

Future options for treatments alternative to the gluten free diet

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The cornerstone of treatment of CD is a lifelong adherence to a strict GFD devoid of proteins from wheat, rye, barley and related cereals. Gluten is, however, a common (and in many countries unlabeled) ingredient in the human diet, presenting a big challenge for CD patients. Gluten-free products are not widely available and are more expensive than their gluten-containing counterparts. Dietary compliance is therefore suboptimal in a large proportion of patients. More than 50% of subjects that embrace a diet for medical reasons (hypertension, obesity, high cholesterol, diabetes, renal failure, etc) fail to comply over time, making any diet therapy a high risk proposition. Furthermore, even when compliance is not an issue, a high percentage of CD subjects on a GFD that are symptom-free and test negative to CD serology show persistence of severe intestinal damage. Progress made in the understanding of the cellular and molecular basis of CD led to the identification of potential targets for therapy.

Enzyme therapy: It has been shown that because of the high proline content, gliadin peptides are highly resistant to digestive processing by pancreatic and brush border proteases. Enzyme supplement therapy with the use of bacterial prolyl endopeptidases has been proposed to promote complete digestion of cereal proteins and thus destroy T-cell multipotent epitopes. It remains to be assessed to what extent such intraluminal digestion may detoxify peptides particularly active in the most proximal part of the small intestine. An alternative approach to reduce gluten toxicity is based on a pretreatment of whole gluten or gluten-containing food with bacterial-derived peptidase. Enzymatic detoxification of gluten has the potential to be an effective method for producing more palatable gluten-free products and possibly treating CD. Proteases of certain lactobacilli present in sourdough are able to proteolyze proline-rich gluten peptides. CD patients

subjected to an acute challenge tolerated breads produced with sourdough (lactobacillus digested) better than those with baker's yeast.

Engineered grains and inhibitory gliadin peptides: Breeding programs and/or transgenic technology may lead to production of wheat that is devoid of biologically active peptide sequences. Site- directed mutagenesis of wheat, which would not affect the baking properties, has also been proposed, although the number and the repetition of such sequences in wheat render this approach difficult. The identification of specific epitopes may also provide a target for immunomodulation of antigenic peptides. According to the nature of amino acid residue in the position interacting with the specific TCR, peptide recognition can turn out in a cellular activation (agonist), ignorance (null peptides), or unresponsiveness, known also as anergy (antagonist). Peptide analogues of gliadin epitope(s) can be engineered with antagonistic effects of native peptide(s). Of course, the chances of success of using analogue peptides to modulate specific immune responses could be hampered by the great heterogeneity of gliadin T-cell epitopes so far identified. Further studies aimed to elucidate the hierarchy of pathogenic gliadin epitopes and their core region would be of crucial importance for engineering peptide-based therapy.

Immunomodulatory strategies: The autoantigenic enzyme tissue transglutaminase (TTG) is mainly expressed in the lamina propria and its expression is upregulated by various stimuli, such as mechanical stress or bacterial/viral infection, during active CD. The enzyme catalyzes transamidation between a glutamine residue of a glutamine-donor protein and a lysine residue of a glutamine-acceptor protein, linking these proteins with a stable intermolecular isopeptide bond and increasing their rate of phagocytosis by antigen-presenting cells. Although the precise molecular details of this interaction in vivo

remain unclear, selective inhibition of TTG in the small intestine might represent a therapeutically useful strategy for countering the immunotoxic response to dietary gluten in CD. The substitution of a glutamine residue with 6-diazo-5-oxo-norleucine (DON) transforms an immunodominant gluten peptide into a potent inhibitor of tissue transglutaminase. DON-modified peptides could be useful for the study and therapy of CD. The efficacy and side effects of TTG inhibitors as a treatment of CD are unknown. The crucial role of the HLA in CD development makes it an obvious target for therapeutic intervention. Blocking of peptide presentation by DQ2 is an attractive approach for a new treatment of CD because DQ2 (or DQ8) is a necessary but insufficient genetic component for disease development. Furthermore, other immunomodulatory targets, including IL-10 are possible alternative tools for promoting tolerance. However, evidence that gluten toxicity is not dependent only on T-cell recognition is growing. In this regard, the mechanism of toxicity of peptide remains unknown. Activation of innate immunity has been demonstrated, and antibodies to IL-15 have been proposed, particularly in the treatment of refractory sprue because of the IEL-activating role of IL-15. Nevertheless, one should realize that treated CD is a benign condition and dietary treatment is safe, although strenuous. Therefore, any immunomodulatory approach must have a safety profile equivalent to that of the GFD, but with the advantage of increased compliance.

