the inflammation, nor the innate immune response in the CD intestine. In the present review, in addition to their importance in the diagnosis, the new observations and scientific data, are summarized, highlighting the multi- functions of those multi-facets ABs.

Anti TG2 ABs Secretion, Binding and Distribution

The anti TG2 autoantibodies are produced in the intestinal mucosa by local committed B cells and plasma cells, a cellular population known to be abundant in the inflamed CD intestine. Plasma cells secreting gluten-specific or TG2-specific IgA and IgM ABs are found in the intestinal lamina propria of the patients. In the same location where colocalization of gliadin peptide p31-49 with IgA was demonstrated. Those ABs have restricted VH and VL combination and usage and limited somatic hypermutations, suggesting that a common factor governs the mutation levels in the plasma cells that produce those autoantibodies [13]. The parallel fluctuation of ABs against gliadin and TG2 in serum and at the intestinal plasma cells, in response to dietary gluten suggest that their production is regulated in a coordinated way. In fact, gluten-specific T cells could provide help to TG2-specific B cells by means of complexes gluten-TG2 acting as hapten-carrier complexes. This model would explain why the TG2 ABs are gluten dependent, and why only individuals positive for HLA-DQ2 or HLA-DQ8, make these ABs [13].

In fact, dozens of non-HLA genes were described in CD but only those two HLAs are found in 95% of the CD affected populations. Upon gluten deamidation by the TG2 enzyme, the post-translated molecule is negatively charged, thus, increasing the affinity binding to the HLA-DQ2/8 grooves and facilitating its presentation to the lamina propria CD⁴⁺ lymphocytes.

Gluten-specific T cells obviously also could provide help to gluten-specific B cells. Using phage AB libraries from the peripheral and intestinal lymphocytes of CD patients, ABs to TG were isolated from all intestinal lymphocytes libraries but not from those obtained from peripheral lymphocytes. This is in contrast to ABs against gliadin, which could be obtained from all libraries, indicating that the humoral

response against TG occurs at the local level, whereas that against gliadin occurs both peripherally and centrally [14].

On GCD the ABs are detected in patients' sera and are deposited extracellularly *in vivo* in the mucosa, below the epithelial basement membrane, in crypt epithelium, around the subepithelial fibroblasts and around mucosal blood vessels, even before passing into circulation. However, on GFD, the serum ABs disappear systemically faster than the intestinal deposits, but they are unuseful in assessing histological recovery on gluten restricted diet [15]. The intestinal IgA-TG2 ABs deposits are associated with the risk of developing frank villous atrophy, and even in seronegative CD patients, the deposits are detected. In IgA-deficient patients, these mucosal autoantibodies appear in the IgM-class instead. Their secretion *in vitro* on gliadin challenge is dependent on their mucosal deposits [16]. The measurement of ABs secreted into intestinal biopsies cultured supernatants has higher sensitivity and specificity than the detection of mucosal deposits and they can predict evolution towards mucosal atrophy [17]. Interestingly, patients with anti IgATG2 ≥ 100 U/ml show more advanced disease and carry a larger number of CD associated HLA-DQ heterodimers [18]. On the contrary, most recently, a Spanish group, found that intestinal $\gamma\delta^+$ intraepithelial lymphocyte cytometric pattern is more accurate than subepithelial deposits of anti -IgA-TG2 ABs for CD diagnosis [19]. Anti TG2 autoantibodies expose a lower avidity compared with alloantibodies directed against gliadin, indicating different regulation or site of initiation of these humeral response [20]. Finally, exploring the celiac antibody binding to TG2, a single conformational TG2 epitope contributed by three domains was described to be critical for the ABs binding and their effects [21].

