Dublin Institute of Technology
ARROW@DIT

Articles

School of Biological Sciences

2007-01-01

Mutagenesis of the catalytic triad of tissue transglutaminase abrogates coeliac disease serum IgA autoantibody binding

Greg Byrne
Dublin Institute of Technology

Fergus Ryan
Dublin Institute of Technology, fergus.x.ryan@dit.ie

John Jackson
St. James' Hospital, Dublin

Con Feighery
St. James' Hospital, Dublin

Jacinta Kelly
St. James' Hospital, Dublin

Follow this and additional works at: http://arrow.dit.ie/scschbioart

Part of the Medical Sciences Commons

Recommended Citation

Byrne, G., Ryan, F., Jackson, J.,Feighery, C., Kelly, J.:Mutagenesis of the catalytic triad of tissue transglutaminase abrogates coeliac disease serum IgA autoantibody binding.Gut, Vol. 56, 2007, pp.336-341

This Article is brought to you for free and open access by the School of Biological Sciences at ARROW@DIT. It has been accepted for inclusion in Articles by an authorized administrator of ARROW@DIT. For more information, please contact yvonne.desmond@dit.ie, arrow.admin@dit.ie.

This work is licensed under a Creative Commons Attribution-Noncommercial-Share Alike 3.0 License

Antenna & High Frequency Research Centre

Articles

Dublin Institute of Technology *Year* 2007

Mutagenesis of the catalytic triad of tissue transglutaminase abrogates coeliac disease serum IgA autoantibody binding

Greg Byrne* Fergus Ryan† John Jackson‡
Con Feighery** Jacinta Kelly††

*Dublin Institute of Technology
†Dublin Institute of technology, fergus.x.ryan@dit.ie
‡St. James's Hospital Dublin
**St. James's Hospital Dublin
††St. James's Hospital Dublin

This paper is posted at ARROW@DIT.
http://arrow.dit.ie/ahfrcart/11

— Use Licence —

Attribution-NonCommercial-ShareAlike 1.0

You are free:

- to copy, distribute, display, and perform the work
- to make derivative works

Under the following conditions:

- Attribution.
  You must give the original author credit.
- Non-Commercial.
  You may not use this work for commercial purposes.
- Share Alike.
  If you alter, transform, or build upon this work, you may distribute the resulting work only under a license identical to this one.

For any reuse or distribution, you must make clear to others the license terms of this work. Any of these conditions can be waived if you get permission from the author.

Your fair use and other rights are in no way affected by the above.

This work is licensed under the Creative Commons Attribution-NonCommercial-ShareAlike License. To view a copy of this license, visit:

- URL (human-readable summary):
  http://creativecommons.org/licenses/by-nc-sa/1.0/
- URL (legal code):
  http://creativecommons.org/worldwide/uk/translated-license

Downloaded from gut.bmj.com on August 11, 2010 - Published by group.bmj.com

Mutagenesis of the catalytic triad of tissue transglutaminase abrogates coeliac disease serum IgA autoantibody binding

Greg Byrne, Fergus Ryan, John Jackson, et al.

Gut 2007 56: 336-341 originally published online August 25, 2006
doi: 10.1136/gut.2006.092908

Updated information and services can be found at:
http://gut.bmj.com/content/56/3/336.full.html

These include:

References This article cites 31 articles, 10 of which can be accessed free at:
http://gut.bmj.com/content/56/3/336.full.html#ref-list-1

Email alerting service Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Topic collections Articles on similar topics can be found in the following collections

Coeliac disease (464 articles)

Notes

To order reprints of this article go to:
http://gut.bmj.com/cgi/reprintform

To subscribe to *Gut* go to:
http://gut.bmj.com/subscriptions

Downloaded from gut.bmj.com on August 11, 2010 - Published by group.bmj.com

COELIAC DISEASE

Mutagenesis of the catalytic triad of tissue transglutaminase abrogates coeliac disease serum IgA autoantibody binding

Greg Byrne, Fergus Ryan, John Jackson, Con Feighery, Jacinta Kelly

Gut 2007;**56**:336–341. doi: 10.1136/gut.2006.092908

See end of article for authors' affiliations

Correspondence to: Dr Jacinta Kelly, School of Biological Sciences, Dublin Institute of Technology, Dublin 8, Ireland; Jacinta.Kelly@dit.ie

Revised 18 May 2006
Accepted 15 August 2006
Published Online First 25 August 2006

Background and aims: Tissue transglutaminase (tTG) is an autoantigen in coeliac disease and the related disorder, dermatitis herpetiformis. The detection of autoantibodies directed against tTG is a highly specific marker of coeliac disease; however, it is unclear if there is a role for these autoantibodies in the disease process. The aim of this study was to investigate whether the catalytic triad of tTG is targeted by coeliac disease autoantibodies.
Methods: A full-length wild-type recombinant tTG and a novel site-directed mutagenic variant lacking the catalytic triad were produced in *Escherichia coli*. Serum samples from 61 biopsy-proven coeliac disease and 10 dermatitis herpetiformis patients were tested for their recognition of both antigens in enzyme-linked immunosorbent assay.
Results: Although IgA autoantibodies from sera of patients with coeliac disease and dermatitis herpetiformis bound wild-type tTG well, a dramatic decrease in binding to the mutant tTG was observed with a mean reduction of 79% in coeliac disease and 58% in dermatitis herpetiformis samples. IgG anti-tTG antibodies did not show a similar pattern of reduction, with no overall difference in recognition of the wild-type or mutant tTGs.
Conclusions: These results suggest that the IgA anti-tTG response in coeliac disease and dermatitis herpetiformis is focused on the region of tTG responsible for its transamidation and deamidation reactions, whereas the IgG response may target other regions of the enzyme.

