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Therapeutic approaches for celiac disease

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Abstract

Celiac disease is a common, lifelong autoimmune disorder for which dietary control is the only accepted form of therapy. A strict gluten-free diet is burdensome to patients and can be limited in efficacy, indicating there is an unmet need for novel therapeutic approaches to supplement or supplant dietary therapy. Many molecular events required for disease pathogenesis have been recently characterized and inspire most current and emerging drug-discovery efforts. Genome-wide association studies (GWAS) confirm the importance of human leukocyte antigen genes in our pathogenic model and identify a number of new risk loci in this complex disease. Here, we review the status of both emerging and potential therapeutic strategies in the context of disease pathophysiology. We conclude with a discussion of how genes identified during GWAS and follow-up studies that enhance susceptibility may offer insight into developing novel therapies.

Keywords

Celiac disease; Genome-wide association study; Gluten-free diet; Investigational therapies; Pathogenesis

Introduction

Celiac disease is a lifelong autoimmune disease characterized by an aberrant inflammatory response to dietary gluten in genetically susceptible individuals. Currently affecting 0.5-1% in most parts of the world, it is one of the most common chronic digestive disorders, with studies showing the prevalence of the disease is increasing [1,2]. The genetic predisposition to celiac disease is strong but complex. Ninety-five percent of patients are HLA-DQ2 or -DQ8 positive, but the presence of these alleles has a low positive predictive value [3]. The majority of the genetic component of celiac disease, as much as 65%, may be caused by over 50 non-HLA genes, with each gene slightly contributing to the risk of celiac disease development [4].

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The celiac lesion is characterized by villous atrophy, crypt hyperplasia, and infiltration of inflammatory cells, both in the small intestinal epithelium and in the lamina propria. The only current treatment for the disease is strict, lifelong adherence to a gluten-free diet, but many celiac patients experience persistent symptoms and enteropathy despite their best efforts to avoid dietary gluten. Additionally, patients with chronic undetected and untreated celiac disease are at an increased risk for developing enteropathy-associated T-cell lymphoma, small bowel adenocarcinoma, and other gastrointestinal cancers [5-7]. Thus, there is an unmet need for novel, non-dietary therapies that improve both health and quality of life for celiac patients [8-10].

Celiac Disease Pathogenesis

The design of novel non-dietary therapies to treat celiac disease requires a mechanistic understanding of disease pathogenesis (Fig. 1). At the broadest level, intestinal enteropathy in celiac disease is caused by genetic, immunological, and environmental factors. Gluten, a proline and glutamine rich glycoprotein, is the most critical environmental driver of the disease, while both human leukocyte antigen (HLA) and non-HLA genes are predisposing hereditary factors. MHC locus is the single most important genetic factor of the disease, with the majority of patients carrying a particular variant of HLA-DQ2 (DQA1*05:01, DQB1*02:01; also known as DQ2.5) [4]. Those who are not DQ2.5+ almost all carry HLA-DQ8 (DQA1*03, DQB1*03:02) or another variant of HLA-DQ2 (DQA1*02:01, DQB1*02:02; also known as DQ2.2) [11]. HLA-DQ2 and HLA-DQ8 predispose patients to celiac disease by preferential presentation of gluten peptides to $CD4^+$ helper T cells in the lamina propria. Activation of these T cells with gluten-derived peptides induces the secretion of various inflammatory cytokines dominated by interferon (IFN)-γ [12]. This, in turn, triggers a cascade of inflammatory reactions that leads to the hallmark intestinal enteropathy of celiac disease.

Given that virtually all patients with celiac disease carry particular HLA variants, HLA can be considered a necessary, but not a sufficient, factor for disease development. This claim is substantiated by the fact that while 40% of Caucasians possess one of the two predisposing haplotypes, only 3% of them develop celiac disease [13]. In addition to HLA genes, genome-wide association studies (GWAS) have identified 57 associated non-HLA variants located in 26 regions, with each locus contributing modestly to the overall genetic risk [14]. The majority of these non-HLA loci identified in GWAS harbor genes involved in the biology of T cells and antigen presenting cells. The story that emerges from the genetics of celiac disease bodes well for ongoing drug discovery efforts, given that most are based on the assumption that gluten-reactive T cells play a central role in controlling disease onset and severity. The other genes identified in GWAS reveal additional potential targets for future celiac disease drug discovery efforts.

The principal environmental driver, dietary gluten, contains a number of distinct disease-specific T-cell epitopes. A common feature of these epitopes is the presence of multiple Pro and Gln residues, with the high Pro content rendering these peptides resistant to proteolytic breakdown by gastric, pancreatic, and intestinal digestive proteases [15]. The result is an elevated intestinal concentration of potentially immunoreactive peptides following gluten

ingestion. Some of the Gln residues of these immunoreactive peptides can be deamidated by the enzyme transglutaminase 2 (TG2), which is also the dominant autoantigen of celiac disease [16]. Deamidation enhances gluten peptide immunogenicity by increasing the affinity of the interactions between the immunoreactive peptides and specific pockets in the ligand-binding sites of HLA-DQ2 or HLA-DQ8 [17,18].

While the HLA-mediated response to gluten-derived antigens in patients with celiac disease is well understood, several features of gluten enteropathy in celiac disease remain unclear at present. First, dietary gluten reversibly increases small intestinal permeability in many patients with celiac disease. It has been proposed that enhanced paracellular intestinal permeability is the consequence of increased expression of zonulin, a protein released by the small intestinal mucosa after gliadin challenge [19]. Additionally, this phenomenon may be caused by IFN-γ and other cytokines produced by gluten-activated $CD4^+$ T cells [12]. Genomic studies of patients with celiac disease also report involvement of genes that control intestinal permeability, including *MAGI2*, *MYO9B*, and *PARD3* [20,21]. Secondly, dietary gluten also appears to activate the innate immune system in patients with celiac disease, leading to production of interleukin-15 (IL-15) both in the lamina propria and in the epithelium. Elevated IL-15 drives two primary effects—expansion of intra-epithelial lymphocytes (IELs) and increased NKG2D expression on IELs, which interacts with MICA and MICB displayed on epithelial cells [22,22,23]. Additionally, unlike IELs in the normal intestine, IELs in patients with celiac disease express another NK receptor called CD94/NKG2C [24]. CD94/NKG2C recognizes HLA-E, a protein that is upregulated in epithelial cells in response to IFN-γ. The interaction of NKG2D with MICA/B and CD94/NKG2C with HLA-E activates the IELs and triggers them to destroy the epithelial cells. Finally, gluten consumption also induces anti-TG2 autoantibody production in individuals with celiac disease. While these antibodies are used to diagnose celiac disease, the cause and pathogenic consequences of autoantibody production remain unclear. Further pursuit of these lines of investigation may reveal important new targets for celiac disease therapy.

