

Celiac Disease: Caught Between a Rock and a Hard Place

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Celiac disease (CD) is an intestinal disorder caused by an intolerance to gluten, proteins in wheat. CD is an HLA-associated disease: virtually all patients express HLA-DQ2 or HLA-DQ8. Recent work has shown that these disease-predisposing HLA-DQ molecules bind enzymatically modified gluten peptides and these HLA-DQ peptide complexes trigger inflammatory T-cell responses in the small intestine that lead to disease. In addition, gluten induces innate immune responses that contribute to the tissue damage that is characteristic for CD. Thus, CD patients are caught between a rock and a hard place: the disease is caused by a combination of adaptive and innate immune responses that both are triggered by gluten. These findings explain the disease-inducing properties of gluten and provide valuable clues for the development of alternative treatment modalities for patients. They also may be of relevance for our understanding of other multifactorial disorders including IBD and HLA-associated autoimmune diseases.

In recent years it has become clear that celiac disease (CD) is far more common than previously thought. Population studies indicate that approximately .5% of the western European and Northern American populations suffer from CD.^{1–4} Because this corresponds to approximately 3 million patients, CD is one of the most common food intolerances known. There is a very strong genetic predisposition to disease development: concordance in monozygotic twins is 80%, although concordance is only 10% in dizygotic twins. Moreover, the vast majority of CD patients express HLA-DQ2 whereas HLA-DQ2–negative patients usually are HLA-DQ8 positive.^{5,6} These HLA-DQ molecules can bind and present gluten-derived peptides to T cells in the intestine.^{7–14} The peptides are generated by enzymatic degradation and modification of gluten in the gastrointestinal tract. The modification by the enzyme tissue transglutaminase (tTG) generates gluten peptides that bind with high affinity to HLA-DQ2 or HLA-DQ8, which explains the strong association between these HLA molecules and CD.^{15,16} In addition to HLA-DQ, other genes are likely to influence disease development. Next to the adaptive gluten-specific T-cell response, 2 other immune responses are set in motion: an antibody response to the

enzyme tTG¹⁷ and an innate response mediated by intraepithelial lymphocytes (IELs).^{18,19} Although the tTG-specific antibody response does not seem to play an important role in the disease process itself, the presence of such antibodies in serum is a highly specific indicator for the disease. Testing for the presence of these antibodies therefore has become an important diagnostic tool.²⁰ In contrast, the innate response contributes to tissue destruction. Gluten exposure leads to increased levels of the cytokine interleukin-15 (IL-15), which up-regulates NKG2D receptor expression on IELs and induces its ligand MICA on enterocytes. Subsequently, the interaction between NKG2D and MICA (MHC class I related A protein) leads to enterocyte killing.^{18,19} CD is thus a prime example of a multifactorial disease in which interactions between several genetic factors and the environment play a major role in disease development.

Wheat Gluten Causes CD

In 1950, Dicke²¹ found that wheat was responsible for causing CD symptoms, which later was attributed to the gluten proteins in wheat. Because of the widespread use of wheat, gluten is found in various food products including many that are not associated directly with wheat. Other cereals, including barley, rye, and oats, contain gluten-like molecules. Gluten is a mixture of gliadin and glutenin proteins. In turn, the gliadins can be subdivided into α/β -gliadins, γ -gliadins, and ω -gliadins, whereas the glutenins consist of low molecular weight and high molecular weight glutenins of which the latter are particularly important for the baking quality of dough.²² Commonly used wheat varieties are tetraploid (pasta wheat) and hexaploid (bread wheat) species that originate from natural hybridizations between diploid species thousands of years ago. Because of their polyploid nature and the presence of several allelic variants of all types of gluten genes in a single wheat variety,

Abbreviations used in this paper: APC, antigen-presenting cells; CD, celiac disease; IL, interleukin; MICA, MHC class I related A protein; tTG, tissue transglutaminase.