Tissue transglutaminase (tTG) is a member of a family of enzymes responsible for the post-translational modification of proteins by the introduction of ε-(γ-glutaminyl)lysine cross-links.[1] As well as transamidation reactions, other roles for tTG have been suggested in apoptosis,[2] extracellular matrix interactions,[3–5] wound healing,[6] $CD8^+$ T cell transendothelial migration,[7] GTP hydrolysis[8] and signal transduction.[9] The catalytic triad of tTG (Cys^{277}, His^{335} & Asp^{358}) performs the cross-linking reaction by attaching a target glutamine to an available lysine in a Ca^{2+}-dependent manner. In the absence of an available lysine residue and in the presence of water, tTG can deamidate its target glutamine residue.

In 1997 Dieterich and colleagues discovered that tTG is the predominant autoantigen in coeliac disease, a malabsorptive disorder of the small intestine caused by wheat gluten ingestion.[10] Coeliac disease affects approximately 1 in 200 Europeans[11] and is characterised by damage to the villous architecture of the upper small intestine with considerable mononuclear cell infiltration. Coeliac disease has a strong genetic component, with most patients expressing the DQ2 heterodimer.[12] In 2002 Vader and colleagues discovered that deamidation of specific glutamine residues in wheat gliadin peptides by tTG significantly enhanced their binding to DQ2 and hence increased their immunogenicity.[13] The mechanisms underlying the autoimmune response directed against tTG in coeliac disease are unknown, but the formation of gliadin-tTG complexes by transglutaminase activity seems to be required for the process.[10] The detection of IgA antibodies directed against tTG is a highly sensitive serological test for diagnosing coeliac disease.[14] However, their role, if any, in the disease process remains unknown. Dermatitis herpetiformis is a related gluten-sensitive enteropathy that is characterised by IgA deposits at the dermoepidermal junction of the skin.[15] It is also associated with IgA anti-tTG reactivity, but in a lower percentage of cases.[16] Epidermal transglutaminase has been established as the predominant autoantigen in dermatitis herpetiformis.[17]

In one recent study investigating autoantibody binding to truncated tTG protein fragments, Sblattero *et al.* (2002) found evidence for a conformation-dependant epitope within the core region of tTG (amino acids 140 to 376), the domain of the enzyme in which the catalytic triad is located.[18] The presence of calcium in enzyme-linked immunosorbent assay (ELISA) coating buffers has been shown to induce a conformational change revealing the catalytic triad[19] and to increase autoantibody binding.[20] Because the catalytic triad is a site of interaction for tTG with gliadin peptides, and possibly a site for intermolecular epitope spreading, this study was designed to establish whether the catalytic triad of tTG is targeted by coeliac disease autoantibodies. To this end, full-length tTG and a novel site-directed mutagenic tTG lacking the Cys–His–Asp catalytic triad were produced and tested as antigen in ELISAs with cohorts of coeliac disease and dermatitis herpetiformis sera to determine the importance of the catalytic core in tTG–autoantibody interactions.

MATERIALS AND METHODS

Serum samples

Seventy-six serum samples from 61 patients (46 females, 15 males; age 15 to 76 years, median = 50) with coeliac disease were used to characterise anti-tTG binding. Diagnosis was based upon histology of duodenal biopsy as well as positivity for anti-tTG and anti-endomysial antibodies. Sera from 10 dermatitis herpetiformis patients (3 females, 7 males; age 19 to 64 years, median = 53) were also included in the study. Forty-nine sera (29 females, 20 males; age 17 to 84, median = 53) from individuals with normal intestinal biopsy and negative anti-tTG serology as determined using the Celikey® ELISA

Abbreviations: tTG, tissue transglutaminase; IPTG, Isopropyl β-D-1-thiogalactopyranoside; rpm, revolutions per minute; PBS, phosphate buffered saline

Downloaded from gut.bmj.com on August 11, 2010 - Published by group.bmj.com

system (Pharmacia Diagnostics) were used to establish cut-off points for ELISAs.

