Occasional viewpoint

Coeliac disease: it takes three to tango!

Coeliac disease (CD), the most common food sensitive enteropathy in humans, is caused by permanent intolerance for dietary gluten.[1 2] Typical symptoms include chronic diarrhoea, abdominal distension, and failure to thrive. These symptoms are the result of a lesion in the upper small bowel characterised by (sub)total villous atrophy, hypertrophic crypts, an increased number of interepithelial lymphocytes, and a chronic inflammatory response of the lamina propria lymphocytes. IgA antibodies against endomysium are specific indicators of CD.[3 4] Yet CD is considered to be primarily a T cell mediated disease because the majority of patients express the HLA-DQ2 [DQ(α1*0501, β1*02)], and/or −DQ8 [DQ(α1*03, β1*0302)] molecules[5 6]; gluten specific HLA-DQ restricted T cells are present at the site of the lesion in the gut[7] and withdrawal of gluten stops the disease process.[2] The identity of potentially disease inducing, gluten derived T cell stimulatory peptides, however, was unknown until recently.

Here we briefly discuss recent work which sheds light on the requirements for an optimal interaction between gluten, HLA-DQ, and T cells, requirements which explain the association of CD with HLA-DQ2/8.

Specificity of the humoral immune response

CD patients generally have high serum levels of antigliadin antibodies. A more specific indicator for the disease, however, is the presence of antiendomysium antibodies.[3 4] Recently, these antibodies have been found to be specific for the enzyme tissue transglutaminase (tTG).[8] tTG is a ubiquitous protein that belongs to a family of structurally and functionally related calcium dependent enzymes. These enzymes cross link proteins by catalysing the formation of isopeptide bonds between γ carboxamide groups of glutamine residues and ε amino groups of lysine residues.[9 10] An important function of tTG is thought to be cross linking of extracellular matrix proteins which stabilises damaged tissue[10 11] but other activities have also been identified.[12–14] Identification of tTG as an autoantigen[8] has fuelled novel concepts about the pathogenesis of CD. Schuppan and colleagues[15] have suggested that anti-tTG antibodies could play a direct role in the pathogenesis of CD as tTG is required for activation of transforming growth factor β (TGF-β).[14] It was proposed that the observed lack of differentiation of villous epithelium in CD patients could be a result of an excess of locally produced anti-tTG antibodies as this would block tTG and thus activation of TGF-β required for epithelial differentiation. Others have suggested that presentation of tTG derived peptides could lead to activation of autoreactive T cells.[16] Finally, it has been suggested that because of the high glutamine content of gliadin, tTG could be involved in the generation of gliadin-tTG complexes thus giving rise to novel epitopes.[17] As discussed previously,[17] the latter hypothesis is particularly attractive as such novel epitopes could play a role in breaking oral tolerance to both tTG and gluten and thus explain the appearance of antiendomysium antibodies that are characteristic of CD. In addition, recent work has established a direct role for tTG in the induction and/or amplification of gluten specific T cell responses (see below).

Specificity of the cellular immune response

Virtually all CD patients express HLA-DQ2 or −DQ8 class II molecules. HLA class II molecules bind and present peptides derived from exogenous protein antigens to CD4+ T cells. These exogenous protein antigens are endocytosed by HLA class II positive antigen presenting cells and degraded in an intracellular endosomal/ lysosomal compartment where the resulting peptides associate with newly synthesised HLA class II molecules. These HLA class II-peptide complexes are subsequently transported to the cell surface for recognition by peptide specific T cells. Because CD is caused by an exogenous protein antigen and is linked to HLA-DQ2/8 expression, this has led to the hypothesis that T cells reactive with gluten peptides bound to HLA-DQ2 or −DQ8 molecules play a major role in disease development. HLA class II molecules bind peptides by making contact with several amino acid side chains within the bound peptide (fig 1A). It is now well established that each class II allele exhibits unique peptide binding characteristics which allow it to bind a unique set of peptides (fig 1A) (for a review see Rammensee and colleagues[18]). Consequently, the definition of such binding characteristics can be used to predict which peptide sequences fit the peptide binding groove and could thus serve as specific ligands for T cells. To facilitate the identification of T cell stimulatory gluten peptides, the peptide binding characteristics of the disease associated HLA-DQ2 and HLA-DQ8 alleles[5 6] have been determined. It was found that both DQ2[19–21] and DQ8[22 23] have a preference for negatively charged amino acids at several positions in the bound peptide. These were puzzling observations as negatively charged residues are extremely rare in gluten proteins. Initially, therefore, these results did not help in the identification of potential T cell stimulatory gluten peptides. Additional information, however, came from another approach: gluten specific HLA-DQ2 and DQ8 restricted T cell clones were generated from small intestinal biopsies of patients and these were used to characterise three T cell stimulatory gluten derived peptides.[24–26] One of these peptides is restricted by DQ2 and represents a fragment of γ gliadin (gdb02, residues 134–153)[25]; the other two peptides are restricted via DQ8 and are derived from α gliadin (gda09, residues 198–232)[24] and glutenin (glt04, residues 707–742).[26] The latter gliadin peptide is immunodominant in adult HLA-DQ8 positive patients.[24]

