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REVIEW ARTICLE

The immunopathogenesis of celiac disease reveals possible therapies beyond the gluten-free diet

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Abstract Celiac disease is a T cell-mediated autoimmune inflammatory disease of the small intestine that is activated by gluten. The diagnosis of celiac disease is challenging as patients display a wide range of symptoms and some are asymptomatic. A lifelong gluten-free diet is the only currently approved treatment of celiac disease. Although the diet is safe and effective, the compliance rates and patient acceptance vary. Furthermore, many patients treated with a gluten-free diet continue to be mildly to severely symptomatic with persistent histological abnormalities, and a small number of patients develop refractory celiac disease. New therapeutic adjuncts and potential alternatives to the gluten-free diet could improve the treatment options for these patients. Advances in understanding the immunopathogenesis of celiac disease have suggested several types of therapeutic strategies that may augment or supplant the gluten-free diet. Some of these strategies attempt to decrease the immunogenicity of gluten-containing grains by manipulating the grain itself or by using oral enzymes to break down immunogenic peptides that normally remain intact during digestion. Other strategies focus on preventing the absorption of these peptides, preventing tissue transglutaminase from rendering gluten peptides more immunogenic, or inhibiting their binding to celiac disease-specific antigen-presenting molecules. Strategies that limit T cell migration to the small intestine or that reestablish mucosal homeostasis and tolerance to gluten antigens are also being explored. Additionally, it is vital to develop new therapeutic options for refractory celiac disease patients. This review highlights therapeutic strategies that may ultimately improve the health and well-being of individuals with celiac disease.

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Introduction

Celiac disease is a T cell-mediated, tissue-specific autoimmune disease that is activated in a subset of genetically susceptible people following dietary exposure to proline and glutamine-rich proteins found in certain cereal grains [1]. The causative proteins are termed gluten in wheat, hordeins in barley, and secalins in rye. For convenience, these proteins are often collectively referred to as "gluten." Celiac disease is characterized by damage to the small intestinal mucosa including partial or total villus shortening, crypt hyperplasia, and increased lymphocyte infiltration of the epithelium [2]. Clinical symptoms can vary considerably between patients. Since celiac disease is most pronounced in the proximal small intestine (duodenum and proximal jejunum), iron and calcium malabsorption is a hallmark of celiac disease. Individuals with celiac disease may have increased rates of mortality, especially if persistent inflammation is present [3, 4].

The past few years have witnessed an explosion of new data that present a more detailed view of the pathogenesis of celiac disease. In this article, we review current concepts of celiac disease pathogenesis with a view toward pinpointing sites in those pathways leading to celiac disease that can serve as possible intervention points for new celiac disease therapies. We also highlight areas of active ongoing investigation in that respect and putative advantages and potential problems in choosing specific targets for therapy.

Celiac disease pathogenesis

Celiac disease occurs in genetically susceptible populations in many regions worldwide, with a prevalence of about 1 % in the USA [5, 6]. The onset of disease requires the presence of dietary gluten and human leukocyte antigen (HLA) class II molecules on antigen-presenting cells that are capable of presenting immunogenic gluten-derived peptides to T cells. Gluten is composed of approximately 15 % proline and 35 % glutamine residues [7]. The high frequency of prolines in gluten proteins combined with the absence of prolyl endopeptidase activity in the human intestinal lumen and brush border makes these proteins resistant to digestion and results in the generation of potentially immunogenic gluten-derived peptides ranging from <10 to almost 50 amino acids in length [8]. Once in the lamina propria, these peptides can be presented to T cells by antigen-presenting cells expressing a particular subset of HLA class II molecules [9, 10]. Activated T cells produce pro-inflammatory cytokines, most notably interferon-γ (IFN-γ) [11], that lead to the release of tissue-damaging proteins including matrix metalloproteinases [12]. Tissue damage is thought to lead to the activation of tissue tranglutaminase (TTG), an enzyme that deamidates glutamine residues, creating negatively charged glutamate residues, which increases the affinity of gluten-derived peptides for celiac-associated HLA molecules [13]. Additionally, IL-15 signaling in celiac lesions results in the upregulation of the natural killer cell marker NKG2D on intraepithelial lymphocytes (IELs) and its ligand MICA on intestinal epithelial cells (IECs), resulting in the killing of IECs [14, 15]. These events subsequently lead to increased T cell influx, crypt hyperplasia, and villous shortening in the proximal small intestine (see Fig. 1 showing a summary of the pathogenesis of celiac disease with potential therapeutic targets).