Polymerase chain reaction amplification, cloning and site-directed mutagenesis of tTG constructs

Total RNA was extracted from peripheral blood mononuclear cells by standard methods. cDNA was generated by a 60 minute reverse transcription reaction using AMV reverse transcriptase (Promega). The full-length tTG sequence was amplified by polymerase chain reaction (PCR) using the following conditions and primers: 40 cycles of 95℃ 30 s, 55℃ 30 s and 72℃ 2 min. (Fwd: 5′-GATCGAATTCATGGCCGAGGAGCTGGTCTTAG-3′ Rev: 5′-GATCCTCGAGTTAGGCGGGGCCAATGATGAC-3′). The tTG PCR product was ligated to the pGEX-4T-1 vector (Amersham Biosciences) by *Eco*RI and *Xho*I restriction sites. Site-directed mutagenesis replaced the Cys–His–Asp catalytic triad with alanine residues using the Quickchange® system (Stratagene). Primers used to perform the site-directed mutagenesis are described in Table 1. DNA sequencing used the GeneReadir 4200 system (LiCor).

tTG expression and purification

Bacteria were grown in nutrient broth to OD_{600} ~0.5, induced to express protein by addition of 1 μM IPTG (Sigma-Aldrich) and grown overnight at room temperature with 200 rpm agitation. The pelleted bacteria were resuspended in 20 mM Tris, 150 mM NaCl, 1 mM EDTA, 1 mM DTT, 15% glycerol pH 8.0 and centrifuged at 3,500×*g* for 15 minutes. The resulting pellets were stored at −80℃. For purification of recombinant tTG, pellets were resuspended in 80 ml CelLytic reagent (Sigma-Aldrich) containing 0.1 mg/ml lysozyme (Sigma-Aldrich) and ~1,000 units DnaseI (Invitrogen). Lysate was incubated at room temperature for 20 minutes and homogenized by syringing. The lysate was then centrifuged at 25,000×*g* for 15 minutes to pellet insoluble material and the resulting supernatant was incubated with 800 μl Glutathione Sepharose slurry (Amersham). Sepharose was washed ×4 in phosphate buffered saline (PBS) and fusion protein was eluted from the beads with elution solution (100 mM Glutathione, 20 mM Tris, pH 8.0). Recombinant tTG was stored in 50% glycerol at −20℃. Protein batches were routinely checked for purity by SDS–polyacrylamide gel electrophoresis (SDS–PAGE).

NanoOrange™ assay for protein quantification

Protein yield was quantified using NanoOrange™ reagent (Molecular Probes). NanoOrange™ is a fluorescent reagent capable of detecting protein concentrations as low as 10 ng/ml, offering a broad, dynamic quantification range and improved sensitivity relative to absorption-based protein solution assays.[21] A standard curve was generated using bovine serum albumin (BSA) ranging from 0.1 to 10 μg/ml in NanoOrange reagent. Analytes were diluted in NanoOrange reagent and read (λ_{ex} 485, λ_{em} 595) in a Spectrafluor Plus instrument (Tecan).

Western blotting

SDS–PAGE was performed according to standard methods. Protein was blotted onto polyvinylidene fluoride membranes (Sigma-Aldrich) using a semi-dry apparatus (Apollo). The membrane was blocked at 4℃ overnight with 5% non-fat dried milk including 0.5% Tween 20 (Sigma-Aldrich). Primary antibodies used were CUB7402/TG100, a monoclonal mouse anti-tTG antibody (Labvision); GST01, a mouse anti-GST antibody (Labvision); and polyclonal rabbit anti-tTG (Roboscreen). All primary antibodies were diluted 1:1000 in 5% non-fat dried milk including 0.5% Tween. After incubation for 2 h at room temperature and thorough washing with PBS containing 0.1% Tween, the membranes were incubated with 1:1000 dilution of either rabbit anti-mouse conjugated to horseradish peroxidase (HRP) or swine anti-rabbit conjugated to HRP (Dako). Blots were visualised using chromogenic substrate 3,3′-diaminobenzidine (Sigma-Aldrich) and hydrogen peroxide (Sigma-Aldrich).

ELISAs

ELISAs were performed by coating certified 96-well Maxisorp® plates (Nunc) with 0.3 μg per well of either purified recombinant tTG or mutant tTG for 16 h at 4℃ in coating buffer (50 mM Tris-HCL, 150 mM NaCl, 5 mM CaCl2, pH 7.5). Wells were blocked with 1% BSA (Diamed) in PBS for 1 h and washed four times with PBS plus 0.1% Tween between each step. Antibodies used in Western blots were also used as primary antibodies in ELISAs as a further quantitative control. Antibodies were diluted as follows: CUB7402/TG100 and GST01 were diluted 1:1000 with PBS plus 0.1% Tween followed by rabbit anti-mouse conjugated with HRP (Dako). Polyclonal anti-tTG was diluted 1:1000 with PBS plus 0.1% Tween followed by 1:1000 swine anti-rabbit conjugated with HRP (Dako). For IgA assays, human sera were diluted 1:20 with PBS plus 0.1% Tween followed by 1:1000 rabbit anti-human IgA conjugated to HRP (Dako). For IgG assays, human sera were diluted 1:500 with PBS plus 0.1% Tween followed by 1:500 rabbit anti-human IgG conjugated to HRP (Dako). All antibody incubations were performed at room temperature for 1 h. ELISAs were developed using 3,3′,5,5′-tetramethylbenzidine liquid substrate system (Sigma-Aldrich) for 7 minutes at room temperature. ELISAs were read at A450 in a Wallac Victor2 multilabel counter. Cut-off points were established by incubating 49 serum samples found to be negative for anti-tTG responses with wild-type and mutant tTGs. Cut-off points were calculated as mean +2 standard deviations.

