Recognition of HLA-DQ gluten is mediated by a relatively conserved set of T-cell receptors that predominate in patients and appear absent in healthy individuals

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Adverse Effects of Wheat Gluten by Frits Koning

Key insights

Celiac disease is a chronic autoimmune enteropathy that is influenced by environmental and genetic factors. It is the only gastrointestinal disorder in which the involvement of gluten has been clearly established. Due to its high proline content, gluten is resistant to degradation in the gastrointestinal tract. A key pathogenic event in celiac disease is the aberrant T-cell responses directed against gluten fragments. These antigenic fragments can activate immune cells in the gut, recruit infiltrating T cells, and initiate proinflammatory signalling events that eventually lead to profound remodelling of the gut tissues.

Current knowledge

Wheat gluten consists of two classes of proteins, the gliadins and the glutenins. The glutenins confer elasticity and viscosity to various food products, but the immunogenic peptide fragments that trigger symptoms in patients with celiac disease come from the gliadin family. The majority of patients with celiac disease express either HLA-DQ2.5 and/or HLA-DQ8. Under normal conditions, these molecules bind and present antigen-derived peptides to T cells. In celiac disease, aberrant T-cell responses are directed towards gluten fragments bound to HLA-DQ2.5 and/or HLA-DQ8. Such immunogenic fragments arise from partial degradation of gluten proteins in the gastrointestinal tract, which are then modified by the tissue transglutaminase (TG2) enzyme.

Summary of the pathogenesis of celiac disease.

Practical implications

Patients with celiac disease should avoid food products that contain wheat, barley or rye. In general, oat is considered safe for patients with celiac disease. Oral supplements of post-proline digesting enzymes (prolyl endopeptidases and prolyl endoproteases) present one strategy for enhancing gluten degradation. Although this alone will not enable patients to consume a normal gluten-containing diet, use of these enzymes may be effective at counteracting the effects of inadvertent gluten exposure.

Recommended reading

Tjon JM, van Bergen J, Koning F: Celiac disease: how complicated can it get? *Immunogenetics* 2010;62:641–651.

Adverse Effects of Wheat Gluten

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Key Messages

- Celiac disease (and the associated dermatitis herpetiformis) is the only (gastrointestinal) disorder where the involvement of gluten has been clearly established.
- The unique properties of gluten proteins explain their disease-inducing properties.
- Safe wheat and safe gluten are not yet within reach, but the current knowledge allows alternative approaches to meet patients' needs.

Key Words

Celiac disease · Wheat · Gluten · Toxicity

Abstract

Man began to consume cereals approximately 10,000 years ago when hunter-gatherers settled in the fertile golden crescent in the Middle East. Gluten has been an integral part of the Western type of diet ever since, and wheat consumption is also common in the Middle East, parts of India and China as well as Australia and Africa. In fact, the food supply in the world heavily depends on the availability of cereal-based food products, with wheat being one of the largest crops in the world. Part of this is due to the unique properties of wheat gluten, which has a high nutritional value and is crucial for the preparation of high-quality dough. In the last 10 years, however, wheat and gluten have received much nega-

tive attention. Many believe that it is inherently bad for our health and try to avoid consumption of gluten-containing cereals; a gluten-low lifestyle so to speak. This is fueled by a series of popular publications like *Wheat Belly*, *Lose the Wheat*, *Lose the Weight*, and *Find Your Path Back to Health*. However, in reality, there is only one condition where gluten is definitively the culprit: celiac disease (CD), affecting approximately 1% of the population in the Western world. Here, I describe the complexity of the cereals from which gluten is derived, the special properties of gluten which make it so widely used in the food industry, the basis for its toxicity in CD patients and the potential for the development of safe gluten and alternatives to the gluten-free diet.

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Wheat, Barley and Rye: Three Gluten-Containing Cereals

Wheat, barley and rye are closely related cereals belonging to the Triticeae genus [1]. Oat is phylogenetically more distant, while rice, maize and sorghum stem from different subfamilies (fig. 1). Consequently, wheat gluten and the gluten-like hordeins and secalins from barley and rye, respectively, are quite similar to each other, and this causes their toxicity for patients with celiac disease (CD). The avenins of oats have lower sequence similarity and a low toxicity profile. Finally, the gluten-like proteins in rice, maize, sorghum and tef are quite distinct from wheat gluten and are devoid of toxicity [2].