Insight into celiac disease pathogenesis has inspired the evaluation of a range of therapeutic strategies (Fig. 1). This chapter will first discuss the approaches undergoing clinical evaluation and then focus on therapeutic modes in the development stage (Table 1), concluding with discussion of potential targets identified during genome-wide association studies.

Therapies in Clinical Trials

Glucocorticoids with Low Systemic Bioavailability

Glucocorticoids are frequently used to induce a remission in or reduce the morbidity of immune-mediated diseases including asthma and Crohn’s disease. They elicit their therapeutic effects by induction of transient lymphopenia and immunosuppression. While significant side effects of systemic glucocorticoids limit their utility in the treatment of lifelong disorders such as celiac disease, it may be possible to utilize topically active glucocorticoids with pharmacological effects that are localized to the gut mucosa. One candidate is budesonide, a glucocorticoid with high-first pass metabolism and poor oral bioavailability that is currently used to treat Crohn’s disease. Three pilot studies in celiac

disease demonstrated that budesonide may provide clinical benefit to those patients with both refractory and non-refractory celiac disease [25-27]. In a separate Phase II pilot study using prednisolone, a glucocorticoid with higher oral bioavailability, celiac patients receiving a 4-week course of the drug experienced a rapid reduction in epithelial apoptosis but a simultaneous suppression of villous regeneration, suggesting short courses of oral prednisolone could benefit specific patient groups [28]. While oral prednisolone may not have acceptable safety characteristics for use in patients with active celiac disease, oral budesonide may; in patients with primary biliary cirrhosis, 6 mg budesonide has been administered daily for up to 3 years with no change in budesonide pharmacokinetics and only minor changes in bone mineral density [29]. One drawback of current formulations of oral budesonide, however, is that they are used to treat illnesses of the lower intestine thereby making them unsuitable for celiac disease. Thus, for oral budesonide to have the greatest therapeutic benefit, a novel formulation is required.

Oral Proteases for Gluten Detoxification

The gluten degrading ability of various bacteria, fungi, and plants has been exploited to develop oral protease therapies for celiac disease. The stability and immunogenicity of gliadin peptides is largely attributable to their high Gln and Pro content—a characteristic that confers resistance to breakdown by pepsin, pancreatic proteases, and intestinal brush border membrane peptidases [15]. Both in people with and without celiac disease, the stability of these epitopes derives primarily from the inability of gastric and pancreatic endoproteases to cleave after Pro or Gln residues coupled with the inability of dipeptidyl peptidase IV and dipeptidyl carboxypeptidase I to cleave long peptides in the brush border membrane. The accumulation of long gliadin peptides in the small intestinal lumen is in turn responsible for eliciting an HLA-DQ2- or -DQ8-restricted T-cell response in patients with celiac disease.

Given that the high Pro content of gluten partially confers resistance to proteases, administration of prolyl endopeptidases (PEPs) has been considered as a strategy for detoxifying gluten peptides given their ability to cleave peptides at proline residues [30]. While PEPs are widely expressed in both mammals and microbes, the levels expressed in humans are insufficient for detoxifying gluten peptides. In contrast, recombinant PEPs from a variety of bacteria and fungi including *Aspergillus niger*, *Flavobacterium meningosepticum*, *Myxococcus xanthus*, and *Sphingomonas capsulata* are able to proteolyze gliadin peptides both *in vitro* and *in vivo* [31,32]. Importantly, these enzymes maintain both structure and function in the pH ranges of the human gastrointestinal tract [33]. In a study with celiac patient-derived gluten-specific T-cell clones, digestion of gluten peptides with a PEP from *A. niger* was able to abrogate T-cell expansion [34]. More significantly, in a randomized, double-blind, cross-over study, pretreatment with PEP prevented gluten-induced fat or carbohydrate malabsorption in over half of patients with celiac disease [35].

The high Gln content of gluten peptides provides an alternative target for oral protease therapy. A Gln-specific cysteine endoprotease B, isoform 2 (EP-B2) from germinating barley seeds is able to rapidly proteolyze gliadin peptides into short polypeptides [36]. Like the fungal and bacterial PEPs, EP-B2 maintains its structure and activity under

gastrointestinal conditions [37]. Early proof-of-concept studies demonstrated EP-B2 effectively digested gluten both in the rat stomach and in gluten-sensitive rhesus macaques in a dose- and time-dependent manner [38].

Given that PEP and EP-B2 have complementary roles in proteolyzing gliadin peptides, one strategy that has been pursued for accelerating gluten detoxification is the administration of both enzymes as a combination therapy. To this end, one potential therapeutic in clinical trials is ALV003, an orally administered mixture of barley EP-B2 and *Sphingomonas capsulata* PEP [39]. The results of two Phase I single, escalating-dose clinical trials demonstrated that all doses of ALV003 were well tolerated, with no observed serious adverse events or allergic reactions [40]. The results of an initial Phase II trial indicated that ALV003 attenuated gluten-induced small intestinal mucosal injury in patients with celiac disease in the context of an everyday gluten-free diet, although symptoms did not significantly differ from the control group [41]. To further investigate efficacy and safety, a Phase IIb, randomized, double-blind, placebo-controlled dose ranging study is being conducted in celiac disease patients with persisting signs and symptoms despite a gluten-free diet.

Clinical trials have been pursued for other proteases as well. A PEP from *Aspergillus niger* (AN-PEP) was shown to degrade gluten in an artificial gastrointestinal model [32]. While a Phase I/II pilot clinical trial in celiac patients showed AN-PEP was both safe and well tolerated, additional trials have not been pursued [42]. Another enzyme cocktail has been shown to have a modest capacity to detoxify gluten [43]. A clinical trial is also underway for the use of Creon, an enzyme cocktail of pancrelipase, for the treatment of celiac patients with low faecal pancreatic elastase.