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gluten is a complex mixture of hundreds of related but distinct proteins.²²

Gluten Triggers Adaptive, T-Cell-Mediated Immune Responses

The large majority of CD patients express HLA-DQ2 and the remainder usually are HLA-DQ8 positive. HLA-DQ2 and -DQ8 are HLA class II molecules that bind and present peptides to CD4⁺ T cells. HLA-DQ molecules are heterodimers consisting of an α and a β chain. Many different HLA-DQ α and β chains exist that can combine in various combinations to form functional heterodimers. Although the HLA-DQ8 dimer can be formed by only 1 particular $\alpha\beta$ chain combination (α^*03 and β^*0302), HLA-DQ2 heterodimers can be formed by 5 combinations (Figure 1): α^*0501 can combine with the β^*0201 and β^*0202 chains, α^*0505 can combine with the β^*0201 and β^*0202 chains, and α^*0201 can combine with β^*0202 chain (Figure 1). Although there are small differences between these 3 α and 2 β chains the first 4 HLA-DQ2 dimers all would have identical peptide-binding properties. Because these are the HLA-DQ2 molecules that predispose to CD development,^{5,6,23,24} this indicates that the peptide-binding properties of HLA-DQ2 are linked directly to disease development. This was corroborated by the observation that HLA-DQ2- or HLA-DQ8-restricted T cells were present in small intestinal biopsy specimens of CD patients.^{7,8} In subsequent studies the T-cell stimulatory

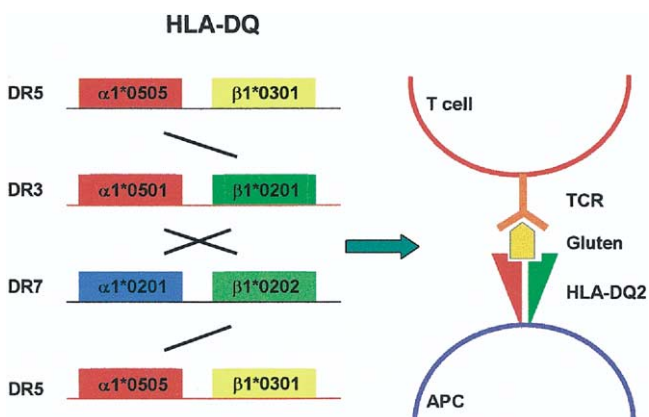


Figure 1. Interaction between the T-cell receptor on T cells and a gluten-derived peptide bound to HLA-DQ2 on APCs leads to T-cell activation. CD-predisposing HLA-DQ2 dimers can be formed in a number of ways. HLA-DQ expression is linked to HLA-DR expression as indicated. The $DQ\alpha^*0501$ and $DQ\alpha^*0505$ chains are very similar and so are the $DQ\beta^*0201$ and $DQ\beta^*0202$ chains. The other HLA-DQ chains shown are distinct. HLA-DQ2 dimers with identical peptide-binding properties thus can be formed by all red-green combinations. Accordingly, CD is observed most frequently in DR3+ and DR5/7+ positive individuals.

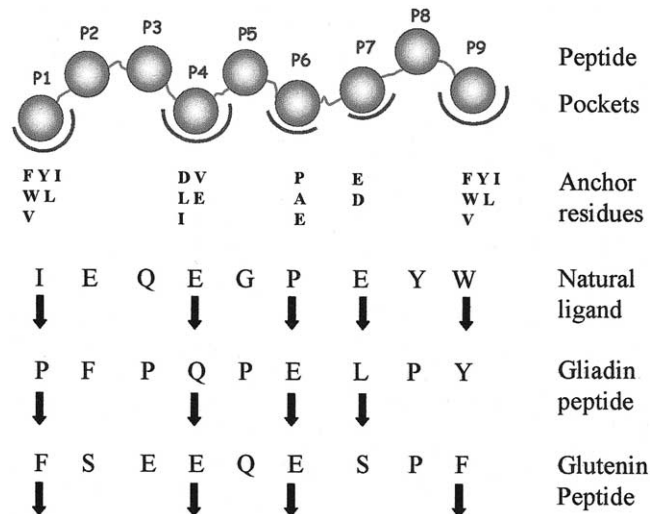


Figure 2. Binding of peptides to HLA-DQ2. HLA-DQ2 has a preference for particular amino acids (anchor residues) at 5 positions in bound peptides: at p1, p4, p6, p7, and p9. In a peptide that is present abundantly in HLA-DQ2 molecules (natural ligand), all 5 anchor residues are present. The HLA-DQ2-gliadin crystal structure indicates that only 4 anchors are used and that not all are optimal.¹⁴ Likewise, in the glutenin peptide only 4 anchors are used.⁶¹ The binding of peptide is facilitated by additional hydrogen bonds between HLA-DQ2 and the peptide backbone (not shown).

