Gluten toxicity, how to get rid of it?

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In 1993 Lundin and colleagues first described the presence of gluten-specific T cells in small intestinal biopsies of coeliac disease (CD) patients [1]. A large number of studies have since established that such T cells can be specific for a large and diverse array of peptides derived from the gliadins as well as the glutenins ([2-5], references in [6]).

Invariably, these peptides can trigger T-cell responses only when bound to the disease predisposing HLA-DQ2 or HLA-DQ8 molecules. This provides an explanation for the well established association between these HLA-molecules and disease development. It also became evident that many of these peptides require modification by the enzyme tissue transglutaminase, a modification that introduces negative charges into gluten peptides, thus enhancing the binding of these peptides to either HLA-DQ2 or HLA-DQ8 [8]. Not only wheat is off-limits to CD patients: barley and rye are also known to contain a variety of proteins that are just as harmful as the gluten proteins from wheat [8, 9]. Oat seems an exception as it is tolerated by most patients, partly due to a low content of gluten-like prolamin proteins [8-10].

Thus, CD patients usually have T cells specific for an array of gluten and gluten-like peptides that originate from all types of gluten proteins and homologues in other cereals. At present the introduction of a gluten-free diet is the only but highly-effective treatment option. This diet, however, has several drawbacks. It is relatively cumbersome, difficult to adhere to, expensive and is deficient in several nutrients and fibers. Many patients feel insecure, especially when eating out or while travelling. Thus, there is an unmet need for alternatives to the gluten-free diet.

With the identification of the harmful sequences in gluten and gluten-like proteins it has become possible to initiate studies aimed at reducing or eliminating the toxicity of such proteins and/or wheat. Early studies indicated that substantial differences existed between wheat varieties regarding their "toxicity profile" [11-13]. Some of those could be attributed to differences in the genetic make-up of the three genomes that comprise the complex hexaploid bread wheat, the A-, B- and D-genome [12, 13]. More recently we embarked on a large-scale study to map the toxicity of the α-gliadins, based on the observation that the α-gliadins are among the most immunogenic gluten proteins and

contain four well-characterized peptides involved in CD [2, 4, 5]. We analyzed over 3,000 α-gliadin genes in the database to determine the full extent of the natural variability that is present in these genes and in the known T-cell stimulatory peptides in these proteins in particular [14]. The results indicated that many natural variants of these immunogenic α-gliadin peptides exist; an example is given in Fig. 1.

Sequence	Occurrence in database
PFPQPQLPY	986
PFLQPQLPY	106
PFSQPQLPY	190
PFPHPQLPY	29
ETC	

FIG. 1. *Many natural vairants of known immunogenic alphy-gliadin peptides*

We could classify all identified variants as belonging to one of the three genomes based on differences in the complete gene sequences. Moreover we synthesized all variants of the four known T-cell peptides that we had identified, and tested these for binding to HLA-DQ2 or HLA-DQ8 as well as for recognition by T cells derived from small intestinal biopsies of CD patients ([14], Fig. 2). The results demonstrated that no α-gliadin proteins exist that lack all T-cell stimulatory epitopes ([14], Fig. 2). Based on these results it can be concluded that it would be impossible to generate CD-safe wheat through conventional breeding programs. Substantial differences, however, were observed between the genes encoded by the three genomes: while the D-genome α-gliadins are by far the most toxic, the B-genome encoded α-gliadins are the least toxic and the A-genome encoded genes have an intermediate toxicity profile.

	DQ2-α1	**DQ2-α2**	**DQ2-α3**	**DQ8-α1**
A	+	-	+	-
B	-	-	-	+
D	+	+	+	+

FIG. 2. *No alpha-gliadin proteins exist that lack all T cell stimulatory epitopes*

Close examination of the results, however, indicated that there are natural variants of all T-cell stimulatory epitopes that do not induce T-cell responses. For example, the A-genome encodes a variant of the DQ2-α2 epitope that is not immunogenic: while the sequence of this epitope on the D-genome is PQPQLPY**P**Q, the A-genome encodes PQPQLPY**S**Q and this P to S substitution results in a peptide that does not induce T-cell responses [14]. Similarly, natural variants of the other three T-cell stimulatory peptides have been identified that lack T-cell stimulatory properties.

These results allow a novel approach to eliminate gluten toxicity: while the D-genome α-gliadin gene encodes four toxic epitopes, it is possible, , by combining the genetic information of the A- and B-genome encoded α-gliadins, to generate a new gene that encodes a that is not toxic for CD patients ([14], Fig. 3). At the peptide level, we have provided proof of principle for this approach and we envisage that similar approaches can be taken to generate non-toxic variants for the other gliadin and glutenin proteins in wheat gluten. In this respect the high molecular weight glutenins are of particular interest as these, to a large extent, determine the baking properties of gluten. Genes encoding such safe-gluten proteins could be introduced into safe cereals for production of safe-gluten that could be used for the fabrication of gluten-free foods with markedly enhanced quality in terms of nutritional value, taste and baking properties.

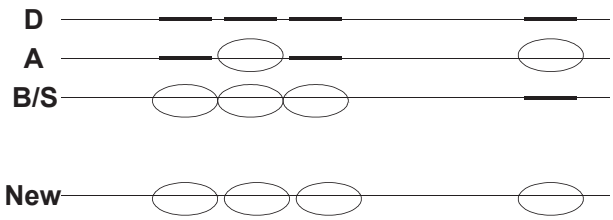

FIG. 3. *New non-toxic gluten gene*

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