Wheat is highly complex: there are over 10,000 varieties known worldwide. However, only a few of those are commonly used for food production, most notably tetraploid durum wheats (pasta wheat) and hexaploid bread wheats (fig. 2). The tetraploid wheats, also known as *Triticum durum*, contain two complete genomes and originate from natural hybridization of two diploid species [1] (fig. 2). Later, hexaploid bread wheat, including the *T. aestivum* and *T. spelta* species, developed spontaneously by another hybridization event [1]. Due to the presence of two and three genomes in the tetraploid and hexaploid wheat varieties, respectively, these produce more gluten proteins, which is favorable for the production of high-quality dough. Conventional breeding technology has been used to further optimize the crop yield over many thousands of years.

Gluten: A Complex Mixture of Proteins

Gluten is a mixture of storage proteins present in the endosperm of seeds from wheat, barley and rye, where it provides nutrition for germinating seeds. Wheat gluten consists of two classes of proteins, the gliadins and the glutenins [1]. The former can be subdivided into the α -, γ -, and ω -gliadins and the latter into the low-molecular-weight (LMW) and high-molecular-weight (HMW) glutenins. Dozens of gliadin genes are expressed in any wheat variety, but much fewer glutenin genes are present. While the glutenins, in particular the HMW glutenins, are most crucial to the formation of high-quality dough with optimal baking properties [1], the gliadins contain most of the toxic fragments that cause symptoms in patients with CD [3]. Gluten provides elasticity and viscosity to dough, which is due to the formation of interchain disulfide bonds between gluten proteins and hydrogen bonds between glutamine residues that are abundantly present in all classes of gluten proteins. The resulting viscoelastic network gives dough its special properties which makes it widely applicable in the food industry.

Rye also contains both gliadin- and HMW-glutenin-like molecules; the former are also known as secalins (from secale; fig. 1). In barley, there are no HMW glutenins, but it does contain the gliadin-like hordeins (from hordeum; fig. 1).

Toxicity of Gluten: It Harbors a Multitude of Immune Stimulatory Fragments

A large number of studies have addressed the toxicity of gluten for patients with CD [4–11]. Virtually all patients express either HLA-DQ2.5 and/or HLA-DQ8 [12,

Fig. 1. Overview of the relatedness of commonly used cereals.

Fig. 2. Natural hybridization of diploid species has led to the formation of tetraploid wheat species. Further hybridization has yielded hexaploid wheat.

13]. These molecules normally bind and present peptides derived from protein antigens to T cells that are surveying the body for the presence of invading pathogens. The detection of peptides derived from proteins from pathogens alerts the immune system, a crucial step towards the eradication of the pathogen. The strong association between HLA-DQ2.5/8 and CD thus indicated that aberrant T-cell responses directed to gluten fragments bound to the HLA-DQ2.5 and/or -DQ8 molecules could underlie the disease pathogenesis. Indeed, gluten-specific T cells can be isolated from small intestinal biopsies of patients with CD but not from controls [3–11]. Also, upon gluten challenge, such T cells can temporarily be detected in the blood [8, 11, 14]. The specificity of these T cells has been investigated in detail, which revealed that immunogenic fragments of gluten are present in all types of gluten proteins. However, some gluten fragments are immunodominant, as T cells specific for these fragments are found in

Fig. 3. Nine amino acid T-cell epitopes in gliadins and homologues in hordeins and secalins indicated by horizontal lines. **a** The 33-mer fragment of α -gliadin that contains six overlapping T-cell epitopes. **b** The length and the number of T-cell epitopes of the ‘33-mer’ are variable. **c** Homologue sequences in gliadins, hordeins and secalins. Amino acid symbols: L = leucine; Q = glutamine; P = proline; Y = tyrosine; F = phenylalanine; W = tryptophan. Q = target for tissue transglutaminase.

virtually all patients [3, 8, 9, 11]. In particular, a proline-rich repetitive sequence in the N-terminal part of the α -gliadins and a homologous sequence in the ω -gliadins is highly immunogenic. This sequence contains six partly overlapping T-cell epitopes (fig. 3a) known as DQ2.5-glia- α 1a, DQ2.5-glia- α 1b and DQ2.5-glia- α 2 [3, 4]. The length of this repetitive N-terminal sequence, however, varies between α -gliadin genes and, consequently, also the copy number of the immunogenic sequences [15] (fig. 3b). In addition, the C-terminal part of the α -gliadins contains DQ8-glia- α 1, an immunodominant sequence in HLA-DQ8-positive patients [6, 16]. Due to the strong sequence homology between the α - and ω -gliadins and between the gliadins and the hordeins and secalins highly similar or even identical sequences are present [15] (fig. 3c). Thus, T cells can respond equally to stimulation with all three cereals. In addition, other T cells can be highly specific for sequences found in one of these cereals only [11].