gluten peptides were characterized. A confounding factor in these studies was the observation that HLA-DQ2 and HLA-DQ8 have a preference for negatively charged amino acids in bound peptides (Figure 2),²⁵⁻²⁷ whereas gluten molecules hardly contain such amino acids.²² This issue was resolved when it was found that the enzyme tTG can convert glutamine residues in gluten peptides into glutamic acid, a negatively charged amino acid (Figure 3), facilitating gluten peptide binding to HLA-

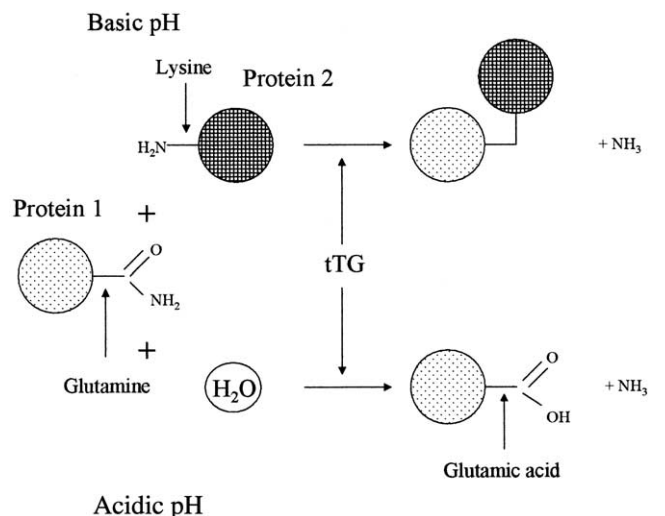


Figure 3. tTG activity either results in cross-linking of proteins by formation of a covalent bond between a glutamine in 1 protein to a lysine in another or to the conversion of glutamine into glutamic acid.

DQ2 (Figure 2) or HLA-DQ8 and subsequent T-cell recognition, both in vitro^{15,16} and in a biopsy culture system.²⁸

tTG is a ubiquitous, Ca²⁺-dependent enzyme that can cross-link extracellular proteins by the formation of a covalent bond between a glutamine residue in 1 protein to a lysine residue in another (Figure 3).²⁰ Intracellularly, tTG is inactive because the Ca²⁺ level is too low. On tissue damage, however, tTG is released and will cross-link extracellular matrix proteins because of the higher Ca²⁺ level, which helps to maintain tissue integrity. In the intestine, tTG is found not only intracellularly but also extracellularly, just below the epithelium and in the brush border.^{15,24} Although cross-linking is the main activity of tTG at a slightly basic pH level, deamidation is favored under the mild acidic conditions in the small intestine (Figure 3),²⁹ leading to the generation of a series of T-cell stimulatory gluten peptides.

T-cell stimulatory gluten-derived peptides have now been identified in the α/β gliadins, the γ gliadins, and the low and high molecular weight glutenins^{9–14,30–34} (Table 1) and patients usually have T cells to several of these peptides.³⁰ Moreover, because of the large diversity in gluten proteins, natural variants of gluten peptides exist, many of which have T-cell stimulatory activity.^{11,30,31,33,34} Similar immunogenic peptides are present in barley and rye, generally considered not to be safe for consumption by patients. Moreover, even though it is well tolerated by most patients,³⁵ oats also have been shown to contain a few of these peptides.^{31,36,37} Another

factor that contributes to the immunogenic properties of gluten and gluten-like molecules is the clustering and repetition of these T-cell stimulatory sequences,^{11,34} which has been shown to enhance the T-cell response.³⁴ Finally, because of the high proline content of gluten, T-cell stimulatory gluten peptides are relatively resistant to enzymatic degradation in the gastrointestinal tract.³⁴ These unique features of gluten distinguish it from other protein sources that are present abundantly in our daily food and contribute to the disease-inducing properties of gluten.