Gluten-Sequestering Polymers

A distinct strategy for attenuating the immunotoxicity of gliadin peptides utilizes orally administered polymeric resins, which sequester gliadin peptides in the small intestinal lumen before they can elicit their immunotoxic effects in the lamina propria. One such polymer is poly(hydroxyethyl methacrylate-co-styrene sulfonate) (P[HEMA-co-SS], BL-7010), which has been shown to bind to gluten proteins under simulated gastric and intestinal conditions *in vitro* [44]. P[HEMA-co-SS] is also able to reverse gliadin-induced alterations to intestinal epithelial cells and to reduce the secretion of the inflammatory cytokine tumor necrosis factor-α (TNF-α) *ex vivo* in mucosal biopsy specimens from patients with celiac disease [45]. The same studies also concluded that P[HEMA-co-SS] could reduce paracellular intestinal permeability and attenuate the systemic immune response to gluten in a variety of mouse models. These data support the clinical evaluation of a luminal polymeric binder as an effective adjunctive therapy to a gluten-free diet. A randomized, double bind Phase I/II clinical trial is currently recruiting celiac patients to evaluate the safety and systemic exposure of single escalating administrations and repeated administration of BL-7010. Much like the oral protease strategy, an important question that remains to be answered is the gluten dose that can be effectively detoxified *in vivo* by a given dose of P[HEMA-co-SS].

Zonulin Antagonists

Following partial gluten digestion in the intestinal lumen, gliadin peptides encounter the epithelial layer. The intestinal epithelial tight junctions function as a barrier that is critical for controlling foreign particle entry into the lamina propria. While the epithelial layer is typically impermeable to macromolecules, both clinical and experimental studies have demonstrated enhanced intestinal permeability upon gluten exposure in untreated celiac patients resulting from abnormal epithelial tight junction protein expression. Compromised barrier function enables elevated transport of the gliadin peptides into the lamina propria. One epithelial tight junction protein that has been implicated in enhancing barrier permeability is zonulin (prehaptoglobin-2) [19,46]. Through the interaction of gliadin peptides with the CXCR3 receptor, gluten is thought to induce overexpression of zonulin in intestinal tissue of patients with celiac disease and simultaneously activate zonulin signaling in tight junctions between epithelial cells, thereby leading to increased intestinal permeability [47]. When exposed to luminal gliadin peptides, intestinal biopsies from celiac patients in remission exhibited sustained luminal zonulin release and increased intestinal permeability, while a far less prominent effect was observed in control biopsies [48]. As a result, antagonists of zonulin have been proposed as a possible therapeutic option for the treatment of celiac disease.

Larazotide acetate (AT-1001) is an octapeptide derived from the zonula occludens toxin from *Vibrio cholerae* that locally inhibits the opening of tight junctions in epithelial cells in the small intestine [49]. Sharing a motif with zonulin, this compound presumably antagonizes zonulin action and is thus believed to prevent gliadin-induced permeability both *ex vivo* and *in vivo* [48,50]. A randomized, placebo-controlled Phase I clinical trial demonstrated AT-1001 was both safe and well tolerated in celiac patients, and suggested AT-1001 was capable of reducing gluten-induced intestinal permeability [51]. Despite this promising initial result, two subsequent clinical trials failed to demonstrate AT-1001 could reduce intestinal permeability. Specifically, in a 2-week and a 6-week study on celiac patients on a gluten-free diet, AT-1001 did not affect gluten-induced intestinal permeability, whereas it did alleviate the severity of gastrointestinal symptoms [52,53]. Recently, Alba Pharmaceuticals completed a 12-week Phase IIb clinical study with 342 patients evaluating the efficacy and safety of larazotide acetate in the treatment of celiac patients with persistent symptoms despite being on a gluten-free diet [54]. This study met its primary endpoint, with a 0.5 mg dose of larazotide acetate improving signs and symptoms of celiac disease compared with placebo by Modified Intention to Treat. The 0.5 mg dose also showed effect on exploratory endpoints, reducing patient reported outcome symptomatic days, average abdominal pain, and non-GI symptoms of headache and tiredness as well as increasing improved symptom days. These results warrant further clinical evaluation of larazotide acetate.

Gluten Tolerization

In contrast to therapies targeting a specific event in celiac disease pathogenesis, an alternative strategy is aimed at inducing gluten tolerance through vaccination. A variety of peptide-based desensitization therapies are undergoing clinical evaluation for the treatment of allergic diseases, including Cat-PAD for cat allergy and AllerT for birch pollen allergy. In

the case of Cat-PAD, peptide vaccination induces CD4+ T cells with regulatory activity, suggesting induction of regulatory T cells is likely the mode of action of this treatment [55,56]. In the case of celiac disease, knowledge of the major HLA-DQ2-restricted T-cell epitopes of gluten has enabled the development of Nexvax2, a vaccine based on immunodominant gluten peptides that is currently undergoing clinical evaluation. A Phase I clinical trial demonstrated Nexvax2 was well tolerated in healthy, HLA-DQ2 positive celiac patients [57]. ImmunsanT, the company developing Nexvax2, is reportedly planning a Phase II clinical study to evaluate efficacy. Key questions that could be addressed by such studies include: (1) does the peptide vaccination tolerize gluten-reactive T cells in the intestinal mucosa, and (2) to what extent does this acquired immunity extend to less immunodominant epitopes? Notably, a distinct vaccine would be required for HLA-DQ8-positive celiac patients, given that immunodominant HLA-DQ2- and HLA-DQ8-restricted T-cell epitopes are non-overlapping.

Probiotics for Gluten Proteolysis

Enteric bacteria serve important immunological roles including the development of gut-associated lymphoid tissues, the polarization of gut-specific immune responses, and the prevention of colonization by pathogens [58]. Dysbiosis has been associated with a variety of autoimmune inflammatory disorders of the intestine, including Crohn's disease, irritable bowel syndrome, and celiac disease [59-61]. Abnormalities in the gut microbiome of celiac patients, the association of specific microorganisms in the regulation of intestinal barrier function and immunity, and the ability of a variety of microbes to enzymatically degrade gluten are factors that have motivated investigation of intestinal bacteria as probiotics for the treatment of celiac disease. Species of *Bifidobacterium* are of interest, given the reduced prevalence of this genus in duodenal biopsies and feces of active as well as well-controlled celiac patients compared to individuals without the disease [61]. The results of *in vitro* studies that demonstrate various *Bifidobacteria* are able to hydrolyze gliadin peptides into less immunogenic peptides [62]. An exploratory clinical study concluded that while the *Bifidobacterium infantis natren* strain had no effect on elevated intestinal permeability or inflammatory protein abnormalities in patients with untreated celiac disease, it may alleviate symptoms in these patients [63].

