

Rapid review

Coeliac disease—a meeting point for genetics, immunology, and protein chemistry

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Context Coeliac disease is caused by a genetically determined, specific immune response to antigens present in wheat gluten. This immune response may be focused on a limited region of the α gliadin component of gluten, and previous studies have suggested that the generation of epitopes for recognition by CD4+ T cells requires deamidation of the protein by tissue transglutaminase. However, it had not previously been shown that candidate epitope peptides could be generated from gluten in vivo, or that these epitopes were selective products of physiological digestion of gluten by tissue transglutaminase.

Starting Point Lu Shan and colleagues (*Science* 2002; **297**: 2275–79) have recently shown that a 33-mer peptide containing known peptide epitopes is generated by digestion with intestinal enzymes in vivo and in vitro, producing a highly stimulatory antigen for CD4+ T cells. The resulting peptide is resistant to further digestion of a gliadin by intestinal brush border enzymes and is a highly specific substrate for deamidation by tissue transglutaminase. Because the 33-mer peptide is not present in cereal proteins that do not cause coeliac disease, Shan and colleagues suggest that generation of this peptide in vivo underlies the specific association between gluten, immune responsiveness, and tissue transglutaminase in coeliac disease. In addition, the 33-mer peptide can also be produced by digestion of gluten by bacterial prolyl endopeptidases, suggesting possible future strategies for generating non-toxic varieties of gluten.

Where next? It is now important to determine whether this 33-mer peptide and this pathway accounts for all immune recognition of wheat gluten in coeliac disease, and to explore if the tissue transglutaminase homologues found in other organisms can be used to produce non-toxic preparations of wheat.

Coeliac disease is one of the commonest immunological diseases, with a prevalence of up to 1% in many Western societies. It is also one of the few such conditions in which both the genetic predisposition and the precipitating antigen are understood to any extent. Disease is entirely dependent on the presence of gluten in the diet and more than 95% patients are *HLA-DQ2* or *HLA-DQ8* positive.^{1,2} The only treatment currently available is lifelong adherence to a gluten-free diet, which is both expensive and of limited palatability. It has recently become clear that coeliac disease is much more prevalent than previously thought³ and there has been an upsurge in research into pathogenesis of the disease. Most of these studies have concentrated on the basis of the immune recognition of the α -gliadin component of gluten by T lymphocytes, but a recent study by Lu Shan and colleagues³ of the structural chemistry of α gliadin has provided novel insights into how this ubiquitous dietary protein can provoke intestinal inflammation in susceptible individuals.

Immune recognition of α gliadin

In most populations, up to 97% of patients with coeliac disease express the *HLA-DQ2* or *HLA-DQ8* genes, coding for a class II MHC molecule comprising a DQA1*0501 chain with a DQB1*0201 or DRB1*0301 chain, or DQA1*0301 with a DRB1*0401 or DQB1*0302 chain.^{1,2} Because the function of class II MHC molecules is to present short peptide-antigens (epitopes) to CD4+ T lymphocytes, this is one of the most important pieces of evidence in favour of the long-held view that coeliac disease represents an aberrant immune response to gluten. Further support for this idea is that α -gliadin-specific CD4+ T cells that produce interferon γ can be isolated from the intestinal mucosa of untreated patients with coeliac disease. Such T cells are not present in normal intestine, or in patients with coeliac disease on a gluten-free diet.⁴ The nature of the antigen that stimulates this T-cell response has been the subject of considerable research for many years and several candidate peptides derived from α gliadin have been suggested from in-vitro studies. A major breakthrough came in 2000, with the publication of two independent studies which used different experimental approaches to identify immunodominant epitope peptides from the 57–75 region of α gliadin.^{5,6} Together with a later report,⁷ these experiments suggested that a large proportion of CD4+ T cells from patients with coeliac disease recognised three overlapping peptides, rich in proline and glutamine (PFPQPQLPY, PQPQLPYPQ, and PYPQPQLPY), which derive from a region of α gliadin known to be recognised by antibodies found in patients with coeliac disease.

Role of tissue transglutaminase

Autoantibodies against tissue transglutaminase are pathognomonic of coeliac disease and each of the three peptide epitopes identified contains at least one glutamine