Impact of the HLA-DQ2 Gene Dose

It is well established that there is a strong HLA-DQ2 gene dose effect. HLA-DQ2 homozygous individuals have an at least 5-fold increased risk for disease development compared with HLA-DQ2 heterozygous individuals.²³ Because HLA-DQ2 heterozygotes express 2 distinct DQα and DQβ chains, they potentially can form 4 distinct HLA-DQ-dimers of which only 1 will be HLA-DQ2 (Figure 4A). In contrast, all HLA-DQ dimers in HLA-DQ2 homozygotes will be of the HLA-DQ2 type (Figure 4A). This has a strong impact on the magnitude of the gluten specific T-cell response. Gluten presented by HLA-DQ2 homozygous antigen-presenting cells (APCs) results in at least a 4-fold higher T-cell response compared with gluten presentation by HLA-DQ2 heterozygous APCs.³⁸ This indicates that the level of HLA-DQ2 expression influences the likelihood of disease development. This can be visualized by assuming that the formation of HLA-DQ2 gluten-peptide complexes is dependent equally on the availability of gluten peptides and HLA-DQ2 molecules (Figure 4B). This implies that HLA-DQ2 homozygous individuals will generate more HLA-DQ2 gluten-peptide complexes compared with HLA-DQ2 heterozygous individuals (Figure 4B). Importantly, the difference between homozygotes and heterozygotes increases progressively with an increase in the available number of gluten peptides (Figure 4B). If we introduce a hypothetical threshold into this model it becomes evident that homozygotes break through this threshold at a lower gluten exposure compared with heterozygotes (Figure 5B). This may have implications for disease prevention (see later).

Table 1. Overview of Characteristics of T-Cell Stimulatory Gluten Peptides

Peptide	Sequence	Protein source	HLA-DQ	tTG-dependent
Glia-α2	PQPQLPYPQ	α-gliadin	DQ2	+++
Glia-α9	PPQPQLPY	α-gliadin	DQ2	+++
Glia-α20	FRPQQPYQP	α-gliadin	DQ2	+++
Glia-α	QGSFQPSQQ	α-gliadin	DQ8	+
Glia-γ2	FPQPQQPF	γ-gliadin	DQ2	+++
Glia-γ1	PQQSFPQQQ	γ-gliadin	DQ2	+++
Glia-γ30	IIQPQQPAQ	γ-gliadin	DQ2	+
Glt-156	FSQQQQSPF	LMW-glutenin	DQ2	+++
Glt-17	FSQQQQQL	LMW-glutenin	DQ2	+++
Glt	QGYYPSPQ	HMW-glutenin	DQ8	–
Glu-5	QLPQQPQQF	Unknown	DQ2	+++

NOTE. In the majority of cases, T-cell reactivity toward these peptides depends on modification by tTG. In a few cases, native gluten peptides can be recognized as well. Glutamine residues that are deamidated by tTG are indicated in **bold**. Deamidation by tTG is determined by the spacing between the glutamine and proline residues in gluten: a glutamine in the sequence QXP (X is any amino acid), but not in QP and QXP, will be modified by tTG.^{36,38} +++, T-cell recognition dependent on deamidation by tTG; +, T-cell recognition is enhanced by deamidation by tTG; –, T-cell recognition independent of deamidation by tTG.

Gluten Triggers Innate IEL-Mediated Immune Responses

At least 1 additional gluten peptide now is thought to play an important role in CD. This peptide, amino acids 31–43 from α gliadin, is not the target of gluten-specific T cells but does induce changes associated