The special amino acid composition of gluten is strongly linked to its disease-inducing capacity. All gluten proteins are glutamine (symbol Q) and proline (symbol P) rich. Together, these two amino acids make up almost 50% of the gluten proteins. Due to their proline-rich nature, the gluten proteins are difficult to degrade, hence they persist in the gastrointestinal tract [4]. Also, many

HLA-DQ2.5-restricted gluten epitopes have a proline at relative position 8 which is crucial for T-cell recognition [17, 18]. However, perhaps most importantly, its proline- and glutamine-rich nature makes gluten a perfect substrate for the enzyme tissue transglutaminase [5], which generates gluten fragments that bind with high affinity to either HLA-DQ2.5 or HLA-DQ8, a prerequisite for T-cell recognition of most immunogenic gluten fragments.

Both HLA-DQ2.5 and HLA-DQ8 preferentially bind peptides with a negatively charged amino acid at relative position 4, 6 or 7 (HLA-DQ2.5) or at position 1 and/or 9 (HLA-DQ8), but gluten does not contain negatively charged amino acids. This discrepancy was solved when it was realized that the immunogenic gluten peptides contained one or more glutamine residues at critical positions and that replacing these amino acids by glutamic acid, which is negatively charged, allowed for high-affinity binding of the modified peptides to either HLA-DQ2.5 or -DQ8. Importantly, it was shown that such a modification of gluten peptides is mediated by the enzyme tissue transglutaminase (TG2), which under normal circumstances is located intracellularly where it is enzymatically inactive but becomes active when it is released upon tissue damage [7, 16]. Moreover, QLPY and QLPP sequences, which are abundantly present in gluten proteins, were found to be an ideal substrate for TG2 and are converted in ELPY and ELPF, where the E stands for the negatively charged glutamic acid [5]. Consequently, in the 33-mer, three Q residues are converted into an E, yielding 6 peptides that bind with high affinity to HLA-DQ2.5 due to the introduction of a negative charge at either position 4 or 6 (fig. 3, 4). Similarly, TG2 can introduce negative charges at position 1 and/or 9 in other gluten peptides, which generates peptides that bind with high affinity to HLA-DQ8, like in the case of the DQ8-glia- α 1 peptide [16] (table 1). Thus, the unique amino acid composition of gluten explains its immunogenic nature.

While it is clear that most gluten toxicity is associated with the α - and ω -gliadins, T-cell epitopes have also been identified in the γ -gliadins and in the LMW and HMW glutenins. In fact, T-cell responses to the γ -gliadins are quite frequently detected in patients, while the glutenins appear to have a much lower toxicity profile. Notably, the identified γ -gliadin and glutenin epitopes are less proline rich (2–3 per epitope) compared to the epitopes in the α - and ω -gliadins (4 per epitope). This may render the γ -gliadin and glutenin epitopes more susceptible to enzymatic degradation which could relate to their lower immunogenicity.

Fig. 4. Tissue transglutaminase (TG2) modification creates gluten epitopes that bind with high affinity to HLA-DQ2/8.

A Biased T-Cell Receptor Repertoire in Patients with CD

Recent studies have investigated the T-cell receptor repertoire expressed by gliadin-specific T cells [19–23]. This has revealed that highly similar T-cell receptors are found in all patients examined (so-called public T-cell receptors). As the potential T-cell receptor repertoire is immense, this indicates that selection and expansion of T cells expressing such public T-cell receptors has taken place in patients. Moreover, as gluten-specific T cells are not found in healthy controls, this indicates that the presence of T cells expressing public T-cell receptors is tightly linked to disease development.

Recent studies have revealed the molecular basis for the interaction between public T-cell receptors and HLA-DQ gluten [21–23]. In both HLA-DQ2 and HLA-DQ8 disease, it was observed that this interaction is mediated by a number of key interactions between the T-cell receptors, HLA-DQ and the bound gluten peptide. Strikingly, in all cases, an arginine residue from the T-cell receptor was found to play a crucial role. Thus, high-affinity recognition of HLA-DQ gluten is mediated by a relatively conserved set of T-cell receptors that predominate in patients and appear absent in healthy individuals. Elimination of such T cells might thus constitute an effective therapeutic option for patients with CD.

