SCIENCE ALERT

Dietary modifications: food dependent autoimmunity in coeliac disease

Molberg O, Mcadam SN, Korner R, *et al*. Tissue transglutaminase selectively modifies gliadin peptides that are recognized by gut-derived T cells in celiac disease. *Nat Med* 1998;**4**:713–17.

Abstract

The action of tissue transglutaminase (TGase) on specific protein-bound glutamine residues plays a critical role in numerous biological processes. Here we provide evidence for a new role of this enzyme in the common, HLA-DQ2 (and DQ8) associated enteropathy, celiac disease (CD). The intestinal inflammation in CD is precipitated by exposure to wheat gliadin in the diet and is associated with increased mucosal activity of TGase. This enzyme has also been identified as the main target for CD-associated anti-endomysium autoantibodies, and is known to accept gliadin as one of its few substrates. We have examined the possibility that TGase could be involved in modulating the reactivity of gliadin specific T cells. This could establish a link between previous reports of the role of TGase in CD and the prevailing view of CD as a T-cell mediated disorder. We found a specific effect of TGase on T-cell recognition of gliadin. This effect was limited to gliadin-specific T cells isolated from intestinal CD lesions. We demonstrate that TGase mediates its effect through an ordered and specific deamidation of gliadins. This deamidation creates an epitope that binds efficiently to DQ2 and is recognized by gut-derived T cells. Generation of epitopes by enzymatic modification is a new mechanism that may be relevant for breaking of tolerance and initiation of autoimmune disease.

Comment

Coeliac disease has long been known to reflect an antigen specific immune response to the gliadin component of wheat protein. Although the exact nature of this response has been disputed, most observers consider that CD4+ T cell mediated immunity is the critical event, with production of cytokines and related effector functions being most important.[1 2] This view is supported by the intense infiltration of the mucosa by activated T cells and by the fact that coeliac disease is strongly associated with certain class II HLA haplotypes, particularly HLA-DQ2.[3 4] In addition, experimental models which reproduce the characteristic pattern of villus atrophy and crypt hyperplasia are dependent on activated CD4+ T cells and cytokine production.[5–8]

Nevertheless, it has always been unclear how an antigen specific immune response directed at a component of the diet can stimulate such severe damage to the tissues of the intestine itself. The assumption that the enteropathy is a direct effect of gliadin specific CD4+ T cells is also challenged by the apparently specific association between coeliac pathology and IgA autoantibodies directed at endomysial antigens. These findings have been interpreted as support for an antibody dependent immunopathology.[9–11] Recent studies have taken this idea further, by showing that the endomysial autoantibodies are directed at the tissue transglutaminase (tTG) enzyme.[12] However, it has been unclear how this unusual pattern of autoreactivity against tissue components can be reconciled with the knowledge that the disease is entirely dependent on exposure to a specific, exogenous antigen. This paradox has now been addressed by a recent paper suggesting that deamidation of gliadin by tTG produces HLA-DQ2 binding peptide epitopes which combine with the tTG enzyme, creating novel antigenic determinants that stimulate an autoantibody response against tTG.

Molberg *et al* show that gliadin specific CD4+ T cell clones derived from the intestinal mucosa of coeliac patients respond better to gliadin in vitro in the presence of tTG. Prior deamidation of gliadin with tTG or by low pH and heat also rendered it more immunogenic for these T cells. The authors conclude that this enzymatic process is in fact crucial for recognition of gliadin by T cells. A molecular solution for the effect is suggested by subsequent experiments in the paper, which show that peptide 134–153 of gliadin can also be deamidated by tTG and that the resulting modified peptide binds much more efficiently to purified HLA-DQ2 molecules in vitro. The authors have found in other studies that this peptide is the dominant epitope recognised by HLA-DQ2 and HLA-DQ8 restricted, gliadin specific T cells and show that the critical modification induced by tTG is deamidation of Gln148. This enables the peptide to bind to one of the main binding pockets in the HLA-DQ molecules, where there is a strong preference for peptides containing a negatively charged amino acid in the appropriate position such as deamidated Gln.[13–15]