Hookworm Infection

An unusual approach that has been pursued for the treatment of a variety of inflammatory conditions is infection with parasitic helminthes—with efforts primarily focused on the use of either the pig whipworm (*Trichuris suis*) or the human hookworm (*Necator americanus*)—given their ability to modulate host immune responses [64,65]. For autoimmune diseases, parasitic helminthes have also been pursued because of the correlation between the disappearance of intestinal parasites and the increasing prevalence of autoimmune diseases in developed countries. In the context of celiac disease, clinical studies have demonstrated that infection with *N. americanus* alone does not suppress disease symptoms sufficiently for it to be a viable therapy, with most subjects developing symptoms and mucosal inflammation immediately following reintroduction of gluten, irrespective of treatment group [66]. Given the ability of *N. americanus* to suppress gluten-induced IFN-γ, IL-17, and IL-23 expression and upregulate IL-10, TGF-β, and IL-22 expression in celiac patients, a 52-

week pilot clinical Phase I/II study was undertaken with twelve patients to evaluate whether *N. americanus* infection could tolerize subjects to trace amounts of gluten [67]. The results indicated that celiac patients inoculated with *N. americanus* did not experience a decline in median villous height-to-crypt depth ratios upon a 1-gram gluten challenge, although they experienced a decrease in mean TG2 IgA titers upon exposure to 3-grams of gluten. It was argued that the approach merited further investigation in celiac patients experiencing occasional gluten exposure.

Preclinical targets

Blocking Transcellular Gliadin Transport

While paracellular intestinal permeability is enhanced in celiac patients, gliadin peptides also undergo transcellular transport across the epithelium. This process is hypothesized to be mediated by secretory IgA (sIgA) via binding to the transferrin receptor CD71, a protein upregulated in the intestinal epithelium of celiac patients [68]. Significantly, the ability of IgA from celiac patients to enhance transcellular passage of gliadin peptides was abolished by inhibition of TG2 enzymatic activity [69]. Thus, inhibition of the gliadin-sIgA-CD71 transport pathway, either through TG2 inhibition or by another mechanism, could be a therapeutic strategy for celiac disease.

Blocking IL-15

The cytokine IL-15 plays a critical role in celiac disease pathogenesis through dysregulation of IELs that leads to villous atrophy. In a transgenic mouse that overexpresses IL-15 in the lamina propria, antibody targeting of IL-15 reversed intestinal damage, suggesting this form of therapy may warrant evaluation in celiac patients [70]. In experiments using small intestinal mucosal biopsies from celiac patients, anti-IL-15 antibodies abrogated gluten-induced overexpression of MICA by epithelial cells and neutralized enterocyte apoptosis [22,71]. Additionally, anti-IL-15 antibodies down-regulated the adaptive immune response in the lamina propria [72]. All of these observations suggest anti-IL-15 therapy could benefit patients with celiac disease, particularly those with a refractory condition. A Phase I clinical study is currently recruiting patients to evaluate the efficacy of the humanized Mik-Beta-1 monoclonal antibody that blocks IL-15 action by binding to the β-subunit of the IL-2/IL-15 receptor in patients with refractory celiac disease. In the past, an anti-IL-15 monoclonal antibody, AMG 714, had undergone Phase II clinical studies in rheumatoid arthritis and psoriasis, however, its development was discontinued given limited efficacy [73].

Inhibition of TG2

Catalytic activity of the primary celiac disease autoantigen transglutaminase 2 (TG2) is an essential step in generating gliadin peptides with high HLA affinity. Inhibition of TG2 thus has potential as a therapy for attenuating the inflammatory response in celiac patients. A number of classes of TG2 inhibitors have been developed, including both active site-directed irreversible inhibitors such as thiadiazoles, epoxides, α,β-unsaturated amides, and dihydroisoxazoles, as well as reversible inhibitors such as thienopyrimidines, cinnamoyl compounds, β-aminoethyl ketones, and acylidine oxindoles. Perhaps the most extensively evaluated are the weakly electrophilic 3-bromo-4,5-dihydroisoxazole class of inhibitors

[74-81]. *In vivo* experiments using a prototypical inhibitor ERW1041E blocked elevated TG2 activity in pulmonary tissue in a mouse model of pulmonary hypertension [82]. In the same study, twice-daily intraperitoneal dosing of this inhibitor at 50 mg/kg was well tolerated over the course of several weeks. Given that extracellular TG2 is predominantly inactive under normal physiologic conditions, and that TG2 knockout mice are developmentally and reproductively normal, TG2 inhibition warrants clinical evaluation as an oral therapy for celiac disease [83,84]. Notably, TG2 is one of nine transglutaminase homologs that catalyze posttranslational modifications of selected Gln residues on target peptides or proteins; therefore, the design of an isozyme-selective TG2 inhibitor is a challenging undertaking. Minimizing cross-reactivity with other transglutaminases could be important, given that (1) TG1 mutations give rise to skin barrier dysfunctions, (2) Factor XIIIa is essential in the blood clotting cascade and is responsible for cross-linking fibrin to form stable clots, and (3) TG3 mutations have been associated with an increased risk of basal cell carcinoma [85-87]. While ERW1041E does not possess sufficient selectivity, other analogs show improved potency and isoform selectivity [88].