Statistics

The Mann–Whitney U test and Wilcoxon ranked sums test were used to compare reactivity to wild-type and mutant tTG antigens.

Table 1 Primers used in site-directed mutagenesis

Primer	Sequence (5′ → 3′)
C277A-F	GTCAAGTATGGCCAGGCTTGGGTCTTCGCCG
C277A-R	CGGCGAAGACCCAAGCCTGGCCATACTTGAC
H335A-F	CGAGATGATCTGGAACTTCGCTTGCTGGGTGGAGTCGTG
H335A-R	CACGACTCCACCCAGCAAGCGAAGTTCCAGATCATCTCG
D358A-F	GCAGGCCCTGGCCCCAACGCCCC
D358A-R	GGGGCGTTGGGGCCAGGGCCTGC

The substituted nucleotides are underlined.
F, forward primer
R, reverse primer

Downloaded from gut.bmj.com on August 11, 2010 - Published by group.bmj.com

Ethical approval

Ethical approval for this study was obtained from the joint ethics committee of St James's and Tallaght hospitals.

RESULTS

Cloning strategy, sequencing and mutagenesis

Recombinant tTG was expressed as a glutathione-S-transferase (GST) fusion protein. The solubility-enhancing properties of the GST tag have been reported to increase yields of difficult proteins.[22] DNA sequencing confirmed the sequence identity, which was homologous with the nucleotide sequence previously characterized by Gentile (1991).[23] The Quickchange system (Stratagene) was used for site-directed mutagenesis on the three amino acids of the catalytic triad of tTG (Cys^{277}, His^{335} and Asp^{358}). The three amino acids were replaced with alanine resides to remove any catalytic or steric properties of the catalytic triad.

Protein production and quantification

Conditions for protein production were optimised at 1 μM IPTG overnight at room temperature. Cells were lysed using CelLytic (Sigma-Aldrich), a detergent-based reagent that releases 95% of soluble proteins from *Escherichia coli*. The purified proteins were examined by SDS–PAGE and shown to be highly pure (fig 1). Since protein yields were consistently low, NanoOrange™ (Molecular Probes), a highly sensitive protein quantification reagent, was used to determine tTG concentrations. Wild-type tTG had an average yield of 1.15 mg/l of bacteria whereas mutant protein preparations yielded, on average, 50% less enzyme then wild-type tTG preparations (data not shown).

Wild-type and mutant tTG characterisation

Reactivity of commercially available antibodies to wild-type and mutant tTG was examined by ELISA (fig 2a). To confirm that equivalent amounts of both fusion proteins were binding ELISA plates, a commercial mouse anti-GST antibody was included in some ELISAs and was found to bind equally to wells coated with both wild-type and mutant tTG. To confirm conformational integrity of both wild-type and mutant tTGs, commercially available antibodies directed against tTG were included in ELISAs. A mixture of two mouse monoclonal antibodies directed against tTG (CUB7402 + TG100 Neomarkers) bound mutant tTG with slightly decreased affinity but this finding did not reach statistical significance (Mann–Whitney U test). Rabbit polyclonal anti-tTG (Roboscreen) bound both mutant and wild-type tTGs with equal affinity. All three commercial antibodies reacted with wild-type and mutant tTGs in Western blots (fig 2b).

Wild-type and mutant tTG recognition by coeliac sera

Coeliac disease serum IgA reactivity to wild-type tTG correlated well with IgA anti-tTG results from the Celikey® tTG ELISA system ($r = 0.804$). However, coeliac disease serum IgA reactivity to mutant tTG was dramatically reduced compared with wild-type tTG (Fig 3a). In fact, although 88.16% of coeliac disease sera were found to be positive in the wild-type antigen assay, only 18.42% of sera exceeded the cut-off point when tested for IgA reactivity to the mutant tTG. There was, on average, a 79% reduction in IgA class autoantibody binding to mutant tTG compared with wild-type tTG.

In IgG ELISAs, sera were used at a higher dilution than in IgA assays and, as a result, AU values were lower. Coeliac disease serum IgG bound wild-type tTG at significantly higher levels than control sera ($p<0.005$, Student's t-test). Interestingly, IgG reactivity did not mirror the pattern observed in IgA assays (Fig 3b) as there was no significant difference between coeliac disease IgG recognition of wild-type or mutant tTG (Wilcoxon signed ranks test).

Wild-type and mutant tTG recognition by dermatitis herpetiformis sera

The IgA wild-type tTG ELISA successfully identified 6 of 10 dermatitis herpetiformis sera as anti-tTG positive (fig 3c). In a similar pattern to the results with sera of patients with coeliac disease, dermatitis herpetiformis serum IgA showed a dramatically reduced binding to mutant tTG. All samples tested for IgA binding to mutant tTG bound the antigen at a level below the threshold for positivity, with a mean percentage reduction in binding of 58% compared with wild-type tTG. Once again, IgG responses did not mirror IgA results. IgG antibodies showed no difference in binding to wild-type or mutant tTGs (Fig 3d).

