Research Focus

Enzymatic gluten detoxification: the proof of the pudding is in the eating!

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Celiac disease is caused by an immune response to the dietary protein gluten. The only available treatment is the strict exclusion of gluten from the diet; however, this is marred by the virtual omnipresence of this protein. The enzymatic degradation of gluten might become an alternative to the gluten-free diet, and recent work indicates that such approaches are getting close to being tested in clinical trials.

Introduction

Celiac disease is a disorder of the small intestine, affecting between 0.5 and 1% of the general population in the western hemisphere. The disease is caused by inflammatory responses to dietary wheat gluten and homologous proteins from barley, rye and possibly oats [1]. The chronic inflammation leads to localized tissue damage in the upper small intestine. Characteristic symptoms are abdominal pain, diarrhoea, malnutrition and a wide variety of other manifestations. Celiac disease has a strong genetic component: 95% of the patients are HLA-DQ2 positive, whereas the remainder are usually HLA-DQ8 positive. This can be explained by the fact that HLA molecules can specifically bind gluten-derived peptides and present them to gluten-specific T cells in the lamina propria of the small intestine of patients. The ensuing T-cell response is characterized by the secretion of pro-inflammatory cytokines such as gamma-interferon. An interesting twist to this story is that most gluten peptides must be modified before they can bind to HLA-DQ2 or HLA-DQ8. This modification, the conversion of glutamine into glutamic acid, is the result of the activity of the enzyme tissue transglutaminase in the small intestine. Tissue transglutaminase preferentially modifies glutamine residues in the sequence Gln–X–Pro [2,3], which is abundant in gluten molecules – they contain up to 35% glutamine and 17% proline residues [4]. Consequently, a large repertoire of gluten peptides that are capable of stimulating T cells exists. The high proline content in gluten also poses a problem when it comes to break down: the proline-rich immunogenic regions of gluten molecules are extremely resistant to degradation in the gastrointestinal tract [5]. Thus, relatively large gluten fragments remain intact, enabling modification by tissue transglutaminase and subsequent binding to HLA-DQ2 or HLA-DQ8, followed by T cell activation. Accelerating the breakdown of gluten might, therefore, be an effective way to eliminate the disease-inducing properties of gluten.

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Oral enzyme supplementation

In 2002, Chaitan Khosla and colleagues proposed the use of a prolyl endopeptidase (PEP) from *Flavobacterium meningosepticum* to accelerate the breakdown of gluten [5]. Oral supplementation with such a post-proline cutting enzyme, administered just prior or together with a gluten-containing meal, might be an effective way to remove gluten toxicity because it would degrade gluten into fragments that can no longer bind to HLA-DQ2 or HLA-DQ8. Indeed, the enzyme was shown to cut toxic gluten-derived peptides *in vitro*. *In vivo* application, however, was hampered by the fact that the enzyme was inactivated by low pH and pepsin, both present in the stomach. Additional limitations of this enzyme result from its relatively low activity and its preference for small peptides as substrates [6]. Bacterial PEP alone would therefore be unable to degrade gluten before it reaches the small intestine, the site where gluten sensitivity manifests itself. In a recent article, Khosla and colleagues now propose a combination therapy to overcome this problem.

Combination therapy

Glutamine-rich gluten and similar cereal proteins are a source of nitrogen for the germinating grains; the mobilization of these reserves requires the degradation of these proteins into small peptides and single amino acids. In barley, EP-B2 is one of the enzymes involved in this process [7]. EP-B2 is a cysteine endoprotease that is produced as a zymogene and is activated by low pH. The enzyme was found to be resistant to pepsin degradation and to be active at a broad pH range, indicating that it might be effective in the harsh environment of the stomach [8]. EP-B2 was found to cut gluten peptides and a recombinant gluten molecule into small fragments, most of which lose the capacity to bind to HLA-DQ2 or HLA-DQ8. EP-B2 shows some specificity for glutamine and, notably, the tissue transglutaminase consensus sequence Gln–X–Pro; it thus destroys many potential sites of gluten modification. Moreover, Khosla and his co-workers demonstrate that by combining EP-B2 with bacterial PEP, gluten could be fully degraded under simulated gastrointestinal conditions [9]. The authors concluded that their results had set the stage for clinical testing.