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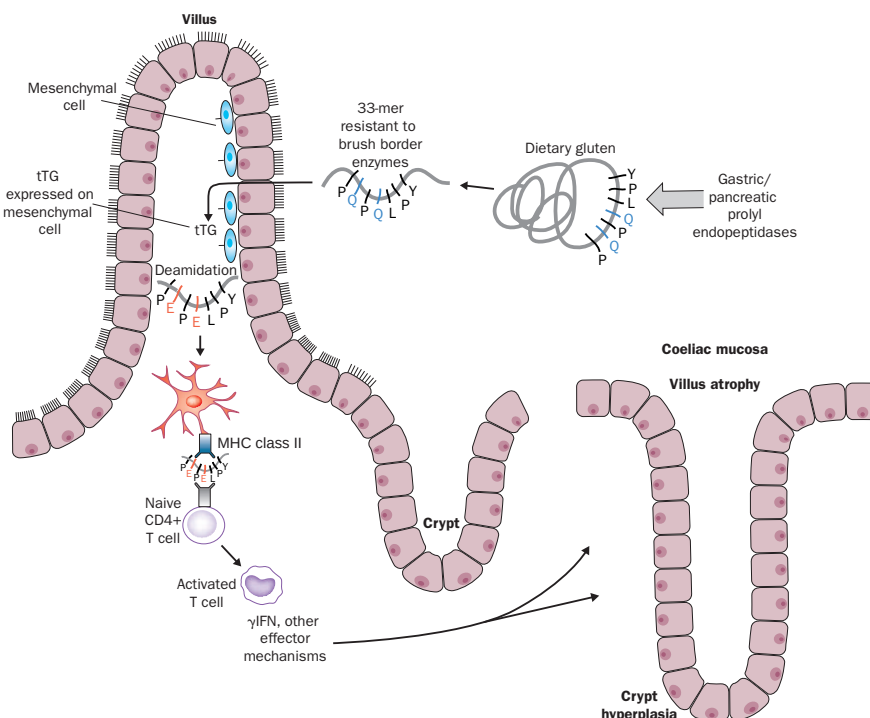
residue which is a substrate for the deamidase activity of tissue transglutaminase. Deamidation of these residues is essential for significant T-cell stimulation, because deamidation exposes negatively charged aminoacids that are an essential part of the structural motif involved in binding to the HLA-DQ2 molecule.⁵⁻⁹ Thus the idea has emerged that tissue transglutaminase plays a critical role in the pathogenesis of coeliac disease by generating the antigenic epitopes present in α gliadin.

Although these studies⁵⁻⁹ provided in-vitro evidence that tissue transglutaminase could modify the antigenicity of α gliadin, the question still remained of whether these epitopes could be produced in vivo. The recent report by Lu Shan and colleagues³ shows that digestion of recombinant $\alpha 2$ gliadin with gastric and pancreatic enzymes in vitro produces a highly stable 33-mer peptide that is rich in proline and glutamine and contains all three previously described T-cell epitopes. This 33-mer peptide is resistant to in-vitro digestion with preparations of brush-border enzymes derived from the small intestine of rats or human beings.

Importantly, the 33-mer peptide is also resistant to proteolysis when perfused into intact gut from the rat, whereas other proteins, including shorter peptides from the 33-mer peptide, are almost completely destroyed by these processes. Taken together, these results indicate that this 33-mer peptide has a selective ability to survive digestion in vivo and may be present in significant amounts in the normal small intestine. Shan and colleagues go on to demonstrate that the 33-mer product has the highest specificity yet recorded for deamidation by tissue transglutaminase and the resulting products are extremely stimulatory for all the HLA-DQ2-restricted α -gliadin-specific T cells they examined, with higher activities than even the purified epitope peptides. Thus a combination of digestion by gastric and pancreatic enzymes, followed by deamidation by tissue transglutaminase (present in the subepithelial layer of the gut) may produce peptide epitopes that can be recognised specifically by HLA-DQ2-restricted T cells and provoke immunopathology in the mucosa of susceptible individuals (figure).

Implications for pathogenesis and therapy

Experiments done over the past 2 years address several important issues in coeliac disease. First and most obviously, they may explain the antigen selectivity of the disease. Although other food proteins such as those in cow's milk and in soya can produce immunologically mediated enteropathies similar to that found in coeliac disease, these conditions are transient and rare.¹⁰ It now appears that gluten is almost unique among dietary proteins in its ability to generate digestion-resistant substrates for tissue transglutaminase, which after



Hypothetical scheme for interaction between intestinal protein processing and the specific immune system in coeliac disease

Dietary gluten is exposed to intraluminal digestive enzymes (including prolyl endopeptidases) producing stable 33-mer peptide containing overlapping proline-rich epitopes with PQQQLPY central motif. 33-mer peptide is absorbed intact by transcellular or paracellular routes into lamina propria and exposure to TTG results in deamidation of glutamine residues to glutamic acid. Modified peptide is presented to specific CD4+ T cells in association with HLA-DQ2 or DQ8. Activated IFN- γ -producing T-effector cells return to mucosa and produce characteristic villus atrophy and crypt hyperplasia.

TTG=tissue transglutaminase, IFN=interferon.

deamidation, can be loaded onto a human class II MHC molecule and so be recognised by CD4+ T cells. The studies by Shan and colleagues are also consistent with the observed clinical toxicity of different food grains in coeliac disease patients. Shan and colleagues searched peptide databases and found homologues of the 33-mer peptide only in wheat, barley, and rye, all of which can provoke coeliac disease. By contrast, rice, oats, and maize do not contain a homologous region and are not toxic in patients with coeliac disease.

