

EDITORIALS

A Search for the Holy Grail: Non-Toxic Gluten for Celiac Patients

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Celiac disease (CD) has recently gained a central stage in the field of autoimmune/gastrointestinal diseases and several factors have contributed in raising its profile. The first is the surprisingly high incidence of CD with a prevalence which is, although some regional variations have been reported, approaching 1:100.¹ However, it is not clear whether such a high incidence of CD is simply due to the new and more accurate serological tests based on the detection of antibodies to transglutaminase 2 (TG2)² or whether it reflects a true rise of incidence in the recent years. In this context, it is now accepted that the last few decades have witnessed a steady increase of prevalence for other autoimmune diseases such as Type 1 diabetes.³ This indicates that environmental factors are involved in the increase of autoimmune diseases in general and this can also be easily hypothesized for celiac disease. Indeed different varieties of wheat, diet style, and modalities of foods preparation have, in the last decades, been radically modified and all of these factors might have contributed to the increase of CD prevalence. As an example, the 1980s CD epidemic in Swedish children which was essentially due to the introduction of gluten in infant food.⁴ It is therefore likely that better diagnostic tools have helped to highlight a genuine incidence increase of CD making the impact of CD even greater.

Another reason why CD has achieved high prominence is the series of reports that have, on the whole, deciphered its pathogenic mechanisms at the basis of CD.⁵ The primary environmental cause of CD is wheat gluten, a mixture of 2 protein families, the gliadins and glutenins.⁶ Both gliadins and glutenins contain “toxic” fragments, and α -gliadin is the most frequently recognized by CD patients, thus serving as archetype to decode the molecular basis of CD.⁵

At the center of CD pathogenesis there is an aberrant and “disproportionate” immune recognition of gluten by lamina propria CD4+ T cells.⁵ That CD4+ T cells had to be involved was unequivocally suggested by the strong MHC class II association, as nearly the totality of CD patients are HLA-DQ2+ with the remaining expressing HLA-DQ8.⁷ The evidence that a relatively small group

of proteins was involved in the pathogenesis of CD was instrumental in uncovering how gluten leads to small intestine damage. It was reported that α -gliadin, as well as other gluten fractions, had a “dangerous liaison” with the enzyme transglutaminase 2 (TG2), caused by the high content of prolines and glutamines, which catalyzed a crucial posttranslational modification by deamidating key glutamines to glutamic acid.^{8,9} This modification exponentially multiplies the ability of such fragments to bind HLA-DQ2/8 molecules, which in turn activate CD4+ T cells ultimately leading to small intestinal damage.¹⁰ The net result of this posttranslational modification is a “qualitative” increase of “toxicity” of gluten. It has to be mentioned that also “quantitative” factors are involved, as homozygosity for HLA-DQ2 can more than quadruple the risk to develop CD.¹¹ All these elements indicate that there is a critical “boundary” for the induction of CD, and that any strategy (qualitative or quantitative) aimed at containing the level of the triggering factor (gluten) below the critical upper limit has valuable therapeutic repercussions. At the moment the therapeutic strategy, the safe and effective gluten-free diet, aims to “quantitatively” curb gluten toxicity. More qualitatively approaches, such as inhibition of TG2, might produce unwanted and dangerous side effects and are still far from general clinical application. As indicated before, the biochemical make-up of gluten/gliadins with a high content of glutamine and proline defines their affinity for TG2. Similarly, the proline richness has a bearing on the resistance of gluten break down by digestive enzymes. This toughness to digestion permits the dangerous portions of gluten to “trespass” the intestinal barrier in significant amounts, triggering the pathogenic immune response. A fragment of α -gliadin (residues 56–88), containing several immunodominant T cell epitopes, epitomizes this resistance to digestive enzymes degradation.¹² The original report describing gliadin’s resistance to enzymatic digestion also indicated that a class of bacterial enzymes, the prolyl endopeptidases (PEP), could break down these gliadin fragments neutralizing gluten toxicity.^{13,14}

Although a gluten-free diet is currently the “safe” and appropriate therapy for CD, this is not always an easy and simple option as “harmful” gluten may contaminate foods during the processing and preparation phases.¹⁵

There are also further social pressures, which might be more pressing for young CD patients, in following a strict gluten-free diet. There is also no consensus on the minimal amount of gluten permitted in foods and this reflects on the clinical experience as celiac patients do not respond in the same way to occasional presence of “toxic” gluten in the diet, with some patients querying the necessity of complying with a very strict gluten-free diet. Even in celiac patients apparently tolerant to gluten, however, persistent gluten challenge can promote a series of life-threatening complications.¹⁶

Therefore, every new realistic method to reduce the “dose” of toxic gluten is worth of consideration. As mentioned above “quantitative” strategies to reduce gluten toxicity are at the moment the front runners as “treatment” of CD. It is apparent that a quantitative diminution of toxic gluten can be achieved in 2 main ways, by reducing the intake as in the case of the gluten-free diet, or by the detoxification of gluten prior its arrival in the small intestine. Two papers^{17,18} in this issue of GASTROENTEROLOGY address these central topics by exploring the viability of enzymatic detoxification of gluten and the search for less toxic varieties of wheat. Both of these studies have obvious scientific implications which might permit a deeper understanding of CD, but more significantly they focus on new strategies to treat CD.

