

Contribution of celiac disease autoantibodies to the disease process

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Evaluation of: Teesalu K, Panarina M, Uibo O, Uibo R, Utt M. Autoantibodies from patients with celiac disease inhibit transglutaminase 2 binding to heparin/heparan sulfate and interfere with intestinal epithelial adhesion. *Amino Acids* doi:10.1007/s00726-011-1020-1 (2011) (Epub ahead of print).

Transglutaminase 2 is a multifunctional protein involved in cellular adhesion. Moreover, transglutaminase 2 has been identified as the autoantigen in celiac disease, and in untreated celiac disease, in addition to being present in the serum, the transglutaminase 2-targeted autoantibodies are bound to their antigen in the basement membrane underlining the small-bowel mucosal epithelium. Furthermore, the disease-specific transglutaminase 2-targeted autoantibodies have been experimentally shown to exert various biological effects on different cell types. Using Caco-2 intestinal epithelial cells, it has now also been demonstrated that serum transglutaminase 2-targeted autoantibodies from untreated celiac patients inhibit the adhesion of these cells. These findings provide an important direction for future research to improve our general understanding of celiac disease pathogenesis and especially the role of the disease-specific autoantibodies during the progression of the disorder.

KEYWORDS: adhesion • autoantibody • celiac disease • epithelium

The mucosal immune response is considered a central event in the pathogenesis of celiac disease, an enteropathy triggered by a single and well-characterized exogenous antigen – gluten – derived from dietary wheat, rye and barley. Ingestion of gluten by celiac patients leads to the development of small intestinal villous atrophy, crypt hyperplasia and inflammation paralleled by the appearance of clinical symptoms such as malabsorption and diarrhea. Both innate and adaptive immune responses are thought to contribute to the development of the disease-characteristic small-bowel mucosal lesion. Gastrointestinal enzymes are not able to completely proteolyse gluten-derived gliadin, and thus even fairly long peptides reach the small intestine. Once they have gained access to the lamina propria, a subset of the gliadin peptides are post-translationally modified, namely deamidated, by an enzyme called transglutaminase 2 (TG2), and this event is considered to be the cornerstone in the activation of the adaptive immune system [1]. Experimental evidence also points to autoimmunity as a contributor in celiac disease. Celiac disease autoimmunity is best exemplified

by the presence of disease-specific endomysial autoantibodies, which have experimentally been shown to target TG2 [2]. The significance of these autoantibodies in the pathogenesis of celiac disease remains a controversial issue, although there are data from cell based experiments demonstrating their ability to induce biological effects in many different cell types [1].

The celiac disease autoantigen, TG2, is a ubiquitously expressed protein with a number of functions in several cellular processes, such as apoptosis, fibrosis, wound healing and adhesion [3]. Its enzymatic functions include the aforementioned deamidation, as well as transamidation. TG2-mediated transamidation reactions can lead to acylation, amine incorporation or protein cross-linking, the latter being involved in cell adhesion by taking part in extracellular matrix protein cross-linking [3]. However, the protein has also been ascribed a role in adhesion independent of its enzymatic functions. TG2 interacts with fibronectin and functions as its integrin-binding adhesion coreceptor [4]. Moreover, TG2 is directly associated with

heparin sulfate chains of syndecan-4, this interaction being of major importance in integrin-independent adhesion [5].

In the small intestinal mucosa, epithelial cells are attached to the basement membrane situated directly beneath them. The subepithelial basement membrane consists of numerous proteins, including fibronectin and TG2, and interestingly, celiac patients have TG2-targeted IgA-class autoantibodies deposited there [6]. In spite of the overall importance of the basement membrane and especially TG2 in adhesion, investigators have not addressed the effect of celiac disease-specific TG2-targeted autoantibodies on intestinal epithelial cell adhesion. A recent study published by Teesalu and coworkers has now addressed this issue and described how celiac patient TG2-autoantibodies influence intestinal epithelial cell adhesion [7].