An additional and crucial insight provided by the most recent work relates to the development of novel therapies for coeliac disease. After the discovery of the specific epitope peptides in the 57–75 region of a gliadin,⁵⁻⁷ it was suggested that these could be used to reinstate specific immune tolerance in patients with coeliac disease, or that non-toxic wheat could be produced by removing the appropriate sequences by genetic modification. However, antigen-specific immunotherapy with peptide epitopes is difficult even in animal models of systemic disease, due to problems of bioavailability. Shan and colleagues show that the small peptide-epitopes defined in previous studies of coeliac disease are readily degraded in the gut and so presumably would be of little use in vivo. However the digestion-resistant 33-mer peptide identified in this recent work could be more useful in this respect.

Genetic modification of wheat proteins may also not be an ideal approach, even if current ethical issues about genetically modified foods can be overcome. Wheat contains a large number of different α gliadins, and several additional proteins in wheat have toxic activity in coeliac disease, or contain immunostimulatory T-cell

epitopes.¹¹ Thus it may be necessary to modify a large number of different genes to create a genuinely non-toxic variety of wheat. However, Shan and colleagues show that the 33-mer α -gliadin peptide is exquisitely sensitive to digestion in vitro and in vivo by a prolyl endopeptidase derived from a flavobacterium. Tissue transglutaminase is unable to generate immunogenic epitopes from the 33-mer peptide after digestion of the peptide by this bacterial enzyme. On the grounds that the only epitopes which will be able to induce pathological T-cell responses in the gut are those that show this unique pattern of resistance to conventional digestion and sensitivity to tissue transglutaminase, it has been suggested that all such epitopes be removed from any dietary protein with specific prolyl endopeptidases of this kind.

Future directions

Despite these major advances in understanding the pathogenesis of coeliac disease at the molecular level, some important questions remain to be answered. One of the most important will be to determine if the model of α -gliadin recognition described above can account for all immune responsiveness in coeliac disease. Although several studies now support the view that only a few peptides dependent on tissue transglutaminase dominate the T-cell response, this idea remains contentious and several epitopes that do not come from the 33-mer peptide have been shown to stimulate T cells from patients with coeliac disease.^{8,9,11,12} Indeed, work by Koning's group suggests that such epitopes may account for a substantial proportion of T-cell stimulation in children with coeliac disease and that some of these epitopes are derived from other wheat proteins such as γ gliadin and glutenin.^{9,11,12} Therefore the immune response to gluten could be much more complex than is suggested by the studies of the tissue-transglutaminase-sensitive 33-mer, and direct comparison of the ability of the different epitopes to be recognised by T cells and to stimulate intestinal pathology in vivo is now essential.

Because coeliac disease occurs in only a small proportion of the 20–30% of the population that express HLA-DQ2 or HLA-DQ8, and HLA genotype contributes only a proportion of the greater than 75% concordance rate in monozygotic twins,¹³ additional factors clearly contribute to the development of the disease. Work in animals and in unaffected human beings indicates that immune recognition of a dietary antigen is not in itself sufficient to induce local inflammation, because the normal immune system becomes specifically unresponsive (tolerant) after such exposure.¹⁴ It is generally believed that disorders like coeliac disease can only occur when this homeostatic process breaks down. This lack of immune tolerance could occur due to genetic polymorphisms in any of the non-MHC molecules involved in the induction, regulation, or expression of mucosal immune responses. Additionally, the studies by Shan and colleagues suggest that genes which control intestinal enzyme activity would be interesting candidates for analysis, although it should be noted that polymorphisms in tissue transglutaminase itself do not appear to play a role.¹⁵ Lastly, environmental factors may play an important role in triggering overt disease in genetically predisposed individuals. Normal immune tolerance to dietary proteins appears to reflect

the fact that such antigens do not stimulate the innate immune system and are presented to naive CD4+ T cells by local antigen-presenting cells (dendritic cells) which are in an inactive or "quiescent" state. These inactive dendritic cells lack the full complement of costimulatory molecules necessary for effective activation of T cells.^{14,16} Sensitisation to gluten could therefore be triggered by any environmental factor which disturbs this homeostatic balance, including the release of inflammatory mediators (so-called "danger signals") or other factors released during local infection, that then activate dendritic cells fully or alter other components of the local microenvironment.¹⁷ Alternatively, such events may influence the activity of tissue transglutaminase or of the other digestive enzymes now predicted to be crucial to the generation of stimulatory epitopes from gliadin.

The interactions between genetics, the environment, and processing of intestinal protein will undoubtedly be the focus of research in coeliac disease in future years.

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