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Previews

A Molecular Warhead and Its Target: Tissue Transglutaminase and Celiac Sprue

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 celiac sprue.

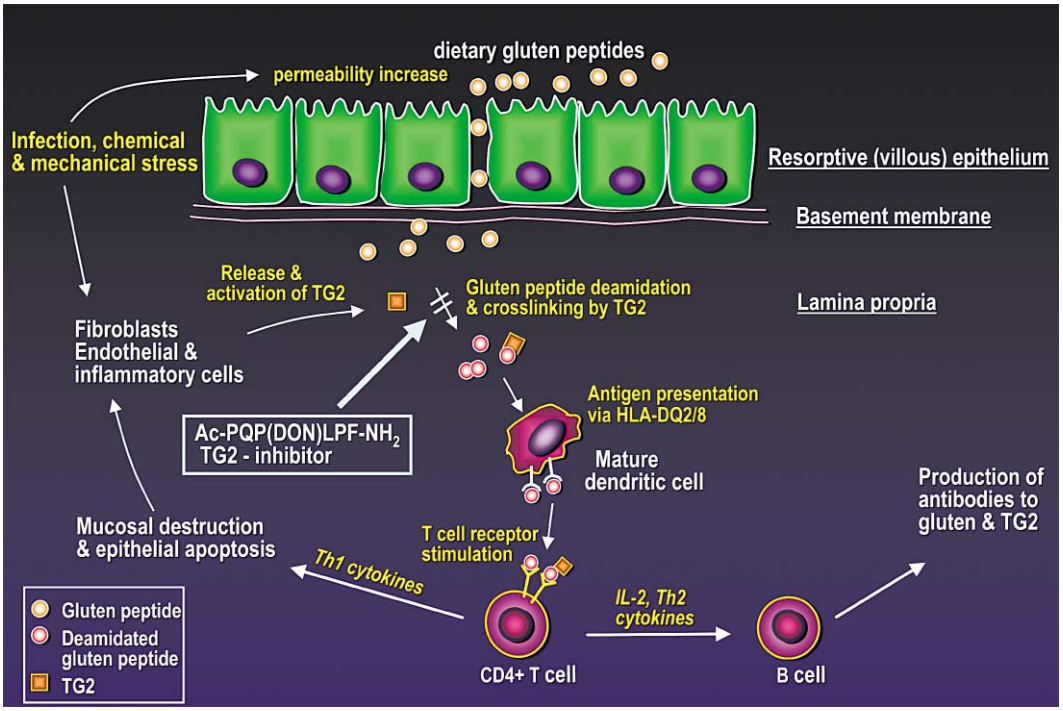
Celiac Sprue is an inflammatory disease of the small intestine characterized by T cell infiltrates in the intestinal epithelium and the underlying lamina propria (supporting connective tissue). This inflammation can lead to destruction of the microscopic small intestinal villi and may drastically reduce the surface and destroy the structure of the resorptive intestinal epithelium [1]. The consequences of these actions are severe malabsorption of nutrients, vitamins, and minerals that can lead to profuse diarrhea, malnutrition, growth retardation, anemia, osteoporosis, or neurological dysfunction. While this classical presentation of Celiac Sprue is relatively rare (ranging from 1:2,000 to 1:10,000 in the Western world), oligo- or even asymptomatic Celiac Sprue is frequent, with an estimated prevalence of 1:200 in Europe and the USA. Nonetheless, these oligo- or asymptomatic Celiac patients appear to run a high risk for later disease exacerbation, secondary autoimmune disorders, or even malignancies, which are associated with untreated Celiac Sprue [2, 3].

The disease has peculiar features, most of which were uncovered only recently, that make it amenable to targeted intervention. The pathogenesis of Celiac Sprue is initiated by the ingestion of gluten, the storage protein of wheat, barley and rye. Key traits of the disease include: (1) it usually remits upon strict dietary avoidance of gluten, (2) it only manifests in patients with a unique genetic background for antigen presentation (specifically, the HLA-class II molecules DQ2 or DQ8), and (3) it is tightly associated with circulating mucosal (IgA-class) autoantibodies to the enzyme tissue transglutaminase (TG2) [4, 5]. These features of the disease are closely linked with one another. Thus, when gluten peptides are presented via HLA-DQ2 or -DQ8 on antigen-presenting cells, such as macrophages, dendritic, or B cells, proliferation and proinflammatory cytokine production is generally elicited in intestinal T cells.

