The aetiology of coeliac disease and its significance for therapy

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ABSTRACT
The enzymopathic hypothesis of the aetiology of coeliac disease (CD) is still relevant as a mechanism operating in this disease and a form of therapy based on the use of enzymes which are deficient in CD is currently being evaluated. This approach is based on the premise that if gluten and other offending proteins are completely digested in the small intestinal mucosa with the aid of supplementary enzymes, an adverse response will not occur. This is comparable to the situation in those who do not suffer from this disease, where there is neither direct toxic action nor an abnormal immunological response to cause damage to the mucosa.

Enzyme therapy offers a way of safeguarding the integrity of the small intestine for patients against traces of gluten ingested whilst attempting to maintain a strict gluten-free diet. Furthermore, in the future, the efficiency of this approach may develop to the point where minor indulgence in some favourite 'foods and beverages, maybe possible.

KEYWORDS: coeliac disease, enzyme therapy, gluten, gliadin peptides, toxicity assays, synthetic peptides.

INTRODUCTION
A great deal of evidence has been now obtained to support the enzymopathic hypothesis of CD. In recent years, considerable efforts has gone into a better understanding of the mechanisms of pathogenesis, but has mostly overlooked the basic cause or aetiology of the disease. There is therefore a need to separate mechanisms of aetiology from those which are important in pathogenesis.

When this is done, it becomes likely that the damage to tissue seen in CD is caused by increased concentrations of undigested peptides derived from wheat, rye and barley resulting from their defective digestion in the small bowel with subsequent initiation of immunologically mediated reactions. There is also the likelihood of direct toxicity resulting from these higher concentrations of peptides.

Background
The background research carried out in order to define the present understanding of the value of enzyme therapy has been achieved by:

1. The fractionation of gluten into components which differ in their toxic properties.
2. In vitro assays of toxicity for fractions.
3. Chemical analysis and differentiation of fractions of gliadin digests.
4. Confirmation of chemical composition and structure by the use of synthetic peptides.
5. Confirmation of toxicity by in vivo methods.
6. Further studies on the mechanism of toxicity.
7. Studies of immunological mechanisms of pathogenesis.

Each of these points will now be discussed.
1. The fractionation of gluten.

The separation of the components of wheat proteins has long been of importance. Since the gliadin fraction has emerged as a source of the toxic substances in wheat (1) much research has been done to determine the most toxic proteins present and this has also extended to peptic-tryptic-pancreatic (PTC) digests of gliadin (2,3). These digests are ideal to work with as they are water soluble and of molecular weight about 1500 Daltons, meaning that peptides are tested which would be of similar size to those presented to the small intestine.

The gliadin fraction of wheat is readily obtained by extraction with 70% (v/v) ethanol, and although the insoluble higher molecular proteins, the glutenins, contribute to toxicity, the gliadin fraction, generally known as the prolamin fraction, is regarded as being the more toxic to coeliacs (5). This fraction also contains the toxic proteins of rye, barley and oats. The gliadins can be separated into electrophoretic fractions α-, β-, γ- and δ-gliadins and ion-exchange procedures can be employed to separate fractions corresponding to these gliadins. However, the PTC digests present the best opportunity for differentiation of toxicity, if we base this on specific amino acid sequences in small peptides.

The fractionation of PTC gliadin digest was achieved by chromatography on SP Sephadex (Pharmacia, Uppsala, Sweden) by the use of phosphate buffers and increasing pH (4).

2. In vitro assays of toxicity

One of the earliest useful assays of activity showed that intestinal mucosa from patients with active coeliac disease recovered histologically after 24 hours in culture in the absence of gluten but remained damaged in the presence of gliadin (6).

This proved to be a useful method for evaluating the toxicity of gliadin fractions. It was shown that one fraction of the PTC gliadin digest (fraction 9) prevented recovery of the mucosa compared to the other 9 fractions of the digest, as shown by electron microscopy (7).

This was supported by another much simpler assay based on disruption of rat-liver lysosomes, where, again, fraction 9 was the most active (8).

Fraction 9 and one of its sub-fractions (9-2) were shown to retain activity to developing foetal rat intestine and cultured atrophic celiac mucosa after preliminary digestion by intestinal mucosa from celiac patients in remission (9). Digestion with normal mucosa abolished this activity, lending strong support to a mucosal deficiency in the celiac patients.

3. Chemical analysis and differentiation of fractions of gliadin digests.

Amino acid analysis of the various fractions revealed that initial fractions were rich in acidic N-pyrrolidone carboxyly peptides, whilst the latter ones such as fraction 9 were rich in proline, glutamine, together with serine, tyrosine and leucine and were thus more neutral in nature. The most important finding was that fraction 9 was the least completely digested by histologically normal remission celiac mucosa of all the fractions. All fractions were equally well digested by mucosa from normal individuals. The undigested peptides were readily seen after chlorination and staining with starch (9).