Blocking HLA-DQ2 or HLA-DQ8

The presence of either HLA-DQ2 or -DQ8 on antigen presenting cells is the most significant genetic factor in predisposing an individual to celiac disease, although neither haplotype is sufficient for disease development. Since homozygosity for HLA alleles does not enhance susceptibility to infection, blocking gliadin peptide presentation by either HLA-DQ2 or -DQ8 represents a potential strategy for reducing the severity of the toxic effects of gluten seen in celiac patients. Efforts to block HLA in other immune-mediated conditions—including rheumatoid arthritis, multiple sclerosis, and type I diabetes—have met with limited success, presumably due to ineffective drug delivery to the disease-relevant antigen presenting cells. If so, then topical delivery to the small intestine in celiac patients should be a considerably easier task. A number of peptides have been synthesized to target HLA-DQ2 [89,90]. While these initial efforts utilized a gliadin peptide scaffold, the ligands showed limited efficacy in reducing T-cell activation. More recent strategies have sought to optimize each amino acid's interaction with the HLA binding cleft [91,92]. Although some of these ligands bind to HLA-DQ2 with greater than 50-fold affinity compared to the immunodominant gluten epitope of DQ2-α-I-gliadin, this affinity may be insufficient for attenuating T-cell activation. Moreover, applicability of this approach to all celiac patients would require the development of analogous HLA-DQ8 blockers.

Suppression of T Cells

Suppression of gluten-specific T-cell mediated immune responses through antibody-based therapies could prove useful in certain circumstances for the treatment of celiac disease. Antibodies against TNF including infliximab, certolizumab and adalimumab have been used in the treatment of Crohn's disease, rheumatoid arthritis, and IBD. Two case studies have evaluated the efficacy of the anti-TNF monoclonal antibody infliximab in treating refractory celiac disease, with both patients experiencing histological improvements [93,94]. Larger scale clinical trials are necessary to demonstrate clinical utility of anti-TNF antibodies for the treatment of refractory celiac disease.

Antibodies against interferon-γ, the dominant inflammatory cytokine produced by gluten reactive T cells, may also prove useful for the treatment of celiac disease. While doses of anti-IFN-γ antibody fontolizumab were well tolerated by patients with Crohn's disease, development was discontinued given limited efficacy [95]. Nevertheless, evaluation of anti-IFN-γ antibodies may still be justifiable for certain patient groups with celiac disease.

A third antibody-based approach to modulate the T-cell response targets the CD3 protein complex, a co-receptor for the T-cell receptor. Given that anti-CD3 therapy is thought to function by both eliminating effector T cells and inducing regulatory T cells and that it is primarily effective in the context of a primed and ongoing immune response, anti-CD3 antibodies are currently being evaluated for the treatment of Crohn's disease, ulcerative colitis, and type 1 diabetes [96-98]. For these same reasons, it may prove to be an effective treatment of celiac disease. One cause for reservation about the potential use of this approach is the fact that effector T cells with active celiac patients become resistant to suppression by regulatory T cells.

Targeting B Cells

In addition to suppressing disease-specific T cells, therapies targeting disease-specific B cells have potential for the treatment of celiac disease. Earlier studies have demonstrated a clinical benefit of depleting B cells with anti-CD20 antibodies in the context of other HLA-associated disorders including rheumatoid arthritis, multiple sclerosis, and type I diabetes [99-101]. B cells may play a role in the pathogenesis of celiac disease, since gluten-specific and TG2-specific B cells should be able to present gliadin peptides to T cells, making CD20 an appealing target [102]. Approaches targeting CD20 in celiac disease have been largely unsuccessful, however, demonstrating that anti-CD20 antibodies are unable to abrogate IgA plasma cells generation in the gut mucosa despite peripheral B-cell depletion [103]. While a number of anti-CD20 antibodies are clinically used, if antibodies produced in the mucosa are pathogenically relevant to celiac disease, they are unlikely to provide a therapeutic benefit to celiac patients.

Blocking Intestinal Homing

Lymphocyte homing to the gastrointestinal mucosa is aided by the chemokine receptor CCR9 and integrin α4β7 in a process mediated by their chemokine ligands CCL25 and MADCAM1, respectively [104]. Celiac patients have elevated CCR9-expressing peripheral blood T cells and MADCAM1 augmentation in the duodenum, suggesting targeting either of these homing mechanisms could be used to treat celiac patients [105,106]. CCX282-B (GSK1605786A) is a selective, orally bioavailable antagonist of human CCR9 that is undergoing clinical trials in Crohn's disease and celiac disease [107]. Numerous clinical trials evaluating CCX282-B for the treatment of Crohn's disease have been withdrawn or terminated early, and a Phase II trial of celiac disease patients initiated in 2007 has been completed but the results have not yet been disclosed. A monoclonal antibody against integrin α4β7, vedolizumab (Entyvio), was recently approved for the treatment of moderately to severely active ulcerative colitis and Crohn's disease, and could be useful for the treatment of subsets of celiac disease patients [108,109]. If any of these drugs are

effective therapeutics for celiac disease, increased monitoring for gastrointestinal infections will be necessary given that this mode of action is not antigen specific.

Future Targets

As mentioned previously, celiac disease is a polygenic disorder having the most important genetic susceptibility determinant located in the class II MHC locus, with HLA-DQ2 and/or HLA-DQ8 being necessary but not sufficient for disease development. These susceptibility alleles only explain 35% of genetic risk, however, and several other genes contribute to celiac disease pathogenesis [110]. Two GWAS and Immunochip analyses performed on celiac disease patients have revealed 39 regions of genetic susceptibility to the disease [4,110]. Many of the genes identified in these studies have been implicated in numerous other immune-modulated diseases (Fig. 2). These studies suggest several possible etiological candidates, with the majority of them related either to T-cell maturation or immune response pathways. Given these genes may modulate gluten sensitivity, future therapies may stem from investigation into the role of these genes in celiac disease pathogenesis. The coding alleles *THEMIS*, *PTPRK*, *FUT2*, *BACH2*, and *RGS1* are reviewed here.