A direct role for tTG in CD

As mentioned, HLA-DQ2 and −DQ8 molecules have a preference for negatively charged residues at several positions in the bound peptides but such amino acids were not present in the native sequences of the identified T cell stimulatory gluten peptides. Several glutamines, however, were present in these peptides and these could potentially

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Abbreviations used in this paper: CD, coeliac disease; tTG, tissue transglutaminase; TGF-β, transforming growth factor β.
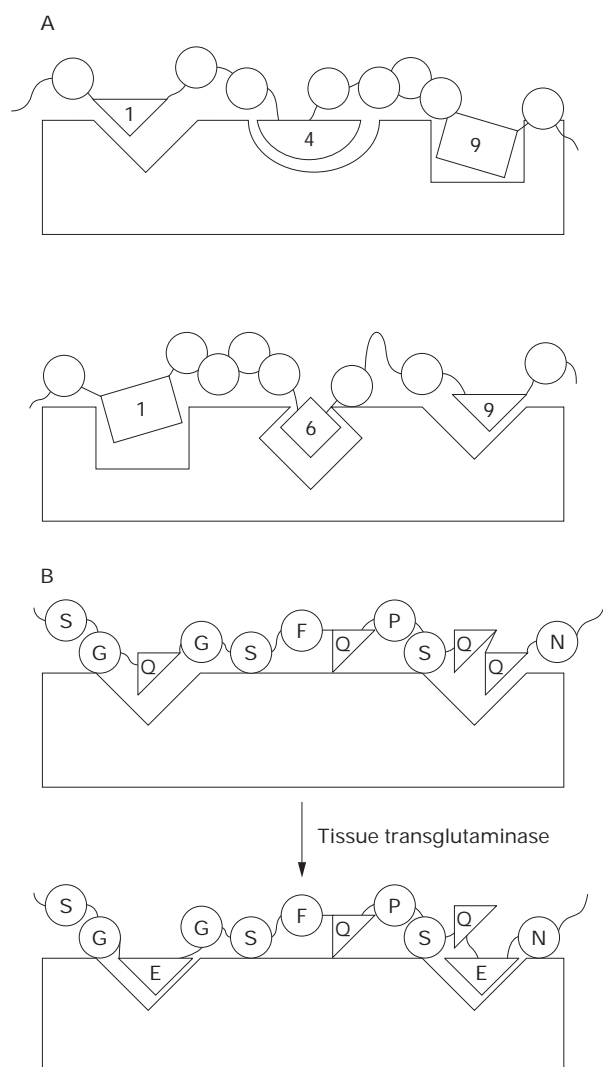
Figure 1   HLA class II peptide binding and effect of tissue transglutaminase (tTG). (A) HLA class II allele specific peptide binding. The side chains of particular amino acids in the class II bound peptide make contact with pockets in the class II molecule. The size, shape, and position of these pockets differ between class II alleles. As a result different class II alleles bind a different peptide repertoire. (B) Because of the activity of tTG, the glutamine (Q) residues at p1 and p9 in the HLA-DQ8 bound gliadin peptide are converted to glutamic acid (E) residues which fit tightly into the p1 and p9 pockets of the HLA-DQ8 molecule. The resulting higher binding affinity increases T cell reactivity.

be converted into glutamic acid (a process called deamidation) by either acid conditions[25] or glutaminase activity. Because it was known that tTG can deamidate glutamine residues,[27] attention focused on the possible involvement of tTG in the modification of gluten peptides. It was first investigated whether replacement of glutamines in the peptides by glutamic acid would influence T cell recognition. The results clearly demonstrated that such replacements strongly enhanced T cell reactivity: 50–100-fold less of the modified peptides was sufficient for induction of maximal T cell proliferation.[28 29] More importantly, it was found that tTG modified particular glutamine residues in gluten peptides and that in the majority of cases this resulted in strongly enhanced T cell reactivity.[28 29] Thus these results indicate that tTG modifies gluten peptides by conversion of glutamine residues to glutamic acid (fig 1B). As a result these peptides now have the appropriate negative charged amino acids for strong binding to

HLA-DQ molecules (fig 1B). This leads to enhanced T cell reactivity.