Diagnosis

Celiac disease is the only autoimmune disease with a known trigger, i.e., gluten. Patients with active celiac disease develop antibodies to TTG, which is the autoantigen in celiac disease [16]. While a causal role for these antibodies in disease has not been established, they are useful in the diagnosis of the disease. The American Gastroenterological Association recommends detection of TTG IgA antibodies by ELISA for the initial screening of patients suspected of having celiac disease [17]. An immunofluorescence assay for antibody to endomysium (EMA) using monkey esophagus or human umbilical cord tissue also detects antibodies to TTG and increases the specificity of screening. Serum antibodies to gluten can be detected by ELISA; however, these antibodies may not be specific to celiac disease [18]. The more recent development of the IgA and IgG deamidated gliadin antibody ELISA may improve the diagnosis of celiac disease when used in combination with the IgA anti-TTG ELISA [19]. Following an initial positive blood test, diagnosis is confirmed by small intestinal biopsy, which remains the gold standard for diagnosis [2, 20].

HLA-DQ2 and DQ8

More than 90 % of celiac disease patients have HLA-DQ2, and almost all of the remaining celiac population possesses HLA-DQ8 molecules [9, 10]. HLA proteins are heterodimers with α- and β-chains, which can be encoded in *cis* or *trans* and present gluten epitopes to T cells. Two common DQ2 isoforms, DQ2.5 and DQ2.2, are found in the general population and in individuals at risk of developing celiac disease. Most celiac disease patients have the HLA-DQ2.5 isoform, which is encoded by a DQA1*0501 α-chain and a DQB1*0201 β-chain [21]. These individuals are at significantly increased risk of developing disease if they are homozygous for DQ2.5 or also have the DQ2.2 isoform, encoded by DQA1*0201 and DQB1*0202 [6, 21]. While in vitro certain gluten peptides can bind to DQ2.2 and trigger T cell activation in a manner similar to DQ2.5 [22], individuals with DQ2.2 alone do not have an increased incidence of disease [23]. In a large blood-based screen of individuals at risk for celiac disease, only 5 % were homozygous for DQ2.5 or had both DQ2.5 and 2.2, but they composed almost 30 % of the EMA-positive blood tests [24]. Further support for a DQ gene-associated risk is provided by in vitro experiments, which have confirmed that the HLA gene type and dose modulate the level of stimulation of gluten-specific T cells [21]. Because HLA-DQ2 and HLA-DQ8 have high affinity for a negative charge at certain residues of gluten peptides, antigen presentation is enhanced by the deamidation of glutamine to glutamate in gluten peptides by TTG [13, 25]. Deamidation of gluten peptides is thought to increase T cell activation by increasing the level of T cell stimulation due to the stronger interactions of gluten peptides to HLA molecules and by increasing the diversity of antigens presented by antigen-presenting cells [13, 25].

Loss of mucosal tolerance to gluten

Under normal conditions, the mucosal immune response to food antigens in the small intestine leads to the development of tolerance to these antigens [26]. This is the result of a combination of anergy and apoptosis of antigen-specific T cells and the active suppression of these cells by regulatory T cells (Tregs). The loss of tolerance to gluten and the activation of gluten-specific $CD4^+$ T cells in the lamina propria are required for the onset of celiac disease [27]. IL-15 is a pro-inflammatory cytokine present in active celiac disease [28] that has been linked with the loss of mucosal tolerance to gluten, providing a mechanism by which oral tolerance to