Oat: The Exception

While wheat, barley and rye belong to the same genus, oat is more distantly related. Consequently, compared to the hordeins and secalins, the gluten-like avenins from oat are less similar to wheat gluten. Indeed, homology searches between gliadins and avenins yield peptides

Table 1. Overview of the presence of three HLA-DQ2.5 fragments and one HLA-DQ8 gliadin fragment in the A-, B-, and D-wheat genomes

Epitope	Sequence	A-genome	B-genome	D-genome
DQ2.5-glia- α 1a	PFPQPQLPY	466	–	520
DQ2.5-glia- α 2	PQPQLPYPQ	–	–	607
DQ2.5-glia- α 3	FRPQQPYPQ	650	–	449
DQ8-glia- α 1	QGSFQPSQQ	–	186	381

Shown is the number of hits within the genomes indicated.

that are quite different [3, 15]. For example, the only known avenin homologs of the DQ2-glia- α 1 sequence PFPQPQLPY are PYPEQQEPPF and PYPEQQQPF, where only four of the nine amino acids are shared, and T cells infrequently cross-react between these gliadin and avenin peptides [15]. In addition, oat contains only a few avenin genes. Consequently, the avenin content of oat is much lower compared to the gluten content of wheat. Altogether, this implies that oat consumption by CD patients leads to a limited exposure to avenin fragments that exhibit a significantly lower T-cell stimulatory potential. This most likely underlies the observation that, even though exceptions have been reported [24], the large majority of patients with CD can safely consume oat provided that it is not contaminated with other gluten-containing cereals [25].

Gluten and the Innate Immune System

Next to the well-established immunogenic nature of gluten there is quite extensive literature on the effects of gluten on the innate immune system, in particular the impact of the α -gliadin-derived p31-43 peptide. This peptide, which is N-terminal from the immunogenic 33-mer, has been implicated in the induction of the expression of CD25, COX-2 and IL-15 as well as to display growth factor activity, amongst others [26, 27]. To date, however, no receptor has been identified to which the p31-43 binds, so the mechanism by which this peptide would induce these biological effects has remained obscure. As I have argued before [28], the effects of the p31-43 peptide may be based on a mechanism that has not yet been investigated properly. It is known that gluten-specific antibodies are frequently found in both patients with CD and controls.

These antibodies are directed to a repetitive sequence in gluten: QPFXQXPY, where X can be any amino acid [29]. Such a sequence is also present in the p31-43 peptide: 31-LGQQQPFPPQPY-43, implying that the 33-peptide could be targeted by gluten-specific antibodies. As the p31-43 peptide is directly adjacent to the immunogenic 33-mer, uptake of large gliadin fragments containing both p31-43 and the 33-mer might thus be facilitated by such antibodies expressed on gliadin-specific B cells or by antigen-presenting cells expressing Fc receptors. In both cases, this would result in intracellular processing of the internalized gliadin fragment, which would allow binding to HLA-DQ and transport of the HLA-DQ-gluten complexes to the cell surface. Thus, the antibody-mediated gliadin uptake would lead to strongly enhanced HLA-DQ-mediated presentation of gliadin to gliadin-specific T cells and promote inflammation. Conversely, the T cells would provide 'help' to the antibody-producing B cells, leading to enhanced antibody production and creating a powerful amplification loop. Thus, the immune modulatory effects of the p31-43 peptide might be indirect and explained by enhanced adaptive T-cell responses.

Detoxification of Gluten

As mentioned, wheat is complex and so are the loci encoding the gliadin genes. Each wheat variety contains dozens of gliadin genes, the actual number depending on the polyploidy. Thousands of such gliadin genes have been sequenced, and their origin can be traced back to the genome from which they originate, the A-, B-, or D-genome. A close analysis of over 3,000 available α -gliadin genes revealed that the gliadins encode a large number of these toxic sequences, yet substantial differences in the presence of the epitopes exist depending on the genome [30, 31] (table 1). The D-genome is clearly the most deleterious, as it encodes all four investigated epitopes, while the A- and B-genomes encode only two and one, respectively. Together with the observation that the length of the '33-mer' differs between α -gliadin genes [15] (fig. 3b), this indicates that not all gliadin genes have a similar toxicity profile. This has fueled hope that 'safe' wheat might be developed by selecting for varieties that contain certain α -gliadins only [31]. Yet, this may be an illusion, as to date

no single gliadin gene has been identified that is truly devoid of immunogenic sequences. Moreover, even though a particular gliadin gene may lack a precise copy of a particular immunogenic sequence, it often harbors a homologous sequence, and T cells may cross-react with such homologous peptides. Hence, it will be extremely difficult, if not impossible, to develop 'wheat' that will be safe for patients with CD through conventional breeding strategies.