A model for CD triggering

Strikingly, these results indicate that tTG, the target for the disease associated humoral response in CD patients, has a direct and profound influence on the cellular immune response to gluten. This suggests two possible scenarios: modification of gluten by tTG may be required for initiation of a disease inducing gluten specific T cell response. Alternatively, the T cell response may first be directed to unmodified gluten and result in tissue damage. This in turn could lead to the release of (cytoplasmic) tTG and generation of more potent T cell stimulatory peptides, amplifying the already existing gluten specific T cell response (fig 2). Both these options are still open at present: one of the gliadin peptides is recognised only after deamidation[25 28] while the other is recognised in its native form but its recognition by T cells is strongly enhanced by tTG treatment.[24 29] There is clear evidence that tTG enhances the reactivity of the majority of gluten specific T cell clones tested so far[24 26 28 29] but it should be noted that these studies were carried out with T cell clones isolated from adult patients. While these observations strongly point to selection of T cell reactivity towards deamidated gluten peptides in adult patients, and thus to an important role of tTG in disease development, it is not yet known if the identified peptides are also involved in the *initiation* of the gluten specific T cell response. To evaluate the role of tTG in disease initiation it will be necessary to identify the nature of the disease *eliciting* peptides and to determine the effect of tTG on the recognition of these peptides. For this purpose we have now isolated HLA-DQ2 restricted, gluten specific T cell clones from biopsies of five recently diagnosed children, T cell clones that are directed to potentially disease *eliciting* peptides. Studies are in progress to determine the specificity of these T cell clones. We anticipate that these studies will shed light on the potential involvement of tTG in disease initiation.

Importantly, the recent studies offer an explanation for the strong association between the occurrence of CD and expression of HLA-DQ2/8. Gluten proteins are peculiar as they contain an unusual high content of glutamine residues (up to 40%). Whereas unmodified gluten peptides lack the proper anchor residues for high affinity binding to HLA-DQ2/8, deamidation by tTG results in generation of negatively charged amino acids at critical positions in the peptides such that they become high affinity ligands for HLA-DQ2 or −DQ8. The association between CD and HLA-DQ2/8 therefore appears to be solely due to the preference of these alleles to bind and present peptides with negatively charged anchor residues, and the likelihood that such peptides are generated by deamidation of the abundant glutamines in gluten. Thus there is now strong evidence that preferential presentation of deamidated gluten peptide antigens by HLA-DQ2 and −DQ8 is the primary mechanism underlying the association of CD with these HLA class II alleles.

Gluten derived T cell epitopes as a tool for immunotherapeutic intervention in CD?

A strict gluten free diet is necessary to prevent the clinical symptoms and histological abnormalities in the gut of coeliac patients. Some adult patients, however, fail to respond to a gluten free diet and immunosuppressive drugs are required to induce remission in these cases. Finally, CD patients have an increased risk of developing T cell lymphoma. For these reasons the development of
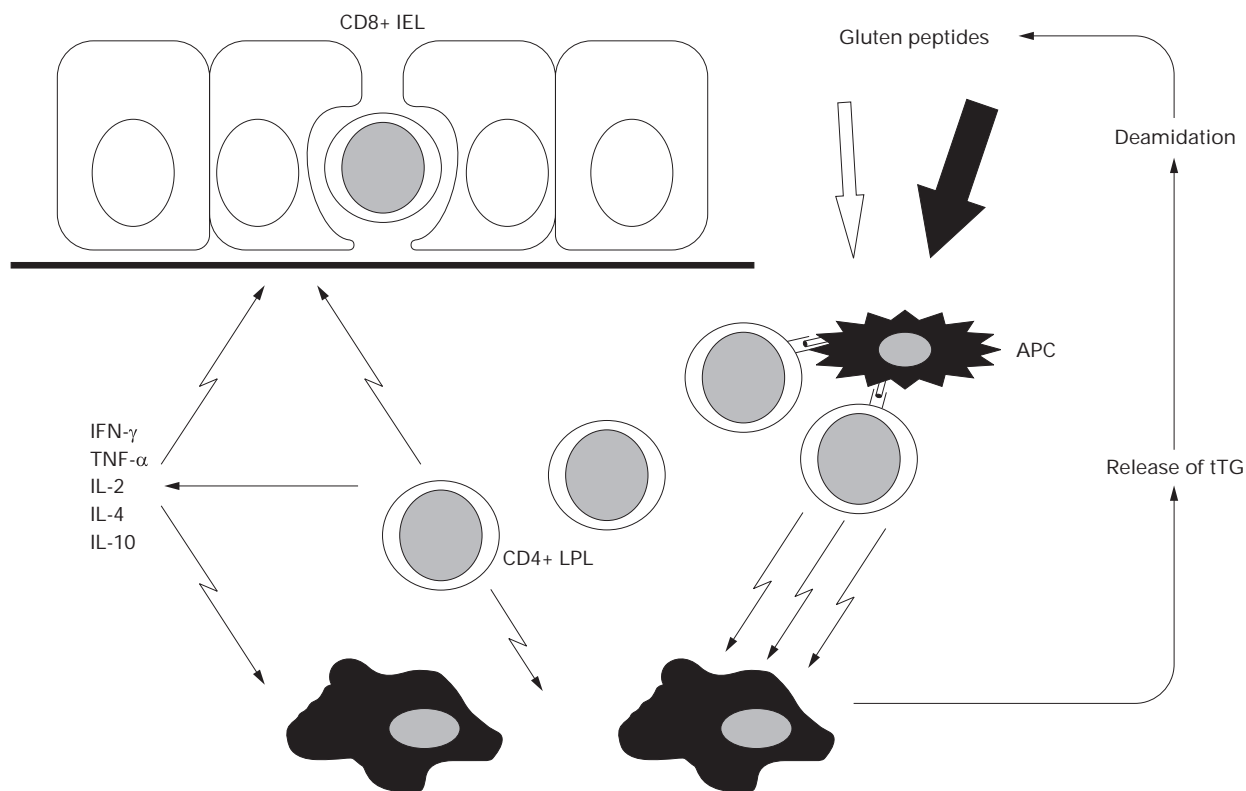
Figure 2 Transglutaminase (tTG) is involved in the initiation and/or amplification of the gluten specific T cell response. Gluten derived peptides are absorbed from the lumen of the gut and are subsequently presented to mucosal CD4+ T cells in the lamina propria (LPL). Secretion of a diverse set of inflammatory cytokines by these activated T cells could directly be involved in the destruction of the gut mucosa. Alternatively, the release of so-called secondary inflammatory mediators such as nitric oxide and metalloproteinases by macrophages and mesenchymal cells might be responsible for the coeliac lesion. On tissue damage, release of tTG takes place. tTG mediated deamidation of gluten increases T cell reactivity due to creation of ligands with a high binding affinity for DQ2/DQ8 molecules. Alternatively, as small amounts of tTG are present in the extracellular environment, the initial T cell activation might also be caused by the deamidated gluten antigens. APC, antigen presenting cell; IEL, intraepithelial lymphocyte.