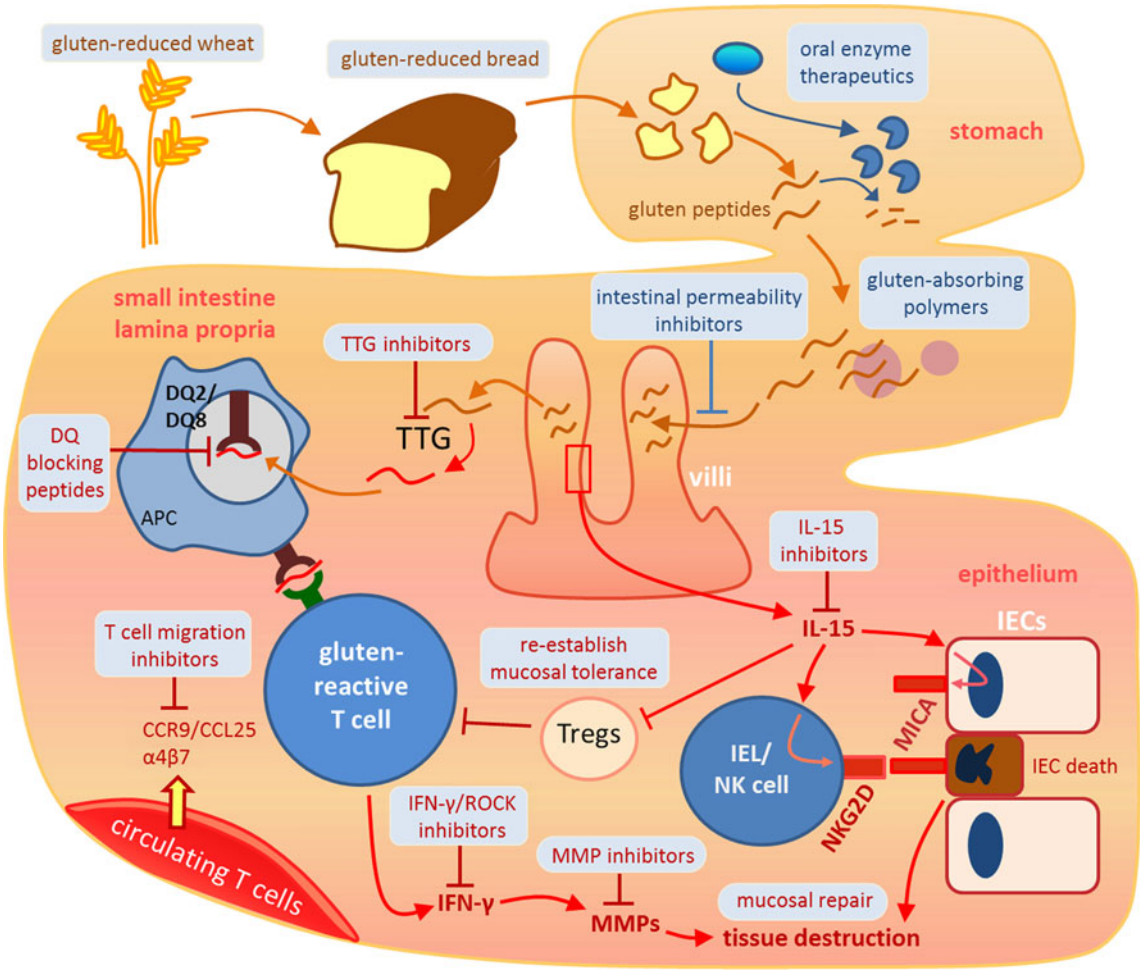

Fig. 1 Celiac disease immunopathogenesis and therapeutic targets. Therapeutic strategies are highlighted above their targets. The digestion of gluten-containing products results in gluten peptides that can be deamidated by tissue transglutaminase (*TTG*) in the small intestinal lamina propria, resulting in peptides with increased affinity for HLA-DQ2 or DQ8 on antigen-presenting cells (*APCs*). This results in an antigen-specific activation of gluten-specific T cells that have migrated to the small intestine using specific surface receptors. Activated T cells produce interferon-γ (*IFN-γ*), which, among other effects, activates matrix metalloproteinases (*MMPs*), which can damage tissues. IL-15 suppresses regulatory T cell (Tregs) activity and induces the expression of NKG2D on intraepithelial lymphocytes (*IELs*) and MICA on intestinal epithelial cells (*IECs*), resulting in directed killing of IECs and corresponding tissue damage

gluten can be overcome. DePaulo et al. [29] demonstrated that IL-15 induces IL-12p70 and IL-23 production by dendritic cells, leading to the suppression of Treg differentiation in mice. This effect is enhanced by retinoic acid and leads to the generation of T helper cell 1 (Th1)-polarized T cells that produce IFN-γ, and even Th17 cells if IL-6 is present.