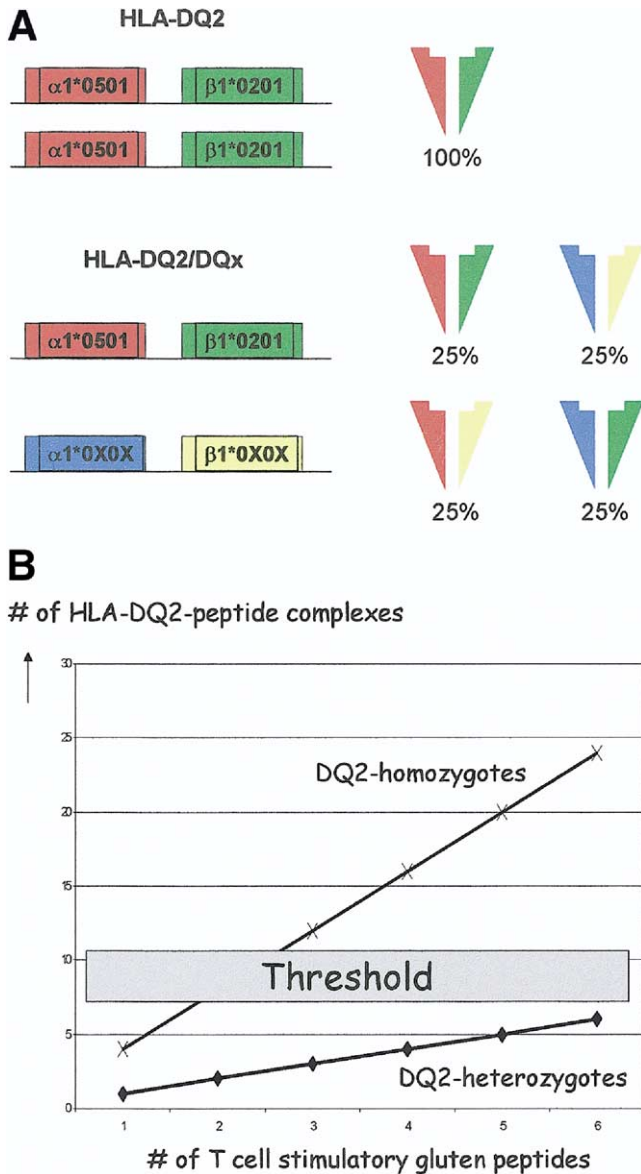


Figure 4. (A) Formation of HLA-DQ molecules in HLA-DQ2 homozygous and HLA-DQ2/DQx heterozygous individuals. The HLA locus is encoded on chromosome 6. HLA-DQ2 homozygous individuals encode identical DQ α and - β chains on both of their chromosome 6 whereas HLA-DQ2/DQx heterozygous individuals will encode HLA-DQ2 on 1 and another HLA-DQ α and - β chains on the other chromosome 6. Consequently, HLA-DQ2 homozygous individuals can form only 1 HLA-DQ dimer whereas HLA-DQ2/DQx heterozygous individuals can form 4. (B) Formation of HLA-DQ2 gluten peptide as a function of the number of available HLA-DQ2 molecules and gluten peptides. HLA-DQ2 homozygotes will be able to form larger numbers of immunogenic complexes and break through the threshold although heterozygotes still are the safe zone. Adapted from Vader et al.³⁸

with CD on administration in vivo and during biopsy challenges in vitro.^{39–41} It also has been found to stimulate cytokine production by a macrophage cell line.⁴² Moreover, it has been shown that preincubation of biopsy specimens of CD patients with the 31–43 peptide enabled T-cell activation by a T-cell stimulatory gluten

peptide.⁴³ These effects of the 31–43 peptide were found to correlate with the induction of IL-15, a cytokine that is crucial for the activation and survival of memory T cells and induces epithelial changes.⁴⁴ More recently, the increase in IL-15 production by peptide 31–43 has been linked to the induction of MICA expression on epithelial enterocytes in the intestine and the up-regulation of NKG2D-receptor expression on IELs (Figure 5).^{18,19} An IEL infiltrate, consisting of CD8+ $\alpha\beta$ T-cell receptor-positive cells and $\gamma\delta$ T-cell receptor-positive cells, is one of the hallmarks of CD.⁴⁵ These IELs can express NKG2D, an activating receptor that has many ligands, including MICA. Importantly, recent reports have shown that the interaction between NKG2D on IELs and MICA on enterocytes is sufficient for T-cell activation and results in enterocyte killing (Figure 5).^{18,19} Thus, gluten induces innate immune responses through the induction of IL-15, which, in turn, induces NKG2D and MICA and leads to direct tissue damage (Figure 5).

It is important to note that there may be a link between the lamina propria and IEL T-cell response. IL-15 production by enterocytes could have an effect on the adaptive immune response to gluten. Vice versa, presentation of gluten peptides by APCs in the lamina propria not only may lead to the activation of gluten-specific T cells, but also to IL-15 secretion by APCs (Figure 5). This could be one of the factors that leads to the increased IL-15 levels in biopsy specimens of CD patients and may contribute directly to NKG2D and MICA expression. This would explain why this mechanism is operational only in CD patients.⁴³