At least two major insights into the pathogenesis of coeliac disease are offered by the work of Molberg *et al*. Firstly, it suggests that a tissue enzyme can modify exogenous antigen such that it is recognised more efficiently by CD4+ T cells. tTG seems to be upregulated in inflammatory sites such as coeliac mucosa,[16] raising the possibility that intestinal infection or other local insults could predispose to the development of gluten sensitivity. This would explain older anecdotal evidence that infection may precede the onset of coeliac disease. Interestingly, postranslationally modified epitopes, perhaps generated in response to inflammation, have also been implicated as T cell targets in other autoimmune disorders such as rheumatoid arthritis.[17]

The second important aspect of the findings is that they may help explain the development of tTG specific autoantibodies in coeliac disease. In addition to binding HLA-DQ2 molecules, it seems that the modified peptide also forms a stable complex with the enzyme itself. The

authors suggest that the tTG–peptide complex then acts as a hapten carrier moiety, with the gliadin peptide being recognised as a carrier determinant by CD4+ T cells which can then provide cognate help for tTG specific autoreactive B cells. Again, this idea is consistent with other autoimmune conditions in which T cells specific for a foreign or modified self antigen can provide help for otherwise unresponsive autoreactive B cells. In addition, it explains the apparent paradox that the production of IgA anti-tTG antibodies is strictly dependent on the presence of gliadin in the diet.

Molberg *et al*'s studies represent a substantial and novel advance in our thinking about coeliac disease, but leave several questions unanswered. Most importantly, it remains unclear whether the tTG specific autoantibodies play a causal role in the intestinal pathology. As tTG appears to play an important role in crosslinking components of the extracellular matrix,[18 19] it seems possible that antibodies directed at the enzyme might interfere with the interactions between mesenchymal cells and the gut epithelium, normally so crucial for many aspects of mucosal architecture and behaviour. This would be consistent with a recent report that IgA anti-tTG antibodies interfere with mesenchyme dependent enterocyte differentiation in vivo.[20] Nevertheless, it should be noted that tTG activity is frequently associated with tissue repair and remodelling,[21 22] suggesting that its overexpression in coeliac disease may be secondary to ongoing inflammation and that the resulting autoantibodies are epiphenomena. This issue may be resolved by determining how tTG activity correlates with the presence of pathology in response to gluten withdrawal and challenge, and if abnormalities in tTG expression are found in other forms of intestinal inflammation.

It would also be informative to examine how the in vitro findings reported by Molberg *et al* can be extrapolated to the in vivo situation. Thus it is not yet fully clear where tTG is expressed in the intestine and whether the levels found under normal or inflamed conditions can approach those required for the gliadin deamidation reactions described in vitro. In addition, although the authors report that tTG is expressed by mononuclear cells in the lamina propria of coeliac mucosa, it is a ubiquitous enzyme with many substrates and most food proteins gain access to the systemic circulation. It therefore seems curious that an autoimmune response directed at tTG would produce pathology restricted to the upper small intestine and only in response to gliadin. The authors tackle this partly by citing their findings that only mucosal CD4+ T cells recognise tTG modified gliadin peptide(s). However, this in itself creates theoretical problems, as there has never been evidence previously that the repertoire of lamina propria CD4+ T cells is substantially different from that of T cells elsewhere in the body. This is clearly one prediction from the current work which warrants further study. Finally, the elegant insights into the HLA restriction of coeliac disease provided by the authors' studies using tTG modified peptide need to be confirmed by direct modelling of the HLA-DQ2/DQ8–peptide complexes. These alleles are expressed commonly in the normal population, many of whom presumably generate appropriate gliadin peptides, yet do not develop coeliac disease. This enigma has long complicated our understanding of autoimmune disease, but the results of Molberg *et al* point the way to novel studies of the interactions between HLA determined genetic predisposition, tissue enzyme activity, exposure to foreign antigen, and the development of immunopathology.

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