On a more positive note, evidence has been provided that natural variants of immunogenic gluten fragments

It will be extremely difficult, if not impossible, to develop 'wheat' that will be safe for patients with CD through conventional breeding strategies

exist that lack T-cell stimulatory properties [17, 18]. In many cases, these variant peptides differ in only one amino acid from their immunogenic counterpart and this often concerns a proline to serine (symbol S) substitution at relative position 8. For example, the DQ2-glia- α 2 epi-

tope PQQPLPYPQ from the D-genome is highly immunogenic, while its naturally occurring counterpart PQQPLPYSQ from the A-genome is not [17, 18]. Thus, gliadin genes could be engineered so that they would no longer encode immunogenic sequences while retaining most of their natural properties. However, due to the repetitive nature of the α -gliadins, multiple proline residues would need to be modified to eliminate all toxicity. This would involve six substitutions in the 33-mer alone, complicating such an approach. Therefore, although strategies to produce safe gluten exist, it would be very expensive and thus at present not a realistic option.

Enzymatic Gluten Degradation

As mentioned, gluten is highly resistant to degradation in the gastrointestinal tract due to its high proline content. To enhance gluten degradation, the use of oral supplementation with post-proline cutting enzymes has been proposed [4]. Such prolyl endopeptidases and prolyl endoproteases (PEP) are not present in the lumen of the gastrointestinal tract but can be isolated from a variety of micro-organisms. The first studied enzyme, PEP from *Flavobacterium meningosepticum*, was indeed capable of degrading gluten peptides [4] but was not active under the low pH values typically observed in the stomach and inactivated by pepsin [32]. Additional enzymes that have been investigated include PEP from *Myxococcus xanthus*, *Sphingomonas capsulate* and *Aspergillus niger* [32–35].

The latter enzyme, AN-PEP, has a favorable pH profile as it is highly active at low pH values and it is resistant to degradation by pepsin [32]. Consequently, it is capable of degrading gluten in the stomach compartment both in vitro and in vivo and it has been found to exert no harmful effects in patients and healthy human volunteers [32, 35–37]. The enzyme is expected to reach the market as an over-the-counter product in 2015. In addition, *S. capsulate* PEP has been combined with a cysteine endoprotease B from barley; the latter cleaves after glutamine [38]. The combined activity of these two enzymes is highly effective in degrading gluten as well, and the efficacy is currently being tested in a phase IIb study.

The use of oral enzymes for enhanced gluten degradation may be of help to patients, in particular when eating out or on holidays

It is doubtful, however, that the availability of such enzyme preparations will allow patients to eat a normal gluten-containing diet. Daily gluten intake varies between 10 and 20 g in adults on a gluten-containing diet. Gluten is present in many food products, and any intake of gluten would have to be accompanied by oral administration of gluten-degrading enzymes. Moreover, evidence has been provided that the efficacy of gluten degradation depends on the context in which gluten is consumed. In the case of AN-PEP, for example, artificial lowering of the pH by adding a carbonated drink enhanced gluten degradation while milk slowed the process [39]. It is therefore hard to imagine that any enzyme will be effective in ensuring complete degradation of the high amounts of gluten protein present in

a normal diet before it reaches the small intestine. The enzymes, however, would be quite effective in degrading relative small amounts of gluten and could thus be used to counteract the deleterious effect of inadvertent gluten exposure, for example when eating out or on holidays.

Conclusions

CD is caused by proinflammatory T-cell responses to gluten fragments bound by the disease-predisposing HLA-DQ2.5 and/or HLA-DQ8 molecules. Such fragments are formed by partial degradation of gluten proteins in the gastrointestinal tract followed by modification by the enzyme TG2, introducing negative charges in gluten fragments required for high-affinity binding to the disease-predisposing HLA-DQ molecules. Gluten toxicity is primarily associated with proline-rich regions in the α - and ω -gliadins and similar regions in the hordeins and secalins in barley and rye, respectively. Oat is safe for the majority of patients. The T-cell receptor repertoire underlying the gluten-specific T-cell response is highly biased and very similar between patients. As such T cells are not found in healthy controls, their appearance and expansion appears to be linked to disease initiation. Detoxification of gluten and/or wheat is currently impossible. The use of oral enzymes for enhanced gluten degradation may be of help to patients, in particular when eating out or on holidays.

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