The papers by Matysiak–Budinik et al¹⁷ and Spaenij–Dekking et al¹⁸, although focusing on a similar aim (reduction of toxic gluten), target 2 different options. Matysiak–Budinik et al¹⁷ explore the efficiency of the process of detoxification operated by PEP in a series of in vitro and ex vivo assays. The authors focus their attention on 2 fragments of α -gliadin, α -gliadin 56–88, which contains a high concentration of T cell epitopes, and a second region (α -gliadin 31–49) which has been reported to be involved in a complementary pathogenic cascade.¹⁹ The data described by the French group are in keeping with previous studies indicating that PEP can digest α -gliadin 56–88, as well as α -gliadin 31–43. However, they also indicate that high doses of PEP (up to 500 mU/ml) are required. At variance from previous studies Matysiak–Budinik et al¹⁷ demonstrate that partially digested fragments of the α -gliadin 56–88 still contain immunodominant T cell epitopes which could trigger pathogenic T cell activation. They further observed that peptide 31–49 was also digested to a shorter but still potentially harmful sequence 31–42. The most interesting part of the study is the demonstration that these partially digested, but still potentially toxic gliadin fragments could cross the epithelial barrier and ultimately reach the lamina propria at concentrations re-

ported to be toxic in vitro and ex vivo.^{12,19} True detoxification was only achieved at PEP doses that might not be easily used in patients. Therefore this study, though confirming the potential use of PEP to detoxify gluten, questions the widespread clinical application of PEP. At present, this therapeutic option appears to be more an “auxiliary” or a “social” alternative to gluten-free diet. This study, however, provides interesting insights on how enzymes supplementation might be used to neutralize gluten toxicity and how “toxic” peptides cross the mucosa in CD.

On the same wavelength of a quantitative therapeutic strategy is the paper by Spaenij–Dekking et al,¹⁸ which focuses on a more steady and long-term therapeutic and potentially preventative approach. In this paper, the authors test a simple question which already other groups, in most cases with limited success, had tried to address: are all wheat varieties equally toxic for celiacs? In this challenging search for less toxic wheat it has even been proposed to detoxify wheat via genetic manipulation to remove or scramble the toxic stretches of gluten. The transgenic crop strategy, although attractive, has had a series of set backs. First, the objective difficulty of the procedure and secondly the considerable public opposition to genetically modified (GM) crops. The authors of this second report therefore choose a “greener” approach to GM crops and explored the toxicity of several wheat varieties. The knowledge gained in these last few years about immunodominant T cell epitopes, the availability of gluten-specific T cell clones and monoclonal antibodies have permitted the authors to perform a more systematic analysis than most of the previous studies. The work of the Dutch group indicates that some wheat varieties are likely to be less toxic for celiacs, according to the screening they apply. However, they also report that some gluten toxic regions are widely expressed in most wheat varieties, indicating that all wheat varieties will have some residual toxicity. Thus, the hope is that less toxic varieties of wheat may be tolerated by celiacs and represent an alternative to other food preparation. The next question that will have to be answered is whether such varieties might become widely used for the production of bread and other celiac foods. There are, however, a series of caveats that have to be considered. The first is to understand whether growing crops of such “less toxic” varieties is a commercially viable option. The second is whether the in vitro results, although obtained with a large, but not complete, repertoire of T cell clones and antibodies, truly predict less toxic wheat varieties. There is, as for the first report, other important information generated by this study. Indeed the work of Spaenij–Dekking et al, in keeping with another recent paper by

Sollid's group,²⁰ indicates that ancient varieties of wheat are apparently less toxic. These findings indicate that environmental pressures evolved over thousands of years have selected the more "toxic" wheat, ultimately leading to the observed incidence of CD. We end this editorial with a provocative note for the whole celiac scientific community. As discussed previously, strategies to control CD are now focussed on *quantitative* rather than *qualitative* strategies to reduce the gluten toxic load. All of these strategies are palliatives as they do not ultimately treat CD. The future challenge has to be a quest for truly therapeutic and safe approaches which will permit celiacs to "peacefully" coexist with gluten. This is a mandatory requirement for the small but sizeable section of patients who have become refractory and thus are not any more responsive to a strict gluten-free diet. The next few years will witness whether this is a challenge that could be taken on.

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