The autoantigenic enzyme TG2 is mainly expressed in the lamina propria (the major DQ2/DQ8-reactive T cell reservoir of the gut), and its expression is upregulated by various stimuli, such as mechanical stress or bacterial/viral infection, during active Celiac Sprue. 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 [6]. TG2 has a particular affinity for the glutamine-rich proteins of gluten (mainly the gliadins), in which glutamine represents 30%–50% of the amino acids. Most significantly, gluten-TG2 complexes can be formed by a process of autocatalysis, and these complexes appear to trigger autoantibody production by B cells via so-called antibody crosspresentation. In addition, TG2 can deamidate some of the glutamine residues in gluten to glutamic acid, especially at low pH. The resulting de novo-introduced negative charge has been shown to increase the binding of gluten epitopes to antigen-presenting HLA-DQ2 or -DQ8 proteins, enhancing their ability for antigenic presentation and potentiating their T cell stimulatory capacity [4, 5, 7]. Various immunodominant gluten peptides that are all substrates of TG2 have been identified [8], and a unique 33-mer peptide within the 266-amino-acid-long α2 gliadin component of gluten, which is resistant to intestinal proteases, was recently shown to harbour six partially overlapping HLA-DQ2 binding and T cell stimulatory peptide epitopes [9].

Since Celiac Sprue is among the most frequent genetically determined disorders (prevalence around 1 in 200), and since at present patients with Celiac Sprue have to maintain a life-long strictly gluten-free diet which exacts a heavy toll on social interactions in our wheat-based societies, pharmacological or immunological treatment alternatives are urgently needed. The paper by Hausch et al. [10] in this issue of *Chemistry & Biology* advances such endeavors one step further by constructing and testing gluten peptide-based inhibitors of TG2, which is an obvious target since it is so intricately involved in Celiac Sprue pathogenesis. The authors substituted the glutamine in the immunodominant peptide substrate Ac-PQPQLPF-NH_2 with a 6-diazo-5-oxo-norleucine (DON) residue to obtain a highly active and tight binding inhibitor of TG2 with low cellular toxicity. Ac-PQP(DON)LPF-NH_2 blocked more than 90% of TG2 activity at submicromolar concentrations and therefore could serve as a lead compound for a novel class of TG2 inhibitors that interrupt the vicious cycle of Celiac Sprue pathogenesis (potentiation of gluten antigenicity via TG2, enhanced T cell activation and inflammation, and finally increased production and release of TG2 as a response to the renewed inflammatory stimulus; see Figure). The overall approach is attractive and is complementary to strategies envisaged by other groups to gain control of Celiac Sprue in the face of continuous gluten exposure as the result of a "normal" Western diet [11].

However, there is still some way to go before therapies for pharmacological prevention of the disease and for treatment become available. In this study, selectivity of the inhibitory peptide was only checked against the relatively unrelated γ-glutaminyl-transpeptidase, whereas other closely related transglutaminases were not considered, including factor XIII, a fundamental protein component of the blood clotting pathway, and TG1, TG3, and TG5, which are essential for dermal epithelialization



Molecular Pathogenesis of Celiac Sprue and the Role of TG2 Inhibition

Infections or mechanical and chemical injury, which lead to an increased permeability of the mucosal epithelium, facilitate the entrance of dietary gluten peptides into subepithelial regions. In the lamina propria, the gluten peptides encounter TG2 which is released from stressed endothelia, fibroblasts, and inflammatory cells residing in the subepithelial region. Crosslinking of gluten by TG2 potentiates its uptake and presentation by antigen-presenting cells, and its deamidation improves the binding to HLA-DQ2/8 molecules. The presentation of the gluten peptides on professional antigen-presenting cells, in particular dendritic cells, triggers a vigorous T cell response that induces inflammation and tissue remodeling (Th1 reaction) or antibody production (Th2 reaction). Inflammation stimulates further release of TG2, thus initiating a vicious cycle that may be interrupted by TG2 inhibitors like Ac-PQP(DON)LPF-NH_2.
A simplified scheme is shown, since both dendritic and T cells circulate to mesenterial lymph nodes (where encounters with T cells occur) and back to the lamina propria.

[12]. Other studies have shown that while the knockout of TG2 alone did not reveal a short-term phenotype [13], abrogation of total TG activity is expected to cause a severe pathology, since TG activity is required for fundamental physiological processes like apoptosis, stabilization of epithelial cell layers and the extracellular matrix, and activation of the key cytokine transforming growth factor β [6, 12]. Therefore, the anticipated effect of inhibition of these transglutaminase could cause pathologies ranging from general, solely dermal, or intestinal, if a strictly enteric drug were devised.