Antibody Response to tTG

The presence of endomysium-specific antibodies of the immunoglobulin A class in serum for a long time has been known to be a specific indicator of CD. In 1997, Dieterich et al¹⁷ showed that these antibodies were specific for tTG. Many studies now have shown that, especially in adults, the presence of tTG-specific antibodies in serum is a highly specific indication of active disease and that antibody titers decrease during a strict gluten-free diet.²⁰ An unresolved question is why these antibodies are such specific indicators of disease. An attractive hypothesis has been put forward by Sollid et al.⁴⁶ It has been shown that tTG can cross-link itself to gluten.⁴⁷ If such complexes are taken up by tTG-specific B cells, they will be degraded and generate gluten peptides that can bind to the HLA-DQ2 molecules of the B cell. When gluten-specific T cells are present, they will detect these DQ-peptide complexes and stimulate the B cell, leading to the secretion of tTG-specific antibodies.⁴⁶ This sce-

nario indicates that these antibodies are secondary to the development of a gluten-specific T-cell response and not involved actively in the disease process itself.

Additional Factors Contribute to CD Development

Approximately 25%–30% of the population in the Western hemisphere expresses HLA-DQ2 and is exposed to high amounts of gluten daily but only a minority develops CD. Also, the α gliadin–derived 31–43 peptide exerts its effect in biopsy specimens of CD patients only.⁴³ This indicates that currently unknown factors must contribute to disease development. Some of those may be environmental. Infections, for example, can lead to interferon- γ production, which would enhance HLA-DQ2 expression and lower the threshold for the development of a gluten-specific T-cell response.³⁸ This is supported by the observation that interferon- α treatment, which promotes interferon- γ production, can result in CD.^{48–50} In addition, there is increasing evidence for the involvement of additional genetic factors. One of these candidate genes is CTLA-4,^{51–53} and there is evidence for the involvement of genes

on chromosome 5 (5q31–33)^{54,55} and 19 (19p13.1).⁵⁶ The identification of these genes ultimately also may provide an explanation for the observed heterogeneity in clinical symptoms and differences in age of onset of the disease.

Perspectives

Can We Prevent CD?

It is well established that oats are tolerated by most CD patients whereas cereals that contain larger numbers of T-cell–stimulatory peptides are not. Also, a double HLA-DQ2 gene dose leads to a 5-fold increased risk.²³ Finally, the Swedish epidemic has shown that an increase in gluten content in infant food resulted in a tripling of CD incidence.⁵⁷ These observations indicate that the level of gluten presentation is linked tightly to the probability of disease development. This may imply that there is a threshold below which gluten consumption is safe, although that threshold likely will not be the same for everyone. In practice, however, we introduce high amounts of gluten when children are 6–7 months old: a 12-month-old child consumes up to 10 g/day of