Methods & results

In the study in question, Teesalu and colleagues tested the effects of untreated celiac patient serum samples and affinity-purified TG2 antibodies on the binding of heparin/heparin sulfate with TG2 *in vitro* [7]. In addition, they investigated whether the same celiac patient-derived samples affected the integrin-dependent and -independent adhesion of Caco-2 intestinal epithelial cells to the fibronectin-TG2 matrix and studied whether these effects correlated with the transamidation activity of TG2.

It emerged that both celiac patient serum and affinity-purified anti-TG2 antibodies with high anti-TG2 antibody titers significantly inhibited TG2 binding to heparin and heparin sulfate, implying that celiac autoantibodies might block the TG2 region responsible for the interaction with heparin or heparin sulfate. As heparin sulfate residues are involved in integrin-independent adhesion to fibronectin-TG2, the authors next investigated whether celiac patient autoantibodies modulate intestinal Caco-2 epithelial cell adhesion. As expected, blocking the integrin $\beta 1/\beta 3/\beta 5$ fibronectin interaction with an arginine-glycine-aspartic acid peptide reduced the amount of intestinal Caco-2 epithelial cells adhering to fibronectin and to a lesser extent to the fibronectin-TG2 surface. Celiac patient serum and serum-derived affinity-purified TG2 autoantibodies with high autoantibody titers further decreased this integrin-independent cell attachment to the fibronectin-TG2 matrix. Interestingly, however, celiac patient serum and serum-derived affinity-purified TG2-autoantibodies also reduced the integrin-dependent adhesion to fibronectin-TG2. It is of note that such inhibitory effects were apparent only when celiac patient samples were preincubated with the fibronectin-TG2 matrix and not the cells. Likewise of note was the absence of correlation of Caco-2 cell attachment with the transamidation activity of TG2 in the presence of celiac patient samples, as neither celiac serum nor affinity-purified serum TG2-autoantibodies affected TG2 activity. Collectively, these data suggest that untreated celiac patient TG-2 autoantibodies reduce both integrin-dependent and -independent intestinal epithelial cell adhesion by targeting extracellular, rather than cell surface, TG2. Moreover, the effects of celiac TG2-autoantibodies on cellular adhesion are not accounted for by the transamidation activity of TG2.

Discussion

In the study under evaluation Teesalu and colleagues demonstrate that celiac patient serum and serum-derived affinity-purified TG2 autoantibodies affect the interaction of TG2 with heparin/heparin sulfate, and further that intestinal epithelial adhesion to fibronectin-TG2 is affected by the antibodies [7].

Celiac patient autoantibodies have previously been reported to modulate several cellular processes, for instance to inhibit angiogenesis and increase vascular permeability [8,9]. In addition, several studies have addressed the effects of the disease antibodies on intestinal epithelial cell behavior. The antibodies have been shown to reduce intestinal epithelial cell endocytosis [10] and differentiation [11], to increase proliferation [11,12], to decrease the intestinal epithelial barrier function in general [13] and to enhance epithelial translocation of gliadin peptides [14]. However, not all of the above effects can be categorically attributed to TG2-targeted autoantibodies, as some of the experiments in question were performed with total serum IgA or other types of affinity-purified antibodies. This notwithstanding, the article by Teesalu and coworkers increases our understanding of the biological effects of celiac patient antibodies on intestinal epithelial cells [7].

The authors report that celiac patient anti-TG2 autoantibodies had no effect on the transamidase activity of TG2, and it is therefore unlikely that the decreased Caco-2 cell adhesion in the presence of celiac autoantibodies is related to TG2 activity. Their results are thus in line with those of previous studies reporting the adhesion-promoting effect of TG2 to be independent of its enzymatic activity [4,15]. In contrast to the authors' findings [7] there are also results showing that celiac patient serum antibodies, as well as TG2-specific celiac patient autoantibodies, either enhance [9,14,16,17] or diminish [10,18,19] the enzymatic activity of TG2. Moreover, some celiac patient serum antibody-induced effects, for example, increased epithelial translocation of gliadin peptides, can be circumvented by enzymatic inhibition of TG2 [14]. It would thus appear that distinct effects of celiac patient serum antibodies on cellular behavior are mediated by different functions of TG2.