DISCUSSION

Recent discoveries in the field of coeliac disease have assigned an important role to tTG in the deamidation of gliadin molecules resulting in strengthening of interactions between DQ2 and gliadin.[13] In the light of these discoveries, a role for the autoimmune response directed against tTG in the pathogenesis of coeliac disease seems more plausible. A previous study has suggested that blockade of tTG by autoantibodies could be responsible for inhibition of epithelial cell differentiation and contribute to the mucosal lesion observed in coeliac disease.[24] In a recent study by Sblattero *et al*. (2002), truncated tTG fragments were applied as antigen to ELISAs with coeliac disease sera to map immunodominant epitopes. Their results suggested that an important conformation-dependent epitope was to be found within the region spanning amino acids 140 to 376.[18] Interestingly, this region encompasses the three amino acids of the catalytic triad of tTG (Cys^{277}, His^{335} and Asp^{358}). Since this region of tTG is only revealed upon Ca^{2+} activation and is a site of tTG–gliadin interaction, it represents a valid target for autoantibody binding studies.

In this study, a full-length recombinant tTG and a mutant variant lacking the catalytic triad were produced to investigate autoantibody binding to this region of tTG while attempting to maintain overall protein conformation. This is the first time site-directed mutagenesis has been used to investigate tTG epitopes. These results demonstrate that the IgA anti-tTG response in coeliac disease sera is specifically targeted towards the region of the enzyme responsible for its transamidation and deamidation reactions. In fact, all coeliac disease sera showed a profound reduction in IgA binding to mutant tTG compared with wild-type tTG, with 79% of sera reacting positively to the wild-type tTG having no reactivity to the mutant tTG. This effect was not limited to antigen coated on ELISA plates because pre-incubation of coeliac disease sera with wild-type tTG in solution caused almost complete depletion of IgA anti-tTG antibodies whereas pre-incubation of coeliac disease sera with mutant tTG had little effect on anti-tTG levels (data not shown).

In similar experiments investigating IgG anti-tTG antibodies, there was no significant difference in reactivity to wild-type or mutant tTGs. Although in the assay conditions employed, IgG anti-tTG levels did not exceed the cut-off levels for positivity, IgG anti-tTG binding was still significantly higher in coeliac disease than control sera ($p<0.005$, Student's t test). The IgG anti-tTG ELISA has been shown to be less sensitive than the IgA assay, with sensitivities as low as 13% being reported previously.[25]

Although mutagenesis could have affected the folding of the mutant tTG and hence, affected autoantibody binding to the mutant tTG, there are three reasons this does not seem to be

Downloaded from gut.bmj.com on August 11, 2010 - Published by group.bmj.com

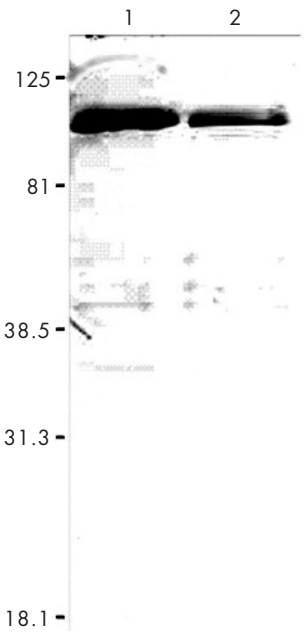

Figure 1 SDS–PAGE of wild-type tTG (1) and mutant tTG (2). tTG is approximately 77 kilodaltons (kDa) and the GST fusion tag is 26 kDa; therefore fusion protein is ~103 kDa. Mutant tTG yields were generally lower than wild-type tTG yields.

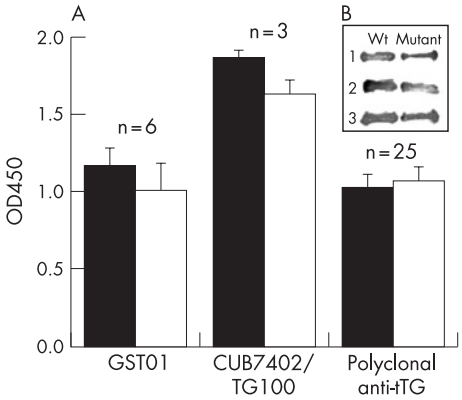

Figure 2 (a) ELISAs comparing commercial antibody binding to wild-type (black bars) and mutant tTG (white bars). (b) Western blots of wild-type and mutant tTG with commercially available antibodies GST01 (1), CUB7402/TG100 (2) and polyclonal anti-tTG (3) are shown in the inset.

the case in these experiments. First, a polyclonal antibody raised against recombinant human tTG bound wild-type and mutant tTGs equally (fig 2a). Second, in silico mutation of the amino acids of the catalytic triad using the three-dimensional position-sensitive scoring matrix (3D-PSSM) program[26] showed no notable difference in secondary structure as a result of the mutations. Third, unlike IgA responses, there was no significant difference between IgG binding of wild-type and mutant tTGs (fig 3).