The mechanism involving the hapten-carrier model for the CD autoantibodies production, was already suggested in the 90th, by the Finish group. This theory was substantiate more recently, by showing that when gliadin peptides are bound to TG2, neo-epitopes of the docked enzyme become uncovered allowing autoantibodies against the active site to be generated [22]. The actual concept being that initially the ABs might be targeted against post-translation modified gliadin peptides and that autoantibodies against TG2 would be produced only after epitope spreading through molecular mimicry [22]. A major question to ask is the place of the neo-epitope anti IgA-TG2 in the immune hierarchy and the sequential chronological appearance. The neo-epitope assays us a covalently cross-linked complex of gliadin peptides docked on human recombinant TG2, in contrast to those assays using only DGP/TG2 [6-11,23]. The docking of the substrate on the enzyme creates new non-self-epitopes that stimulates the CD patient's humoral immune system to produce the anti neo-epitope-IgA- TG2 autoantibodies. It seems that those ABs appear early in the disease progression, having a predictive value. In fact, in 34 subjects who were anti-TG2 negative but neo-TG2 positive were reexamined 6 months latter: 47% of them carried a haplotype compatible with CD and two of these also become positive for anti-TG2, on follow-up [24]. More recently, assessing a range of CD associated serological test, the neo-epitope-TG2 autoantibodies were found to be the most reliable for not only the diagnosis but also for monitoring GFD compliance and as predictors of intestinal pathology [25].

The Anti-Tg2 Abs' Deposits are Associated with Extraintestinal Manifestations

TG2 IgA deposits seem to be a constant feature in overt CD patients and are frequently detectable in the intestine of other gluten-related conditions like dermatitis herpetiformis and gluten-ataxia [26,27]. The

TG2 autoantibodies do not bind to papillary skin structures, suggesting that the circulating ABs are not directed against dermal targets. This does not hold for gluten ataxia. Distinct CD patient-derived anti TG2 ABs recognize neuron and cross react with both TG3 and TG6, the autoantigens of dermatitis herpetiformis and gluten ataxia, respectively. The pathogenicity of those anti TG2/3/6 was substantiated when injected intraventricularly to mice, they induce ataxia [27].

As summarized in the systemic review [26], the vast majority of potential CD patients express TG2 deposits at the intestinal level, but no sufficient data are available to exactly define their prognostic role as a marker of evolution toward overt CD. Additionally, TG2 deposits in the intestinal mucosa of the majority of patients with type 1 diabetes were described, but only those with elevated serum levels of anti-TG2 ABs showed the VH usage that is typical for CD ABs [28]. Anti TG2 ABs can bind TG2 in thyroid tissue and their titer correlate with anti thyroidperoxidase ABs, suggesting that anti TG2 ABs could contribute to the development of thyroid disease in CD [29]. Finally, several other extraintestinal conditions or targets were associated with anti TG2 AB binding or deposition: liver of untreated CD, placenta, human primary throphoblasts, bone and heart muscle tissue [27,30].

Are Anti TG2 Autoantibodies bystander, Protective or Pathogenic in CD?

TG2 AB titers are playing a prime role in CD diagnosis but the question remains what are their part in CD induction, establishment and progression.

They have long been considered to be innocent bystanders in celiac disease. Lately, however, evidence has accumulated on the role of anti TG2 antibodies as modulators of the small-intestinal mucosal biology, impacting extraintestinal pathophysiological manifestations and modifiers of TG2 enzyme functions. In fact, accumulating data, from cell based *in vitro* and *ex-vivo* experiments, has demonstrated their ability to induce biological effects in many different cell type [31]. Since TG2 post translational deamidation of gliadin peptides is considered to be a cornerstone in the activation of the adaptive immune system, and since TG2 has multiple effects on major players in the intestinal innate immune system, and CD anti-TG2 antibodies were shown to inhibit TG2 enzymatic activity, those antibodies might be considered as pathogenic [22,27,31-34]. They destabilize the integrity of the intestinal epithelium, thus may contribute to CD establishment and progression [35]. A CD total IgA was documented to increased transepithelial passage of gliadin peptides, in caco2 cell-line. However, the patients' sera was positive to anti TG2 AB, leaving their specific contribution unanswered [36]. It should be emphasized that till recently no *in vivo* studies were available, documenting the multiple pathogenic effect of the ABs, found *in vitro*. Mice experiments, using viral vector-mediated expression of TG2 ABs nor immunization with TG2 autoantigen, failed to induce the CD intestinal phenotype. However, only recently the Finish group demonstrated that intraperitoneal injection of CD patient sera or immunoglobulins to Hsd: Athymic Nude-Foxn1nu mice, induced a condition mimicking early developing CD [37].