Pursuing this work further, it was shown that there were peptide residues from fraction 9 after digestion with mucosa from some first degree relatives of the coeliac who did not have coeliac disease according to histological criteria (10).

These experiments pointed to a peptidase deficiency in coeliac disease and a partial deficiency in the first degree relatives of those with this disease.

4. Confirmation of chemical structure by the use of synthetic peptides.

A huge breakthrough occurred with the elucidation of the amino acid sequence of A-gliadin, representative of the structure of α-gliadins (11). Based on this structure, de Ritis et al. (12) found that there were key sequences of four amino acids associated with toxic fractions prepared from A-gliadin, these being QQQP and PSQQ.

It was important to determine the amino acid sequences of peptides in fractions of native gliadin. Fraction 9 was subjected to reverse-phase HPLC, the resulting fractions analysed for amino acid composition and the major one chromatographed and then sequenced (13). The most abundant peptide corresponded to residues 75-86 of A-gliadin (RPQQPYFPQQQ). Fraction 9 and this peptide, prepared synthetically, stimulated the production of γ-interferon in peripheral blood from coeliac patients in remission to a greater degree than in blood from normals (14). It was thought that the key sequence in this peptide could be a tetra...
and a 35% reduction on average (23).

Other fractions except fraction 9, no reduction in urinary xylose was observed, but after fraction 9 was fed there was about a 36% reduction on average (23).

6. Further studies on the mechanism of toxicity

Most of the studies undertaken by other groups concentrate on the pathogenesis of the disease and tend to neglect the reason why the peptides are present in such significant concentrations. Certainly, the presentation of gliadin peptides to T-cells can occur in the proximal small intestine of normal individuals as well as in celiacs, but in normals, the enzymes present would quickly lower the concentrations of toxic peptides, whereas this does not appear to happen with remission celiacs. Evidence for this comes from mucosal digestion studies of synthetic toxic peptides (25,26). In both cases where serine-containing peptides (6-18; 11-18) and tyrosine-containing peptides (73-85; 76-86) were used, residues of peptides such as octa-peptides were obtained in the undigested residues, suggesting that the active motifs in the peptides (PSQ, QQPY respectively) remained undigested, supporting the importance of these motifs to toxicity. The residual peptides corresponded mainly to NSPSQQPQ and QQPYPPQ in each case.

An explanation for the different types of toxicity present in the serine-containing and tyrosine-containing peptides has been provided using molecular modeling (27).
Serine-containing peptides like peptide 11-19 are stabilized by hydrogen bonding into a helical structure which has been shown to be important in cytotoxicity (28), the prominent type of activity measured in the chick assay. On the other hand, tyrosine-containing peptides like 75-86 appear to be random coil type, with much less hydrogen bonding, are more immunogenic and can bring about cytolytic production and other sequelae through interaction with T-cells. What is common to both types of peptides is their inability to be completely digested by remission coeliac mucosa, thus providing the trigger for tissue damage.

7. Studies of immunological mechanisms of pathogenesis

CD is associated mainly with the HLA class II extended haplotypes DR3-DQ2 or DR57-DQ2. The DQ2 molecule is an αβ heterodimer and plays an important role in the immune response. Most patients with CD but also 20% of the general population carry the risk allele encoding for DQ2 (29). Hence, other susceptibility genes of quite different function are possible and it could turn out that those relating to defective peptide synthesis may be discovered.

The immunological data suggest that pathogenesis of CD results from the way in which gliadin peptides are presented to T-cells. The peptides are presented by CD-specific HLA-DQ2-positive antigen-presenting cells and reactions which damage the lamina propria are potentiated by tissue transglutaminase (30). HLA-DQ8-positive antigen-presenting cells are also involved in some patients.

Obviously, in the case of immune mechanisms which are involved in the pathology of CD, the presentation of gliadin peptides by the HLA-DQ2 and HLA-DQ8 coeliac-specific positive antigen-presenting cells must be considered as a primary event in pathogenesis (31). It appears that increases in the affinity of these molecules for these peptides can be induced by selective deamidation by transglutaminase (32), leading to epitopes which can be recognized by T-cells when presented to DQ2 molecules. Molberg et al. (31) provided evidence for TG in CD as having a role in selectively deamidating gliadin peptides that are recognized by T-cells in the small intestine. However, this is not to say that high levels of transglutaminase could be a basic cause of CD, but rather that they would amplify the immunological response.