These results suggest a difference in specificity between IgA and IgG class anti-tTG responses in coeliac disease. The IgA anti-tTG response is focused on the active region of the enzyme whereas the IgG antibodies appear to recognise other sites on the protein and are largely unaffected by mutation of the catalytic triad. The targeting of the active core of tTG by IgA antibodies suggests the possibility that these autoantibodies may affect the enzyme's function. Although it might be expected that inhibition of such a multifunctional enzyme may be associated with some systemic consequences, it is possible that tTG inhibition occurs primarily in the gut and is mediated by the mucosal immunoglobulin class, IgA. These antibodies could, therefore, contribute to the local coeliac disease lesion. Using phage antibody libraries, Marzari and colleagues (2001) demonstrated that unlike those against gliadin, IgA antibodies directed against tTG can be isolated from intestinal libraries but not from peripheral blood libraries.[27] These results suggest that the anti-tTG response is generated at a local level whereas anti-gliadin responses are systemic.

Several studies have shown some level of inhibition of tTG by coeliac disease autoantibodies.[28 29] These studies did not preincubate tTG with antibodies at 37℃, possible because of the instability of tTG preparations. The observation that tTG is capable of continuing its cross-linking function to some extent even in the presence of autoantibodies is not surprising because antibody–antigen interactions are non-covalent and reversible.[30] Furthermore, because substrate is in excess in these artificial systems and the enzyme–substrate affinity is highly evolved, substrate is likely to compete well with autoantibodies for interaction with the catalytic triad.

In experiments investigating IgA anti-tTG antibodies from patients with dermatitis herpetiformis, 6 out of 10 samples were found to react to wild-type tTG. This is in keeping with reports that a lower proportion of sera from patients with dermatitis herpetiformis show positive anti-tTG serology compared with coeliac disease sera.[16] IgA and IgG class anti-tTG autoantibodies from dermatitis herpetiformis patients reacted to wild-type and mutant tTGs in a similar fashion to those from coeliac disease sera. Epidermal transglutaminase has recently been identified as the predominant autoantigen in dermatitis herpetiformis. It has been suggested that dermatitis herpetiformis patients may produce populations of antibodies that react with tTG only, epidermal transglutaminase only, and cross-reactive antibodies that target epitopes common to both enzymes.[17] Results from this study suggest that IgA anti-tTG antibodies in dermatitis herpetiformis are very similar to those found in coeliac disease. It is possible that the autoimmune response in this disorder is initiated in the gut against tTG with subsequent intermolecular epitope spreading to epidermal transglutaminase.

Whether the potential inhibition of tTG activity by autoantibodies has any significance in vivo is yet to be determined. However, the blockade of enzyme activity by autoantibodies is not unprecedented. In Wegener's granulomatosis, inhibition of proteinase 3 by autoantibodies has been described[31], whereas in autoimmune atrophic gastritis the inhibition of the H^+, K^+-ATPase proton pump by parietal cell autoantibodies has been well characterised.[32] It is worth noting that although antibody-mediated enzyme inhibition is thought to play a role in both of these conditions, total blockade of enzyme activity is not reported. The highly specific nature of IgA anti-tTG targeting could have implications for the activity of tTG at a local level by two possible mechanisms. First, tTG has been shown to have a role in the wound healing process, and its blockade by IgA anti-tTG could interfere with coeliac lesion repair.[6] A second possibility is the prevention of villous crypt-cell differentiation: tTG is involved in the activation of TGF-β, a cytokine required for maturation of crypt cells into enterocytes.[33] It has been shown that IgA from patients with coeliac disease can block the differentiation of T84 intestinal crypt cells in vitro.[24] Given the prevalence of IgA deficiency in coeliac disease patients,[34] it seems unlikely that these autoantibodies are involved in coeliac disease aetiology but perhaps contribute to failure to repair the gut lesion.

The discovery that tTG may have a pivotal role in coeliac disease pathogenesis has led to speculation about a role for the