An alternative explanation for IL-15-dependent loss of tolerance to gluten peptides was described by Hmida et al. [30] who utilized duodenal biopsy specimens. They demonstrated that lamina propria lymphocytes of celiac disease patients contained elevated levels of Tregs, but IELs from these patients were resistant to the suppressive effects of the Tregs. Additionally, lamina propria lymphocytes and peripheral blood lymphocytes from patients with villous atrophy and elevated IL-15 were resistant to Treg suppression. A similar increase in Tregs in biopsies from patients with active celiac disease compared with treated celiacs was observed by Zanzi et al. [31], who also found IL-15-mediated suppression of Tregs.

Genetic factors

While approximately 35 % of the Caucasian population has HLA-DQ2 or DQ8 [32], only a small percentage develop celiac disease, suggesting that additional genetic as well as environmental factors are required for disease. Additional evidence for a genetic basis for susceptibility comes from the high concordance rate for celiac disease of >70 % in monozygotic twins compared to a concordance rate of ~9 % in dizygotic twins, the latter being similar to the concordance rate in non-twin siblings of celiac disease patients [33]. Although a causal relationship has only been demonstrated for

the HLA-DQ2 and DQ8 loci [9, 10], genome-wide association studies have identified other genetic loci that may contribute to celiac disease. A 500-kb region including the IL-2 and IL-21 genes was estimated to contribute <1 % to the familial risk [34] compared to an approximately 40 % contribution of HLA-DQ2 and DQ8 genes to the disease [35, 36]. Additional loci of non-HLA genes were estimated to explain about 5 % of celiac disease heritability, with each additional gene contributing a fraction of a percent [35, 37]. Thirteen new loci, including many immunological candidates, have recently been identified using a dense genotyping strategy that has identified both common and rare genetic risk variants, bringing the number of known celiac disease risk loci to 40 [38].

Some of the pathways discovered include genes that encode proteins involved in T cell development in the thymus, viral RNA detection, T and B cell co-stimulation, and immune signaling molecules including cytokines and chemokines [37]. Genetic screens of "potential" celiacs with DQ2 or DQ8 and anti-TTG antibodies, but no intestinal lesions, may provide insight into the genes required for patients to progress to full-blown celiac disease [39]. Although many of the identified genes can be placed into a model of celiac disease based upon previous studies using human intestinal biopsy samples [6], information from genetic studies has the potential to identify novel pathways involved in disease, leading the way to the development of new therapeutic strategies.

Clinical and genetic studies have shown an association of celiac disease with other autoimmune diseases including type I diabetes and autoimmune thyroiditis [35, 40]. As with many autoimmune diseases, celiac disease is more common in women than men [41]. Celiac disease and diabetes share seven genetic loci including the HLA-DQB1 locus [42], and type I diabetics are at increased risk of developing celiac disease [43]. The mounting evidence indicates that celiac disease has much in common with autoimmune T cell-mediated diseases [6], which may be important when considering therapeutic interventions.

Potential environmental triggers

In addition to gluten, other environmental triggers may be important for the onset of the disease. A relationship between breastfeeding and celiac disease has been observed in most studies on the subject [44]. Reduced risk of developing celiac disease was associated with both the duration of breastfeeding and breastfeeding during gluten introduction. The cause of this effect is unknown. Breastfed and formula-fed infants have differences in their microbiota [45], and microbiota differences also appear to also be impacted by HLA genotype [46]. The microbiota as well as nutritional- and immune system-supporting factors in breast milk may contribute to the reduced gastrointestinal illness experienced by breastfed infants, and this reduction in infections extended beyond the time of breastfeeding [47].

Interestingly, infection by a variety of pathogens, including adenovirus 12 and hepatitis C virus (HCV), has been associated with celiac disease [48]. An increased risk rate for celiac disease diagnosis was observed for children with celiac disease-associated HLA genes after one or more rotavirus infections [49], and there are other descriptions of the onset of celiac disease following rotavirus infections [50]. Epidemiological studies have noted that patients with celiac disease have an increased likelihood of being born in the summer months, leaving food introduction around 6 months of age to occur at the seasonal peak in gastrointestinal illnesses in the winter [51, 52]. Although a causal relationship between infection and disease onset has not been demonstrated, rotavirus and other intestinal pathogens can create a pro-inflammatory environment and increase intestinal permeability [53] that may enhance the immune response to dietary antigens. IFN-α, a type I IFN commonly produced in response to viral infection, has been shown to increase the activation of the Th1 response to anti-CD3 in the small intestine, resulting in increased crypt hyperplasia [54]. Further support for a role for type I IFNs in disease induction is provided by numerous cases where patients with HCV being treated with IFN-α therapy have developed celiac disease [55, 56]. Since celiac disease results from a complex interplay of environmental and genetic factors, further studies that elucidate additional environmental and genetic risk factors for disease may suggest new therapeutic strategies to prevent disease onset.