Regardless of the precise mechanism by which the celiac patient anti-TG2-autoantibodies reduce intestinal epithelial cell adhesion, the findings of Teesalu and colleagues have important implications for disease pathogenesis [7]. It has been reported that in the small-bowel mucosa of untreated celiac patients, the epithelium tends to blister or totally detach from the basement membrane [20], and the authors here provide a possible explanation for this phenomenon [7]. As they themselves hypothesize, it can be envisaged that the binding of celiac patient TG2-specific autoantibodies to basement membrane TG2 could hinder the interaction of TG2 and cell surface heparin sulfate proteoglycans such as syndecans. This could lead to loosened adhesion of the epithelium to the underlying basement membrane and eventually to epithelial blistering.

Five-year view

Celiac disease is a relatively well-characterized autoimmune disease, since the environmental trigger (i.e., gluten), autoantigen

(TG2) and the function of the major genetic component (i.e., the HLA molecules) in the pathogenesis are already known. However, although there are publications available demonstrating that the disease-specific TG2-autoantibodies exert biological effects in several cell types, their precise contribution to the pathogenetic process is not yet completely understood. In the next few years, the increase in the number of available research tools and methods will presumably serve to increase knowledge of the effects of the disease-specific autoantibodies. The paper by Teesalu and colleagues demonstrating that celiac disease-specific TG2-targeted autoantibodies affect intestinal epithelial cell adhesion has indeed already provided new insight in this regard [7]. Issues arising from the article and calling for future attention include, for instance, the question of whether the defective adhesion induced by celiac autoantibodies modulates the composition of the extracellular matrix or the basement membrane and what the pathways by which the autoantibodies exert their functions are.

All in all, our understanding of celiac disease pathogenesis, and of the contribution of the disease-specific autoantibodies, which may well be substantially increased within the next 5 years, will provide a foundation for the design and development of novel treatment options for celiac disease. At the same time the basic scientific findings can be translated in the long-run to the clinical care of celiac disease patients.

Financial & competing interests disclosure

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Key issues

- Untreated celiac patient serum and affinity-purified serum transglutaminase 2 (TG2)-autoantibodies with high antibody titers reduce TG2 binding to heparin/heparin sulfate.
- Intestinal Caco-2 epithelial cell adhesion to fibronectin-TG2 is reduced in the presence of serum and TG2-autoantibodies derived from untreated celiac patients.
- Celiac patient autoantibodies targeting TG2 may contribute to the epithelial blistering/detachment known to occur in celiac disease.