alternative ways to interfere with or prevent the disease process is highly desirable.

Because of recent discoveries, a number of possible strategies can now be envisaged: construction of wheat deficient in T cell stimulatory peptides, induction of peptide specific T cell tolerance, and interference with tTG activity in the intestine. Based on the available data it seems that the T cell response in established CD patients is directed to multiple peptides[24 26 30] (unpublished observations). Moreover, one of the peptides identified is a repetitive sequence in glutenins.[26] If similar observations are made in recently diagnosed children this will make it extremely difficult to construct non-toxic wheat strains. The apparent heterogeneity of the gluten specific T cell response is also a formidable barrier when induction of tolerance is considered as a diverse array of T cell receptors with specificity for a number of peptides would all have to be tolerated. Finally, blocking the activity of tTG may have serious side affects as tTG is most likely involved in controlling tissue damage in the intestine. At present, therefore, these new insights offer little hope for patients. This may change when a detailed analysis of the gluten specific T cell response in children is completed and the nature of the *disease eliciting* peptides is determined. It is well established that after initiation of an antigen specific T cell response the repertoire broadens through a process called epitope spreading, for example a response to a limited number of peptides becomes a response to a much larger number of related peptides in time. Once the *disease eliciting* peptides have been identified, and these prove to be only a few, there may be possibilities to interfere. At the very least this may create possibilities to prevent disease induction in the future.

Concluding remarks

Considerable progress has been made in the identification of both T and B cell epitopes in CD. Elucidation of tTG as the target of antiendomysial antibodies and the observed effects of this enzyme on the gluten specific T cell response suggest a dual role for tTG in the aberrant immunological response in CD. Furthermore, a molecular explanation for the HLA disease association has been provided as tTG induced deamidation results in T cell stimulatory peptides that display high binding affinity for DQ2 and DQ8. Thus apart from HLA-DQ and gluten, tTG is required for the formation of highly antigenic complexes: it takes three to tango. Characterisation of additional potentially *disease eliciting* peptides can be expected in the near future and this may offer new possibilities for the prevention of CD or the development of alternative treatment modalities.

Clearly, our current knowledge does not explain why only a small percentage of HLA-DQ2 and −DQ8 positive individuals develop the disease. For this, other factors that contribute to disease development need to be determined. Interesting candidates are the stress induced MHC class I-like MICA/B antigens as recent results have demonstrated that these molecules specifically activate γδ T cells from the gut,[31] and the number of γδ T cells is increased in the small bowel of CD patients.[32 33] To further understand the gluten driven immunological processes in the gut of patients it may be necessary to study the interplay between the various subsets of T cells in the gut. In any case, an important part of the coeliac puzzle has now been resolved. As a result, CD is likely to become the first HLA associated disease for which the molecular basis will be fully unravelled.

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