THEMIS

Healthy individuals mount immune responses to exogenous pathogens while avoiding autoimmune attacks on normal tissue. In this regard, the ability to recognize self from non-self is essential. A diverse pool of functional T cells with immunological tolerance is generated through positive selection while overly self-reactive T cells are removed by negative selection during ontogeny. Thymocytes arrive at these cell fate decisions through interactions with ligands, where a narrow range of affinities distinguishes positive from negative selection. At this selection threshold, a small increase in ligand affinity for the T-cell receptor leads to the induction of negative selection. In celiac disease-specific GWAS, the peak on 6q22.33 includes the gene *thymocyte-expressed molecule involved in selection* (*THEMIS*). An association study in a north Indian population showed a SNP on intron 2 of THEMIS was associated with celiac disease, although the functional significance remains unknown [111]. *THEMIS* encodes a 73-kDa protein expressed in the thymus, spleen, and lymph nodes that is involved in T-cell maturation and in regulating lineage commitment of thymocytes into CD4+ or CD8+ cells [112]. More specifically, *THEMIS* enables thymocyte positive selection through its ability to attenuate TCR signals via SHP1 recruitment and activation in response to low- but not high-affinity TCR engagement [113]. Functionally it converts TCR affinity into a selection outcome and thus determines the affinity threshold for activation. Recent work in a *THEMIS* knockout rat has linked the function of this gene to the suppressive function of CD4+ regulatory T cells and suggested that the defect is involved in intestinal inflammation [114]. Previous studies have demonstrated that impaired thymic negative selection can result in autoimmune disease. THEMIS has also been implicated in Crohn's disease and multiple sclerosis [115,116]. In celiac patients, work evaluating the expression levels of *THEMIS* in duodenal mucosa of active and treated celiac disease patients and in controls found higher expression in active patients compared with treated patients and controls [117]. It is thus possible that higher expression of THEMIS in celiac

patients could lead to dysregulation of thymocyte selection, thereby contributing to the pathogenesis of the disease.

PTPRK

In addition to *THEMIS*, the celiac GWAS peak on 6q22.33 also included the gene *protein tyrosine phosphatase, receptor type, kappa* (*PTPRK*) encoding RPTPκ. RPTPκ is a ubiquitous transmembrane protein tyrosine phosphatase whose expression is induced by TGF-β [118]. One hypothesis is that RPTPκ is involved in the pathogenesis of celiac disease through the modulation of T-cell development. An important role for RPTPκ in T-cell development was hypothesized following the discovery that *PTPRK* is deleted in the LEC rat, a model characterized by hypoplasia of the thymus and spleen, reduced levels of IgG, selective deficiency in CD4+ T cells, and strongly reduced T helper function [119]. Of note, however, was the discovery that the LEC rat also lacks the THEMIS gene calling into question the role of *PTPRK* in T-cell development [120]. A more plausible role for RPTPκ in celiac disease is through the dysregulation of intestinal cell junctions. Numerous studies have validated the role of the extracellular domain of *PTPRK* in promoting cell-cell adhesion through homophilic binding, a process that is critical for intestinal barrier function [121]. The GWAS showed that two independently associated sets of SNPs were finely localized around the 3' untranslated region of *PTPRK*, with one located in a predicted binding site for the microRNA hsa-miR-1910 [14]. Consistent with this observation was the finding that *PTPRK* showed lower expression in active celiac disease compared with treated patients and controls [117]. In addition to celiac disease, GWAS have also implicated *PTPRK* in other autoimmune diseases characterized by intestinal permeability including Crohn's disease and multiple sclerosis [115,122]. Future studies are warranted to elucidate the role of *PTPRK* in celiac disease and to determine if this gene could represent a novel therapeutic target.

FUT2

The FUT2 gene present on 19q13.33 encodes α-1,2-fucosyltransferase, an enzyme that controls the expression of A-, B- and H- blood group antigens in mucus and other body secretions [123]. Specifically, it catalyzes the conversion of type 1 precursor to H antigen type 1 by adding a terminal α-1,2-linked fucose to galactose on type 1 precursor, where the H antigen type 1 is the precursor for the further synthesis of A and B blood group antigens. These antigens are also expressed in the gastrointestinal mucosa, serving as anchors for various microbes. Previous studies have demonstrated that FUT2 can influence the composition of gut microbiota, in turn protecting the host from pathogenic microbes and affect the development of the mucosal immune system [124]. In 20% of Caucasians, FUT2 is rendered nonfunctional by a nonsense mutation (*rs601338-AA*) that introduces a premature stop codon, where individuals homozygous for this mutation are called non-secretors. While a number of studies have linked secretor status to either risk or protection from a variety of disease, important here is the evidence that the FUT2 non-secretor status has been associated with increased risk for celiac disease as well as Crohn's disease and type I diabetes [125-127]. In the context of celiac disease, past studies have demonstrated that secretor status is strongly associated with the diversity and composition of intestinal microbiota while others have shown microbiota can induce marked changes in monocyte

derived dendritic cell morphology and simultaneously increase the production of inflammatory cytokines including IFN-γ, TNF-α, and IL-12 [124,128-130]. Taken together, these results suggest *FUT2* may indirectly, by means of microbiota composition, be involved in the manifestation of celiac disease.

BACH2

Polymorphisms within the gene encoding BTB and CNC homolog 2 (*BACH2*) on 6q15 have been associated with numerous allergic and autoimmune diseases including including asthma, Crohn's disease, vitiligo, multiple sclerosis, type I diabetes, autoimmune thyroid disease, and celiac disease [110,122,131-134]. BACH2 is a BTB-leucine zipper family transcription factor that has been identified as a B cell-specific transcriptional repressor responsible for regulating plasma cell differentiation, immunoglobulin class switching, and somatic hypermutation [135]. More recent work has demonstrated that *BACH2* is expressed in T cells and is critical for the formation of regulatory T cells and maintenance of naive T-cell state [136]. Importantly, *BACH2* represses genes with effector-lineage-specific functions in a manner that constrains full effector differentiation within T_H1, T_H2, and T_H17 cell lineages. Consistent with this observation is the fact that absence of *BACH2* during regulatory T-cell polarization results in inappropriate diversion to effector lineages. *BACH2* knockout mice have limited viability resulting from severe inflammation in the gut, lungs, and other tissues [136]. Taken together, these results identify *BACH2* as an essential regulator of T-cell differentiation that can prevent inflammatory disease by maintaining immune homeostasis. Concordant with this conclusion, the SNP identified in the celiac disease GWAS is associated with BACH2 down-regulation [137]. While specific targeting of BACH2 represents a significant challenge, further investigation into the role of this gene in celiac disease pathogenesis may identify novel therapeutic targets.