Furthermore, although improved binding of antigenic gluten peptides to HLA-DQ2 or -DQ8 by TG2-catalyzed deamidation of glutamine has been unequivocally demonstrated to enhance stimulation of T cells from patients with Celiac Sprue in vitro, the opposite, i.e., immunological silencing, may occur in vivo. Reports have shown that in the case of autoimmune diabetes (another HLA-DQ-linked and T cell mediated disease), it is not the tightly bound but rather the weakly bound HLA-DQ peptides of the diabetes autoantigen glutamic acid decarboxylase (GAD) that cause disease [14].

Therefore, the jury is still out about the beneficial effects of gut- and Celiac Sprue-specific TG inhibitors, and in vivo testing is essential to predict whether this approach is viable. Nonetheless, this report by Hausch et al. [10] is a good example of translational research, which uses the knowledge of the pathological mechanisms of a disease to generate targeted treatment strategies.

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Bridging the Synthetic and Biopolymer Worlds with Peptide-Drug Conjugates

Roberts and Li break the standard library mold by generating hybrid libraries of small molecules tethered to peptides. Hybrid libraries harness larger chemical and structural diversities and thus represent a new frontier in lead drug discovery.

At first glance, the report by Li and Roberts published in this issue of *Chemistry & Biology* would appear to be a move in the wrong direction [1]. Who would want to mess up a perfectly good small molecule by tethering it to a peptide with the expected effective loss in pharmacokinetics? The answer is affinity. The two researchers have developed a clever way to boost drug affinities by tapping into the massive combinatorial potential of peptide libraries. The technique holds promise for rapidly optimizing the binding affinities of lead molecules without being confronted with the sometimes excruciating task of preparing large libraries of small molecule analogs.

To accomplish their goal, Li and Roberts literally had to bridge two important but distinct areas of drug lead discovery: biopolymer libraries and small molecules. Biopolymer libraries, which can offer dazzling molecular diversities approaching a trillion or more different compounds, are central to this new technology. Libraries of biopolymers, such as peptides, RNA, or DNA, are readily accessible to researchers and are a standard tool used to generate ligands for a particular target or to screen for a lead compound or polypeptide drug with improved affinity. The tremendous diversity of such libraries can often provide a starting point for such screens with relatively little synthetic effort [2]. On the other hand, combinatorial libraries of small molecules are much more laborious to prepare and rarely approach the levels of diversity possible with biopolymers. However, one crucial advantage of using small molecules is the ability to explore the physical and chemical parameters of complementary molecular diversity well beyond the 20 naturally occurring amino acids or 5 nucleotides. Until now, combinatorial small molecule and biopolymer libraries have been considered separate approaches to generate molecular diversity, but this perception will change as a consequence of this innovative research producing libraries comprised of small molecule-peptide hybrids.

Though many methods for constructing peptide libraries are available, the best physically connect each peptide with its encoding DNA. Phage display is one example of this methodology. Here, for each individual constituent of the library, the peptide is attached to the surface of a bacteriophage, a virus that infects bacteria, and the DNA encoding the displayed peptide is encapsulated by the phage particle (a convenient vehicle for site-directed mutagenesis and sequencing to determine the identity of the displayed peptide). Library diversity is also vital for its value as a research tool. An underappreciated source for generating diversity, whether DNA, RNA, or peptide, is chemically synthesized degenerate oligonucleotides, made possible by phenomenally efficient phosphoramidite coupling reactions. However, when library diversities grow larger than about one million different peptides, assaying or screening each peptide individually becomes impractical, and at this stage strategies are required to select peptides with desirable properties (e.g., binding to a particular receptor) before amplifying the selectants. Another advantage of phage display is that peptides selected for their desired properties can be readily amplified in an *E. coli* host. By repeating the process of peptide selection and amplification multiple times, the staggering varieties of peptides found in the original library can be narrowed down to a few peptides with sought-after characteristics.

Li and Roberts use an alternative to phage display in this study. They utilized the mRNA display format that traces its roots to studies of "polysomes" (multiple ribosomes translating an mRNA transcript) carried out in the 1970s. This technology was improved in a number of ways in the 1990s, including the optimization of mRNA 5′ and 3′ end sequences and the addition of chaperones to the in vitro translation mixture, to facilitate the synthesis of peptide libraries with diversities greater than 10^{12} different peptides [3, 4]. Perhaps the most significant advance in the context of library synthesis was made by Roberts and Szostak, who developed a technique to covalently connect an mRNA to its peptide translation product [5]. To this end, the antibiotic puromycin and a short DNA linker are first appended to the 3′ end of synthetic mRNAs. When the ribosome translates this modified mRNA, it stalls upon reaching the DNA linker, and the puromycin at the tail of the mRNA/DNA hybrid