Downloaded from gut.bmj.com on August 11, 2010 - Published by group.bmj.com

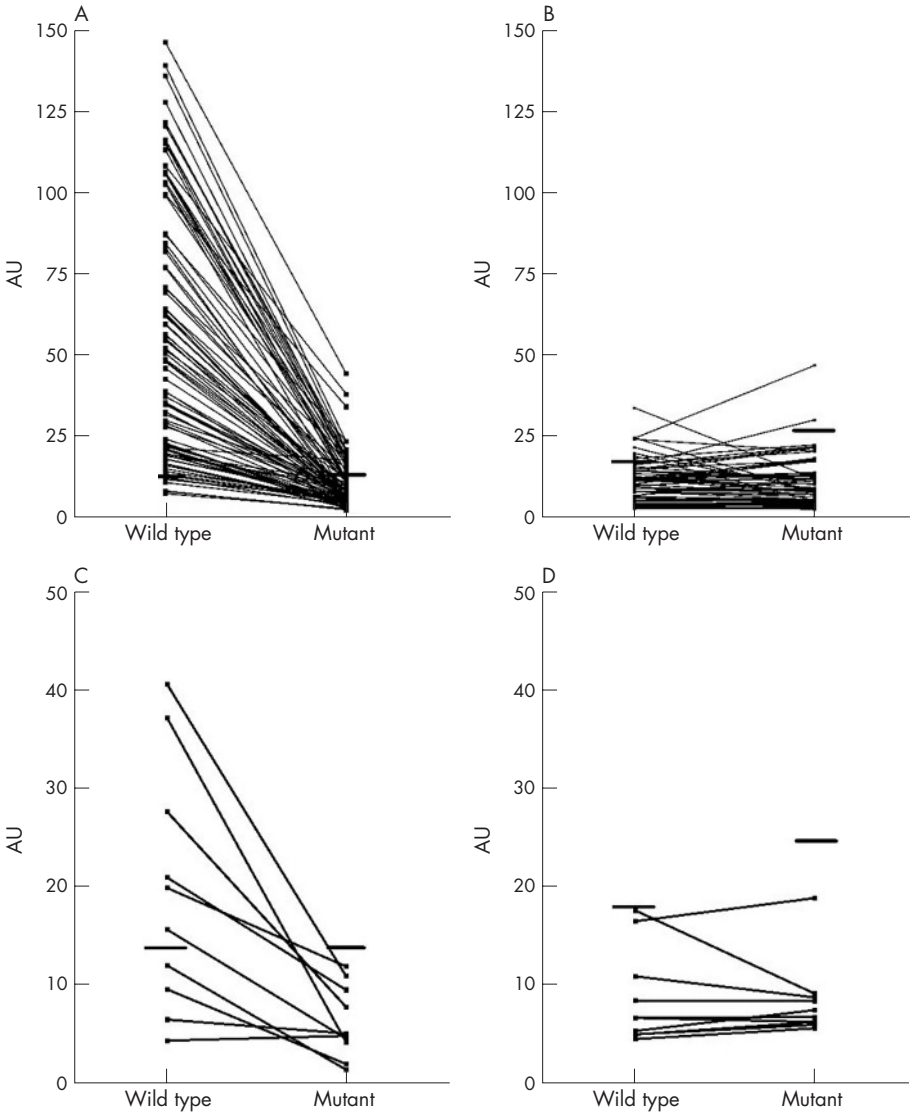

Figure 3 Coeliac disease serum IgA (n = 76) (a) and IgG (n = 64) (b) were analysed for their ability to bind wild-type and mutant tTGs. Dermatitis herpetiformis serum IgA (c) and IgG (d) were also analysed. Cut-off points are represented by bold lines.

anti-tTG autoantibody component of this multifactorial disease. In this study, evidence for the highly specific targeting of the active site of tTG by IgA autoantibodies has been discovered, whereas IgG autoantibodies seem unaffected by mutagenesis of this catalytic triad. These findings suggest that anti-tTG autoantibodies of mucosal origin could, at least partly, inhibit tTG activity. These results help further dissect the role of the autoantibody response against tTG in coeliac disease. Future work could involve the investigation of the inhibitory capacity of coeliac disease IgA anti-tTG on the cross-linking function of tTG.

Authors' affiliations

G Byrne, J Jackson, C Feighery, J Kelly, Department of Immunology, St James's Hospital, James's St, Dublin 8, Ireland
G Byrne, F Ryan, J Kelly, School of Biological Sciences, Dublin Institute of Technology, Kevin St, Dublin 8, Ireland

Competing interest: none declared

REFERENCES

1 **Griffin M**, Casadio R, Bergamini CM. Transglutaminases: nature's biological glues. *Biochem J* 2002;**368**:377–96.
2 **Fesus L**, Thomazy V, Falus A. Induction and activation of tissue transglutaminase during programmed cell death. *FEBS Lett* 1987;**224**:104–8.
3 **Gaudry CA**, Verderio E, Aeschlimann D, *et al*. Cell surface localization of tissue transglutaminase is dependent on a fibronectin-binding site in its N-terminal beta-sandwich domain. *J Biol Chem* 1999;**274**:30707–14.
4 **Balklava Z**, Verderio E, Collighan R, *et al*. Analysis of tissue transglutaminase function in the migration of Swiss 3T3 fibroblasts: the active-state conformation of the enzyme does not affect cell motility but is important for its secretion. *J Biol Chem* 2002;**277**:16567–75.
5 **Gaudry CA**, Verderio E, Jones RA, *et al*. Tissue transglutaminase is an important player at the surface of human endothelial cells: evidence for its externalization and its colocalization with the beta(1) integrin. *Exp Cell Res* 1999;**252**:104–13.
6 **Verderio EA**, Johnson T, Griffin M. Tissue transglutaminase in normal and abnormal wound healing: review article. *Amino Acids* 2004;**26**:387–404.
7 **Mohan K**, Pinto D, Issekutz TB. Identification of tissue transglutaminase as a novel molecule involved in human CD8+ T cell transendothelial migration. *J Immunol* 2003;**171**:3179–86.
8 **Iismaa SE**, Chung L, Wu MJ, *et al*. The core domain of the tissue transglutaminase Gh hydrolyzes GTP and ATP. *Biochemistry* 1997;**36**:11655–64.
9 **Mhaouty-Kodja S**. Ghalpha/tissue transglutaminase 2: an emerging G protein in signal transduction. *Biol Cell* 2004;**96**:363–7.