References

- Lindfors K, Mäki M, Kaukinen K. Transglutaminase 2-targeted autoantibodies in celiac disease: pathogenetic players in addition to diagnostic tools? *Autoimmun. Rev.* 9(11), 744–749 (2010).
- Korponay-Szabó IR, Sulkkanen S, Halttunen T *et al.* Tissue transglutaminase is the target in both rodent and primate tissues for celiac disease-specific autoantibodies. *J. Pediatr. Gastroenterol. Nutr.* 31(5), 520–527 (2000).
- Belkin AM. Extracellular TG2: emerging functions and regulation. *FEBS J.* 278(24), 4704–4716 (2011).
- Akimov SS, Krylov D, Fleischman LF, Belkin AM. Tissue transglutaminase is an integrin-binding adhesion coreceptor for fibronectin. *J. Cell Biol.* 148(4), 825–838 (2000).
- Telci D, Wang Z, Li X *et al.* Fibronectin-tissue transglutaminase matrix rescues RGD-impaired cell adhesion through syndecan-4 and $\beta 1$ integrin co-signaling. *J. Biol. Chem.* 283(30), 20937–20947 (2008).
- Koskinen O, Collin P, Lindfors K, Laurila K, Mäki M, Kaukinen K. Usefulness of small-bowel mucosal transglutaminase-2 specific autoantibody deposits in the diagnosis and follow-up of celiac disease. *J. Clin. Gastroenterol.* 44(7), 483–488 (2010).
- Teesalu K, Panarina M, Uibo O, Uibo R, Utt M. Autoantibodies from patients with celiac disease inhibit transglutaminase 2 binding to heparin/heparan sulfate and interfere with intestinal epithelial cell adhesion. *Amino Acids* doi:10.1007/s00726-011-1020-1021-1 (2011) (Epub ahead of print).
- Myrsky E, Kaukinen K, Syrjänen M, Korponay-Szabó IR, Mäki M, Lindfors K. Coeliac disease-specific autoantibodies targeted against transglutaminase 2 disturb angiogenesis. *Clin. Exp. Immunol.* 152(1), 111–119 (2008).
- Myrsky E, Cava S, Simon-Vecsei Z *et al.* Celiac disease IgA modulates vascular permeability in vitro through the activity of transglutaminase 2 and RhoA. *Cell. Mol. Life Sci.* 66(20), 3375–3385 (2009).
- Caputo I, Barone MV, Lepretti M *et al.* Celiac anti-tissue transglutaminase antibodies interfere with the uptake of α gliadin peptide 31–43 but not of peptide 57–68 by epithelial cells. *Biochim. Biophys. Acta* 1802(9), 717–727 (2010).
- Halttunen T, Mäki M. Serum immunoglobulin A from patients with celiac disease inhibits human T84 intestinal crypt epithelial cell differentiation. *Gastroenterology* 116(3), 566–572 (1999).
- Barone MV, Caputo I, Ribocco MT *et al.* Humoral immune response to tissue transglutaminase is related to epithelial cell proliferation in celiac disease. *Gastroenterology* 132(4), 1245–1253 (2007).
- Zanoni G, Navone R, Lunardi C *et al.* In celiac disease, a subset of autoantibodies against transglutaminase binds toll-like receptor 4 and induces activation of monocytes. *PLoS Med.* 3(9), e358 (2006).
- Rauhavirta T, Qiao SW, Jiang Z *et al.* Epithelial transport and deamidation of gliadin peptides: a role for coeliac disease patient immunoglobulin A. *Clin. Exp. Immunol.* 164(1), 127–136 (2011).
- Verderio EA, Telci D, Okoye A, Melino G, Griffin M. A novel RGD-independent cell adhesion pathway mediated by fibronectin-bound tissue transglutaminase rescues cells from anoikis. *J. Biol. Chem.* 278(43), 42604–42614 (2003).
- Király R, Vecsei Z, Deményi T, Korponay-Szabó IR, Fésüs L. Coeliac autoantibodies can enhance transamidating and inhibit GTPase activity of tissue transglutaminase: dependence on reaction environment and enzyme fitness. *J. Autoimmun.* 26(4), 278–287 (2006).

- 17 Caja S, Myrsky E, Korponay-Szabo IR *et al.* Inhibition of transglutaminase 2 enzymatic activity ameliorates the anti-angiogenic effects of coeliac disease autoantibodies. *Scand. J. Gastroenterol.* 45(4), 421–427 (2010).
- 18 Esposito C, Paparo F, Caputo I *et al.* Anti-tissue transglutaminase antibodies from coeliac patients inhibit transglutaminase activity both *in vitro* and *in situ*. *Gut* 51(2), 177–181 (2002).
- 19 Dieterich W, Trapp D, Esslinger B *et al.* Autoantibodies of patients with coeliac disease are insufficient to block tissue transglutaminase activity. *Gut* 52(11), 1562–1566 (2003).
- 20 Kainulainen H, Rantala I, Collin P *et al.* Blisters in small intestinal mucosa of coeliac patients contain T cells positive for cyclooxygenase-2. *Gut* 50(1), 81–89 (2002).

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