Correction of the intestinal barrier defect: The primary functions of the gastrointestinal tract have traditionally been perceived to be limited to the digestion and absorption of nutrients and electrolytes, and to water homeostasis. A more attentive analysis of the anatomic and functional arrangement of the gastrointestinal tract,

however, suggests that its barrier function and ability to regulate the trafficking of macromolecules between the environment and the host is another extremely important function of this organ. Together with the gut associated lymphoid tissue and the neuroendocrine network, the intestinal epithelial barrier, with its intercellular tight junctions, controls the equilibrium between tolerance and immunity to non-self antigens. When the finely tuned trafficking of macromolecules is dysregulated in genetically susceptible individuals, both intestinal and extra-intestinal autoimmune disorders can occur. This new paradigm subverts traditional theories underlying the development of autoimmunity, which are based on molecular mimicry and/or the bystander effect, and suggests that the autoimmune process can be arrested if the interplay between genes and environmental triggers is prevented by re-establishing the intestinal barrier function.

A common denominator of autoimmune diseases is the presence of several preexisting conditions that lead to an autoimmune process. The first is the genetic susceptibility of the host immune system to recognize, and potentially misinterpret, an environmental antigen presented within the gastrointestinal tract. Second, the host must be exposed to the antigen. Finally, the antigen must be presented to the gastrointestinal mucosal immune system following its paracellular passage from the intestinal lumen to the gut submucosa, which is normally prevented by competent tight junctions. In many cases, increased intestinal permeability seems to precede disease and causes an abnormality in antigen delivery that triggers the multiorgan process leading to the autoimmune response.

Taking the information above into consideration, it is conceivable to propose that the pathogenesis of autoimmune diseases, including CD, can now be described by the following three key points. First, autoimmune diseases involve a miscommunication

between innate and adaptive immunity. Second, molecular mimicry or bystander effects alone might not explain entirely the complex events involved in the pathogenesis of autoimmune diseases. Rather, the continuous stimulation by nonself antigens (environmental triggers) seems to be necessary to perpetuate the process. Contrary to general belief, this concept implies that the autoimmune response can theoretically be stopped and perhaps reversed if the interplay between genes predisposing individuals to the development of autoimmunity and environmental triggers is prevented or eliminated. Third, in addition to genetic predisposition and exposure to triggering nonself antigens, the loss of the protective function of mucosal barriers that interface with the environment (mainly the gastrointestinal and lung mucosa) is necessary for autoimmunity to develop. Based on this theory, it is possible to conceptualize that the removal of any of the three elements necessary to develop autoimmunity (i.e., genetic predisposition, exposure to the environmental trigger (s), or defect of the intestinal barrier function) would be a valid therapeutic option. Given that elimination of the predisposing genes is not a valuable option and that the removal of the trigger antigen (an option available only for CD) has its own challenges (see above), the correction of the intestinal barrier defects may represent an innovative therapeutic alternative. Small intestinal permeability abnormalities are seen in untreated CD patients, which return to normal on a GFD. The use of the zonulin inhibitor AT1001 to correct intestinal barrier defects has been already successfully explored in an animal model of autoimmunity. More recently, AT1001 has been tested in an inpatient, double-blind, randomized placebo controlled human clinical trial to determine its safety, tolerability, and preliminary efficacy. No increase in adverse events was recorded among patients exposed to AT-1001 as

compared to placebo. Following acute gluten exposure, a 70% increase in intestinal permeability was detected in the placebo group, while no changes were seen in the AT-1001 group [32]. After gluten exposure, IFN- γ levels increased in 4 out of 7 patients (57.1%) of the placebo-group, but only in 4 out of 14 patients (28.6%) of the AT-1001-group. Gastrointestinal symptoms were significantly more frequent among patients of the placebo group as compared to the AT-1001 group. Combined, these data suggest that AT-1001 is well tolerated and appears to reduce gluten-induced intestinal barrier dysfunction, pro-inflammatory cytokine production, and gastrointestinal symptoms in celiac patients.