Despite several studies showing no effect of anti TG2 on TG2 activity, the ABs were also shown to exercise their effects by inducing gain of the TG2 enzymes' functions. They were shown to enhance transamidation, modulate vascular permeability and ameliorate anti-angiogenesis, [36,38-40]. Despite the physiological TG2 importance in

cell biology and survival, its gain of functions induced by its specific autoantibody results in pathological processes enhancing CD intestinal damage.

Furthermore, O-glycans of the disease-specific TG2 IgA1 autoantibodies in celiac patients exhibited elevated galactose deficiency, decreasing upon GFD. Thus, an additional post translational modification is occurring in CD development and galactose deficiency in IgA1-TG2 could be an aggravating factor [41].

Further complexity is added by the recent observations that Th17 and type 1 regulatory cells and CD²⁵⁺CD⁴⁺ regulatory T cells are involved in CD progression, thus having a potential impact on anti 2 autoantigen secretion and behavior [42].

Multiple environmental factors, in addition to gluten, affect CD. Infections, stress, formula feed, microbiome alterations toward increase diversity and the most recently described microbial transglutaminase [43-45].

For simplicity, Table 1 describes the biological effects of anti TG2 ABs in CD. As shown, the effects are divided to pathogenic or protective and whether found *in vitro* or *in vivo*.

Pathogenic functions	<i>In vitro/vivo</i>
Inhibit epithelial differentiation	vitro
Induce epithelial proliferation	vitro
Increase epithelial permeability	vitro
Inhibit angiogenesis	vitro
Increase vascular permeability	vitro
Reduce cell adhesion	vitro
Induce neuronal apoptosis	vitro
Activate monocytes	Vitro
Increase intracellular Ca ²⁺	vitro
Bind to placenta tissue	vitro
Induce trophoblast apoptosis	vitro
Increase transepithelial passage of gliadin peptides (total IgA, anti TG ²⁺)	vitro
Reduce enterocyte endocytosis	vitro
Interfere with gliadin peptide 31-43 uptake	vitro
Induce ataxia	vivo
Enhance transamidation	vitro
Contribute to epithelial blistering/detachment	vitro

Table 1: the biological effects of anti TG2 ABs in CD [22,27,32-34,36,38-40,46,47].

Conclusion

TG2 is a multifunctional enzyme, exerting intra and extracellular, enzymatic and non-enzymatic, Ca²⁺ dependent and independent functions. Its specific autoantibody, the anti TG2, is similarly multifunctional, affecting some of the corresponding enzyme activities.

It seems that most of them are due to loss of function of TG2, the minority being gain of function. To our knowledge, no beneficial protective effects were assigned to those CD associated anti TG2 mounted autoantibodies. Taken together, celiac ABs could collectively promote small bowel intestinal or extraintestinal damage. Yet, most of the TG2 ABs' activities were explored *in vitro* and *ex-vivo*, very few in animal model but none *in vivo*, in human. The recently derived adult stem cells, exhibiting functional human intestinal tissue comprising all major cell types of the intestine, can resolve some of the moral aspect of human research [48]. The CD serum contains numerous autoantibodies, IgA-TG2 is only one of them and up till today its differential role in CD induction and maintenance is far from being unraveled. Unraveling them might open some new therapeutic strategies for CD.

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