RGS1

SNPs in RGS1 have been associated with a variety of autoimmune diseases including type I diabetes, multiple sclerosis, and celiac disease [138-140]. In celiac disease, the SNPs for *RGS1* show association in the 5'-UTR region—specifically in the first exon and 10 kb upstream of it—suggesting these SNPs affect its transcriptional regulation. *RGS1* encodes regulator of G-protein signaling 1, a protein that modulates chemokine-induced GPCR activity. It has long been recognized that RGS1 can regulate B cell chemotaxis, although splenic and lymph node T cells from RGS1−/− mice show seemingly normal T-cell chemotaxis [141,142]. Recent work has demonstrated that *RGS1* mRNA is highly enriched in murine gut versus lymphoid T cells, and that *RGS1* expression is substantially higher in T cells from the human gut compared to peripheral blood in a manner that is exacerbated during intestinal inflammation [143]. A significant observation has been that elevated *RGS1* levels markedly reduce T-cell migration in response to lymphoid-homing cytokines, while *RGS1* depletion enhances gut T-cell egress in a manner that impairs colitogenic potential [143]. While it cannot be predicted whether *RGS1* is necessarily involved in either celiac disease initiation and/or progression, given the critical role the IELs play in the villous atrophy characteristic of celiac disease, further investigation into the therapeutic potential of modulating *RGS1* is warranted.

Conclusions

The importance of developing alternative, non-dietary therapies for the treatment of celiac disease is underscored by the increasing prevalence of the disease and the growing awareness that a substantial fraction of treated celiac patients show lingering evidence of disease activity. Despite the pursuit of numerous strategies for combating the disease, development of successful therapies has proved to be challenging given the complexity of this autoimmune disorder. Recent GWAS and follow-up studies have increased our understanding of the genetic factors that enhance susceptibility to celiac disease that offer insight into the underlying molecular pathways and mechanisms. Evidence linking polymorphisms in these genes to this and other autoimmune and inflammatory conditions warrants investigation into their target for therapeutic relief.

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Practice Points

- Celiac disease is a lifelong autoimmune disease, where intestinal enteropathy is caused by genetic, immunological, and environmental factors
- Dietary control through the avoidance of gluten is the only accepted form of therapy
- Previous and ongoing clinical trials for celiac disease include treatment with glucocorticoids, oral proteases, gluten-sequestering polymers, zonulin antagonists, gluten vaccines, probiotics, and hookworms
- Genome-wide association studies have identified a number of new risk loci

Research Agenda

- The lack of a non-dietary therapy demands pursuit of therapies currently undergoing clinical evaluation
- Strategies including blocking transcellular gliadin transport, IL-15, HLA–DQ2 or –DQ8, and intestinal homing in addition to inhibiting TG2, suppressing T cells, and targeting B cells are promising preclinical targets
- GWAS and Immunochip analyses suggest several possible etiological candidates that warrant investigation into their value as future targets for therapeutic relief

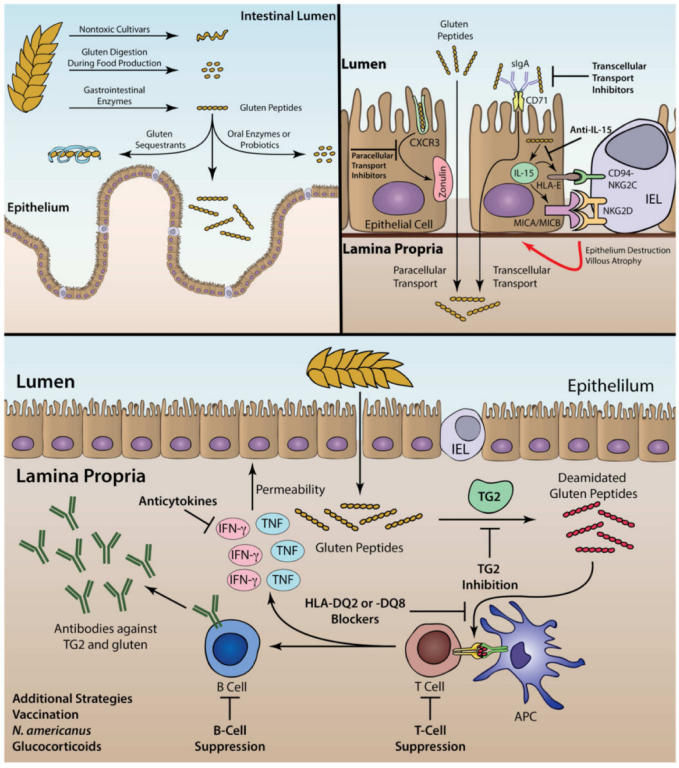

Fig. 1.

Investigational approaches targeting the factors contributing to celiac disease pathogenesis. Long, Pro- and Gln-rich fragments of gluten survive gastrointestinal breakdown by luminal and brush border enzymes and consequently enter the lamina propria. Production of non-toxic cereals lacking antigenic peptides or sourdough fermentation during baking could avoid the ensuing immune response. Alternatively, gluten-sequestering polymers, oral proteases, and probiotics may reduce the exposure to immunogenic gluten peptides. At the intestinal epithelium, compromised epithelial barrier function enables paracellular and transcellular transport of gluten peptides. Antagonists of zonulin and inhibitors of the peptide-sIgA-CD71 transport pathway could reduce this paracellular and transcellular permeability, respectively. Gluten peptides induce epithelial and other cells to secrete IL-15, resulting in an increase in the number of IELs. These IELs are subsequently activated by epithelial MICA/B-NKG2D and CD94/NKG2C-HLA-E interactions that stimulating cytotoxic effects on epithelial cells. Neutralizing IL-15-mediated effects could provide a therapeutic benefit for celiac patients. Most gluten peptides survive gastric digestion and are excellent substrates of TG2 in the lamina propria. The resulting deamidated gluten peptides are then recognized by CD4+ T cells in the context of HLA-DQ2 or –DQ8 on antigen presenting cells. Thus, either TG2 inhibitors or HLA blockers are potential drug candidates. The T-cell response in the mucosa could be suppressed by anti-CD3-antibodies or peptide vaccination, or by blocking T-cell homing with anti-integrin $\alpha 4\beta 7$ or CCR9 antagonists. Upon activation, gluten reactive T cells secrete inflammatory cytokines including IFN-γ and TNF that contribute to enteropathy. Anti-IFN-γ– and –TNF-antibodies could be considered as therapeutic targets. Finally, through interactions with T cells, B cells differentiate into plasma cells that produce autoantibodies against TG2. B-cell depletion with anti-CD20

antibodies is yet another possible therapeutic strategy. Further means to prevent or treat celiac disease in the future is modifying the inflammatory immune response by hookworm infection or with steroid therapy. *Abbreviations*: sIgA, secretory IgA. TG2, transglutaminase 2; HLA, human leukocyte antigen; IFN-γ, *interferon gamma; TNF, tumor necrosis factor.*