It is of importance to note that after selective deamidation of the immunoreactive peptide of Anderson et al. (32), the tyrosine-containing motif PYPQ is still present, which has been associated with the toxicity of several peptides from A-gliadin such as QQPY/QPYP (peptide 7-84 of A-gliadin). Immunoreactive peptides such as peptide 57-73 (32) and peptide 45-50 from A-gliadin (33) also contain tyrosine and are often present as 4-mer motifs such as QQPY or QPYP. Peptide 31-49 (20), shown to be toxic in vivo to patients with CD, has both these motifs, but also has QQQP, one of two 4-mer motifs suggested by de Ritis et al. (12), the other being PSQQ, commonly found associated with in vitro activity and shown to be toxic in vitro as peptide 206-217 (21). The disruption of this PSQQ motif associated with toxicity is not achieved by remission coeliac mucosa (25) but would be achieved by prolyl endopeptidase, which attack proline residues on their C- terminal side. The same applies to peptides containing the QQPY/QPYP motif, thus reinforcing the notion of a specific peptidase deficiency of this type in CD (28).

8. Clinical trials of therapeutic products

The discovery of an enzyme deficiency in coeliac disease has opened the way for therapy using enzyme supplementation. A genetic defect is likely in CD which prevents the biosynthesis of enzymes, especially peptidases. The results of mucosal digestion studies suggest that endopeptidases which are needed to split the key motifs of toxic fractions of gliadin are required in coeliac mucosa before complete digestion can occur. If such a product could be introduced into the proximal small intestine intact i.e. avoiding digestion by pepsin, trypsin etc., it could complete the digestion of the gliadin peptides to amino acids and very small peptides which are incapable of harming intestinal cells and tissue. After all, an amino acid mixture corresponding to the wheat proteins is not toxic to coeliac patients. Such a product has been developed by Cornell and Steimauk (34) and has undergone successful clinical trials (35). It is in the form of an enterically coated capsule containing an animal intestinal extract. Such extracts can be prepared from pig, cow or sheep intestine, which is in keeping with the finding that higher
animals do not suffer from CD. These animals have been eating cereal crops for much longer periods of time than humans and have thus acquired the necessary enzymes.

Endopeptidases have been found in the brush border of human enterocytes (30) and Hausch et al. (37) have found that a bacterial prolyl endopeptidase reduces the antigenicity of a gliadin peptide. These enzymes (EC 3.4.21.20) cleave peptide bonds on the C-terminal side of proline residues (30) and would thus cleave the motifs of both the active serine-containing and tyrosine-containing peptides (PSQQ and QQPY/QPPP respectively).

Work is proceeding to identify the enzymes in the cow and pig responsible for detoxifying the gliadin and has shown that the form of enzyme in both animals has a molecular weight between 170 and 200 kD. This fraction from gel permeation chromatography of the intestinal extract is by far the most active for abolishing the activity of PTC gliadin digests towards rat liver lysosomes. These tests need to be extended to toxic peptides of both the active serine-containing and tyrosine-containing peptides (PSQQ and QQPY/QPPP respectively).

Enzyme therapy is a safeguard against the serious effects of long-termingestion of small amounts of gluten. At present the product will counteract only effects of long-termingestion of small amounts of these cereals. At present the product will counteract only effects of long-termingestion of small amounts of these cereals. At present the product will counteract only effects of long-termingestion of small amounts of these cereals. At present the product will counteract only effects of long-termingestion of small amounts of these cereals. At present the product will counteract only effects of long-termingestion of small amounts of these cereals. At present the product will counteract only effects of long-termingestion of small amounts of these cereals. At present the product will counteract only effects of long-termingestion of small amounts of these cereals. At present the product will counteract only effects of long-termingestion of small amounts of these cereals. At present the product will counteract only effects of long-termingestion of small amounts of these cereals. At present the product will counteract only effects of long-termingestion of small amounts of these cereals. At present the product will counteract only effects of long-termingestion of small amounts of these cereals. At present the product will counteract only effects of long-termingestion of small amounts of these cereals. At present the product will counteract only effects of long-termingestion of small amounts of these cereals. At present the product will counteract only effects of long-termingestion of small amounts of these cereals. At present the product will counteract only effects of long-termingestion of small amounts of these cereals. At present the product will counteract only effects of long-termingestion of small amounts of these cereals. At present the product will counteract only effects of long-termingestion of small amounts of these cereals. At present the product will counteract only effects of long-termingestion of small amounts of these cereals. At present the product will counteract only effects of long-termingestion of small amounts of these cereals. At present the product will counteract only effects of long-termingestion of small amounts of these cereals. At present the product will counteract only effects of long-termingestion of small amounts of these cereals. At present the product will counteract only effects of long-termingestion of small amounts of these cereals. At present the product will counteract only effects of long-termingestion of small amounts of these cereals. At present the product will counteract only effects of long-termingestion of small amounts of these cereals.

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