Downloaded from gut.bmj.com on August 11, 2010 - Published by group.bmj.com

10 **Dieterich W**, Ehnis T, Bauer M, *et al*. Identification of tissue transglutaminase as the autoantigen of celiac disease. *Nat Med* 1997;**3**:797–801.
11 **Sollid LM**. Molecular basis of celiac disease. *Annu Rev Immunol* 2000;**18**:53–81.
12 **Fasano A**. Clinical presentation of celiac disease in the pediatric population. *Gastroenterology* 2005;**128**:S68–73.
13 **Vader LW**, de Ru A, van der Wal Y, *et al*. Specificity of tissue transglutaminase explains cereal toxicity in celiac disease. *J Exp Med* 2002;**195**:643–9.
14 **Collin P**, Kaukinen K, Vogelsang H, *et al*. Antiendomysial and antihuman recombinant tissue transglutaminase antibodies in the diagnosis of coeliac disease: a biopsy-proven European multicentre study. *Eur J Gastroenterol Hepatol* 2005;**17**:85–91.
15 **Nicolas ME**, Krause PK, Gibson LE, *et al*. Dermatitis herpetiformis. *Int J Dermatol* 2003;**42**:588–600.
16 **Koop I**, Ilchmann R, Izzi L, *et al*. Detection of autoantibodies against tissue transglutaminase in patients with celiac disease and dermatitis herpetiformis. *Am J Gastroenterol* 2000;**95**:2009–14.
17 **Sardy M**, Karpati S, Merkl B, *et al*. Epidermal transglutaminase (TGase 3) is the autoantigen of dermatitis herpetiformis. *J Exp Med* 2002;**195**:747–57.
18 **Sblattero D**, Florian F, Azzoni E, *et al*. The analysis of the fine specificity of celiac disease antibodies using tissue transglutaminase fragments. *Eur J Biochem* 2002;**269**:5175–81.
19 **Mariani P**, Carsughi F, Spinozzi F, *et al*. Ligand-induced conformational changes in tissue transglutaminase: Monte Carlo analysis of small-angle scattering data. *Biophys J* 2000;**78**:3240–51.
20 **Roth EB**, Sjoberg K, Stenberg P. Biochemical and immuno-pathological aspects of tissue transglutaminase in coeliac disease. *Autoimmunity* 2003;**36**:221–6.
21 **Jones LJ**, Haugland RP, Singer VL. Development and characterization of the NanoOrange protein quantitation assay: a fluorescence-based assay of proteins in solution. *Biotechniques*. 2003;34: 850–4, 856, 858).
22 **Smyth DR**, Mrozkiewicz MK, McGrath WJ, *et al*. Crystal structures of fusion proteins with large-affinity tags. *Protein Sci* 2003;**12**:1313–22.
23 **Gentile V**, Saydak M, Chiocca EA, *et al*. Isolation and characterization of cDNA clones to mouse macrophage and human endothelial cell tissue transglutaminases. *J Biol Chem* 1991;**266**:478–83.
24 **Halttunen T**, Maki M. Serum immunoglobulin A from patients with celiac disease inhibits human T84 intestinal crypt epithelial cell differentiation. *Gastroenterology* 1999;**116**:566–72.
25 **Feighery L**, Collins C, *et al*. Anti-transglutaminase antibodies and the serological diagnosis of coeliac disease. *Br J Biomed Sci* 2003;**60**:14–8.
26 **Kelley LA**, MacCallum RM, *et al*. Enhanced genome annotation using structural profiles in the program 3D-PSSM. *J Mol Biol* 2000;**299**:499–520.
27 **Marzari R**, Sblattero D, Florian F, *et al*. Molecular dissection of the tissue transglutaminase autoantibody response in celiac disease. *J Immunol* 2001;**166**:4170–6.
28 **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* 2002;**51**:177–181.
29 **Dieterich W**, Trapp D, Esslinger B, *et al*. Autoantibodies of patients with coeliac disease are insufficient to block tissue transglutaminase activity. *Gut* 2003;**52**:1562–6.
30 **Janeway CA**, Travers P, Walport M, *et al*. *Immunobiology*. New York: Churchill Livingstone, 2004:110–5.
31 **van der Geld YM**, Tool AT, *et al*. Interference of PR3-ANCA with the enzymatic activity of PR3: differences in patients during active disease or remission of Wegener's granulomatosis. *Clin Exp Immunol* 2002;**129**:562–70.
32 **Burman P**, Mardh S, *et al*. Parietal cell antibodies in pernicious anemia inhibit H+, K+-adenosine triphosphatase, the proton pump of the stomach. *Gastroenterology* 1989;**96**:1434–8.
33 **Barnard JA**, Beauchamp RD, Coffey RJ, *et al*. Regulation of intestinal epithelial cell growth by transforming growth factor type beta. *Proc Natl Acad Sci U S A* 1989;**86**:1578–82.
34 **Lenhardt A**, Plebani A, Marchetti F, *et al*. Role of human-tissue transglutaminase IgG and anti-gliadin IgG antibodies in the diagnosis of coeliac disease in patients with selective immunoglobulin A deficiency. *Dig Liver Dis* 2004;**36**:730–4.

BNF for Children 2006, second annual edition

In a single resource:
- guidance on drug management of common childhood conditions
- hands-on information on prescribing, monitoring and administering medicines to children
- comprehensive guidance covering neonates to adolescents

For more information please go to **bnfc.org**