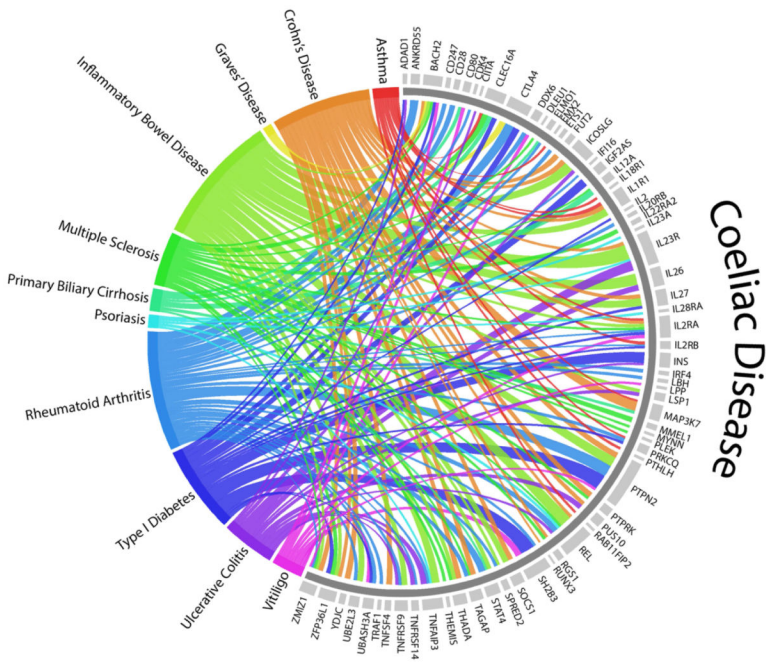

Fig. 2.

Non-HLA susceptibility regions shared between celiac disease and select immune-modulated diseases [144,145]. A genome-wide significance threshold was set at $P = 5 \times 10^{-8}$. FUT2 was included despite not being identified in a celiac disease-specific GWAS.

Table 1

Potential therapies for celiac disease.

Mode of action	Compound	Compound Class	Company/University	Status
Topical steroid	Budesonide	Small molecule	Generic drug	Approved
Topical steroid	Prednisolone	Small molecule	Generic drug	Phase II
Glutenase	ALV003	Enzyme	Alvine, USA	Phase IIb
Glutenase	AN-PEP	Enzyme	DSM, Netherlands	Phase I + II
Glutenase	STAN1 (enzyme supplements)	Enzyme	Heim Pal Childrens Hospital, Hungary	Phase I + II
Zonulin antagonist	AT-1001	Peptide	Alba, USA	Phase IIb
CCR9 antagonist	CCX282-B	Small molecule	ChemoCentryx, USA	Phase II
Immune modulation	*Necator americanus*	Parasite	Princess Alexandra Hospital, Australia	Phase II
Peptide vaccination	Nexvax2	Peptide	Nexpep, Australia	Phase I
Anti-IL-15	AMG 714	Monoclonal antibody	Amgen, USA	Phase II in RA, psoriasis, (discont.)
Anti-IFN-γ	Fontolizumab	Monoclonal antibody	PDL and Biogen Idec, USA	Phase II in IBD, (discont.)
Anti-CD3	Visilizumab	Monoclonal antibody	Facet, USA	Phase II in UC, GvHD (discont.)
Anti-CD3	Teplizumab	Monoclonal antibody	MacroGenics, USA	Phase II in T1D
Anti-CD3	Otelixizumab	Monoclonal antibody	Tolerx, USA	Phase III in T1D
Anti-CD20	Rituximab	Monoclonal antibody	Biogen Idec, USA	Approved
Anti-CD20	Tositumab	Monoclonal antibody	GlaxoSmithKline, USA	Approved
Anti-CD20	Ibritumomab	Monoclonal antibody	Spectrum, USA	Approved
Anti-TNF-α	Infliximab	Monoclonal antibody	Janssen Biotech, USA	Approved
Anti-TNF-α	Certolizumab	Monoclonal antibody	UCB, USA	Approved
Anti-TNF-α	Adalimumab	Monoclonal antibody	AbbVie, USA	Approved
Anti-integrin α4β7	Vedolizumab	Monoclonal antibody	Millennium Pharmaceuticals, USA	Approved
IL-2/IL-15R Beta	Hu-Mik- Beta-1	Monoclonal antibody	National Cancer Institute, USA	Phase I
Anti-gluten	AGY	Polyclonal antibody	Igy, Canada	Phase I
Pancrelipase	Creon	Enzyme cocktail	Sheffield Teaching Hospitals, UK	Phase 4
TG2 inhibitor	Dihydroisoxazoles	Small molecule	Sitari Pharmaceuticals, USA	Discovery
TG2 inhibitor	ZED-101	Small molecule	Zedira, Germany	Discovery
TG2 inhibitor	Cinnamoyl triazoles	Small molecule	University of Montreal, Canada	Discovery
HLA-DQ2 blocker	Dimeric analogue of gluten peptide	Peptide	Stanford University, USA & University of Oslo, Norway	Discovery
HLA-DQ2 blocker	Azidoproline analogue of gluten peptide	Peptide	Leiden University, Netherlands	Discovery
Gluten tolerization	Bifidobacterium Infantis	Probiotic	Universidad de Buenos Aires, Argentina	Exploratory
Gluten tolerization	Genetically modified Lactococcus lactis	Probiotic	ActoGeniX, Belgium	Discovery
Gluten-sequestering polymers	P(HEMA-co-SS) (BL-7010)	Polymer resin	University of Montreal, Canada	Phase I + II

RA, rheumatoid arthritis; HLA, human leucocyte antigen; IBD, inflammatory bowel disease; UC, ulcerative colitis; GvHD, graft versus host disease; TG2, transglutaminase 2; T1D, type 1 diabetes; P(HEMA-co-SS), poly(hydroxyethyl methacrylate-co-styrene sulphonate); Approved, Approved in other diseases.