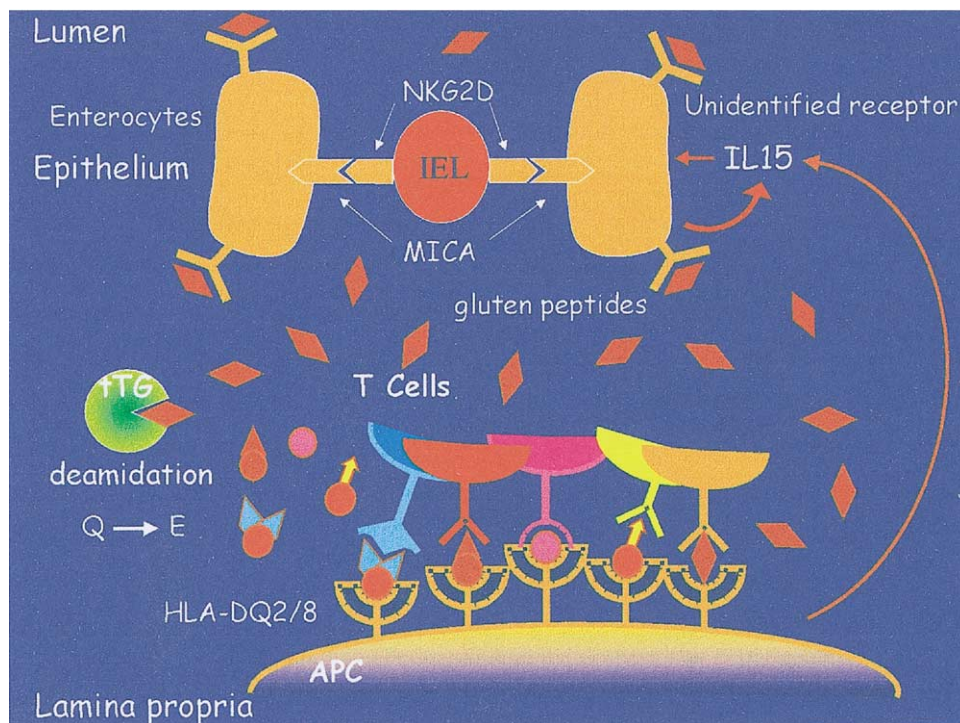


Figure 5. Caught between a rock and a hard place. In the lamina propria, T cells respond to multiple gluten peptides bound to HLA-DQ2 and/or HLA-DQ8 on APCs, which results in a release of inflammatory cytokines, including interferon- γ . Interferon- γ –induced up-regulation of HLA-DQ expression will enhance gluten–peptide binding. Cellular damage caused by the inflammation will release intracellular tTG. This will result in additional gluten modification, contributing to enhanced T-cell reactivity toward gluten. APC activation also may lead to an increase in IL-15 production. Simultaneously and independent of the gluten-specific T-cell response, gluten can induce IL-15 production through an unidentified mechanism (unidentified receptor on enterocytes?). The increase in IL-15 levels leads to NKG2D and MICA up-regulation on IELs and enterocytes, respectively, and results in enterocyte destruction.

gluten. This abrupt introduction of a large quantity of protein with potent immunogenic properties may be fatal particularly for individuals at high risk: children of CD patients and DQ2 homozygous individuals. The possibility that a less-abrupt introduction of lower amounts of gluten may prevent CD development thus deserves investigation. In this respect it is imperative also to take into consideration the recently observed impact of the timing of gluten introduction on the risk for developing type I diabetes.^{58,59}

Can We Improve Diagnosis?

Although an intestinal biopsy examination still is considered to be required for a proper diagnosis, the presence of tTG-specific antibodies is a highly specific indicator of disease. Novel diagnostic tools may become available when the additional genetic factors that predispose to CD have been identified.

Can We Devise Alternative Therapy?

Degradation of gluten by prolylendopeptidases, enzymes that cleave peptides after a proline and would degrade gluten into harmless fragments, has been suggested.³⁴ The feasibility of this approach, however, has yet to be shown. Alternatively, blockers of tTG and/or HLA-DQ might be effective. Although blockers of tTG may have (serious) side effects because the normal functions of tTG also would be blocked, this may not be the case for HLA-DQ blockers because HLA-DQ may have a limited function in the adaptive immune response to pathogens. Finally, compounds that interfere in the NKG2D-MICA interaction, or neutralize IL-15, also might diminish symptoms.

Can We Generate Safer Foods?

Once CD has developed, patients can be sensitive to trace amounts of gluten in food. A monoclonal antibody-based assay has been developed that detects α and γ gliadin-derived T-cell stimulatory peptides⁶⁰ and can be used to better guarantee food safety for CD patients. This current knowledge also can be used to detoxify gluten by the introduction of amino acid substitutions that abolish the immunogenic nature of gluten.³⁵ The removal of all toxicity, however, would require extensive modification and the resulting proteins may have lost important gluten-specific properties.

Conclusions

Gluten has unique properties that set it apart from other food proteins. It is a degradation-resistant mixture of proteins that is modified efficiently by the

enzyme tTG. The result is the generation of a series of immunogenic peptides that can trigger T-cell responses (Figure 5). Clustering of these epitopes leads to multivalency, which enhances immunogenicity. Similar peptides are found in other cereals, in particular barley and rye. The likelihood of the initiation of a gluten-specific T-cell response is influenced by the level of HLA-DQ2 expression. The combination of high HLA-DQ2 expression and high gluten exposure is thus a dangerous mix. In addition, gluten has an impact on the innate immune system. It is capable of inducing IL-15 expression that triggers both NKG2D-receptor expression on IELs and MICA on enterocytes. The NKG2D-MICA interaction results in enterocyte damage and thus contributes to the intestinal damage that is typical for CD (Figure 5).

Although these observations give insight into the immune processes that are operational in patients, they do not explain why only a minority of the individuals at risk develop CD. Additional environmental and genetic factors are likely to determine the outcome of gluten exposure and future research should reveal their identity and mode of action. In the meantime, our understanding of the fatal interaction between gluten and the adaptive and innate immune system does offer opportunities for the development of prevention strategies, novel therapies, and safer foods for patients.

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