Monotherapy

An alternative to this approach relies on the application of a single enzyme such as prolyl endoprotease from *Aspergillus niger*, a food-grade organism [10]. This enzyme, termed AN-PEP, is unrelated to the above-mentioned bacterial PEP, it works optimally at acidic pH and is also resistant to pepsin. In a recent study we have shown that it effectively degrades all T-cell-stimulatory gluten peptides tested under conditions that mimic those in the stomach [11]. Moreover, the enzyme breaks down intact gluten molecules into fragments that can no longer bind to HLA-DQ2 and HLA-DQ8. Finally, the enzyme is food grade, extremely stable and can be produced cost effectively. We conclude that AN-PEP is a potential prime candidate for testing in clinical trials [11].

Will oral supplementation work *in vivo*?

The proof of the pudding is in the eating: in this case a pudding that contains gluten. But will these approaches be effective in patients? It should be pointed out that the above-mentioned studies have been carried out with gluten peptides, recombinant gluten molecules and crude gluten, which are not the same as the gluten present in our day-to-day foodstuffs, where it has often been baked or cooked and is mixed with other diverse food components. Therefore, the gluten in normal food might be less readily accessible to enzymes and harder to degrade. Furthermore, because there are no good animal models for celiac disease, clinical trials will ultimately have to demonstrate if oral enzyme supplementation can be developed into an effective treatment. Oral supplementation might not only allow gluten consumption by celiac patients but also could help to control the detrimental effects resulting from contamination of *bona fide* gluten-free products: in both cases, it would have a positive impact on the quality of life of the patients. It is also plausible that enzymatic gluten degradation might be useful for the production of gluten-free foods. Two approaches, combination therapy and monotherapy, can now be explored. Obviously, from the point of view of the patients, it does not matter which of these two approaches proves most effective as long as it works, is affordable, easy to handle and safe.

In conclusion, a novel approach to treat celiac disease is going to be tested in clinical trials soon. If successful, this would be the first time patients are provided with an alternative to a life-long gluten-free diet.

References

1 Koning, F. (2005) Celiac disease: caught between a rock and a hard place. *Gastroenterology* 129, 1294–1301

2 van de Wal, Y. *et al.* (1998) Selective deamidation by tissue transglutaminase strongly enhances gliadin-specific T cell reactivity. *J. Immunol.* 161, 1585–1588

3 Molberg, O. *et al.* (1998) Tissue transglutaminase selectively modifies gliadin peptides that are recognized by gut-derived T cells in celiac disease. *Nat. Med.* 4, 713–717

4 Wieser, H. *et al.* (1980) Vergleichende Untersuchungen über partielle Aminosäuresequenzen von Prolaminen und Glutelinen verschiedener Getreidearten. II. Fraktionierung der Gluteline (Comparative investigations of partial amino acid sequences of prolamines and glutelins of different cereals. II. Fractionation of glutelins) *Z. Lebensm. Unters. Forsch.* 171, 430–436

5 Shan, L. *et al.* (2002) Structural basis for gluten intolerance in celiac sprue. *Science* 297, 2275–2279

6 Shan, L. *et al.* (2004) Comparative biochemical analysis of three bacterial prolyl endopeptidases: implications for coeliac sprue. *Biochem. J.* 383, 311–318

7 Davy, A. *et al.* (1998) Substrate specificity of barley cysteine endoproteases EP-A and EP-B. *Plant Physiol.* 117, 255–261

8 Bethune, M.T. *et al.* (2006) Heterologous expression, purification, refolding, and structural-functional characterization of EP-B2, a self-activating barley cysteine endoprotease. *Chem. Biol.* 13, 637–647

9 Siegel, M. *et al.* (2006) Rational design of combination enzyme therapy for celiac sprue. *Chem. Biol.* 13, 649–658

10 Edens, L. *et al.* (2005) Extracellular prolyl endoprotease from *Aspergillus niger* and its use in the debittering of protein hydrolysates. *J. Agric. Food Chem.* 53, 7950–7957

11 Stepniak, D. *et al.* (2006) Highly efficient gluten degradation with a newly identified prolyl endoprotease: implications for celiac disease. *Am. J. Physiol. Gastrointest. Liver Physiol.* Epub ahead of print doi:10.1152/ajpgi.00034.2006 (http://ajpgi.physiology.org)