Refractory celiac disease

Currently, the gluten-free diet (GFD), discussed below, is the only effective treatment for celiac disease. More than 95 % of celiac disease patients have uncomplicated disease that resolves, at least to a certain extent with the GFD [57]. Unfortunately, 2–5 % of patients develop refractory celiac disease (RCD) and retain symptoms and villous atrophy despite 6–12 months on a proper GFD [58–60]. RCD is divided into two subtypes, RCD I and RCD II, which may represent different stages of disease [59]. RCD I patients have villous atrophy similar to untreated celiac disease, with elevated numbers of IELs expressing normal CD3, CD8, and T cell receptor (TCR)-β surface markers [58]. These patients are at increased risk of a variety of infectious and noninfectious diseases and may have a slight increase in mortality. RCD II is a far more serious disease, with fewer than half of patients surviving more than 5 years after diagnosis [61]. RCD II is diagnosed by histological analysis of small intestinal biopsies containing abnormal IELs that lack standard T cell markers, including surface CD4, CD8, and TCR-α/β, and

contain intracellular CD3ε. These IELs also express a clonal TCR-γ chain gene rearrangement and appear to be the precursor to T cell lymphomas that commonly develop in these patients. While RCD II patients are at highest risk of overt enteropathy-associated T cell lymphoma (EATL), RCD I patients can develop this as well [61].

The gluten-free diet

History

The current gold standard therapy for celiac disease is a GFD. Nearly 60 years ago, Willem Dicke, a Dutch pediatrician, along with colleagues Weijers and van der Kamer, demonstrated that a "wheat factor" was the causative agent of celiac disease [62]. They recognized that this "wheat factor" was not wheat starch, and other starches including rice, corn, and potatoes, which did not worsen disease. By fractionating wheat flour components and systematically feeding them to children with celiac disease, they demonstrated that the gluten, and more specifically the gliadin, fraction of wheat flour contained the harmful factor [63]. Subsequently, others demonstrated the effectiveness of a gluten-free diet in adults [64].

Gluten tolerance levels

The daily gluten consumption in the general population has been estimated at approximately 10–20 g [65], and multiple studies have demonstrated that daily doses of <1 g/day are sufficient to induce mucosal damage in celiacs. It is estimated also that celiacs can safely ingest 10 mg, and possibly up to 100 mg, of gluten each day without inducing mucosal damage [66]. Catassi et al. [67] demonstrated in a prospective, double-blind, placebo-controlled study that 50 mg of gluten per day for 90 days was sufficient to decrease the villus height-to-crypt depth ratio in celiac disease patients, although no changes in IEL numbers occurred. In that study, a daily dose of 10 mg gluten did not induce a significant change in the mucosa of the patients. To ensure that this level is not surpassed, gluten-free foods that are devoid of cross-contamination must be eaten, requiring accurate determination of the gluten content of foods labeled gluten-free. Cross-contamination of foods labeled gluten free with gluten is estimated to be 5–50 mg/day [67].

Governmental agencies are currently working to establish safe levels of gluten in foods labeled gluten-free. The Codex Alimentarius Commission, run by the World Health Organization and Food and Agriculture Organization, began setting standards for gluten in 1979. The level of gluten in food designated gluten-free was set at 200 ppm (milligrams per kilogram) gluten in 1983; the level was decreased in 2008 to 20 ppm [68]. Twenty parts per million gluten would result in a daily exposure to between 8 and 20 mg gluten based upon calculations using the daily consumption of gluten-free foods in certain European countries [69]. In the USA, the Food and Drug Administration is considering labeling requirements that allow a food to be labeled "gluten-free" if it does not contain wheat, barley, or rye, or contains <20 ppm gluten [70]. The European Commission adopted Commission Regulation No. 41/2009 requiring <20 ppm gluten to be labeled "gluten-free" and <100 ppm to be labeled "very low gluten" if the product contained wheat, barley, rye, or oats and was processed to reduce gluten content [71]. Additional certification organizations exist, including the Gluten-free Certification Organization, an independent program of the Gluten Intolerance Group of North America which accepts 10 ppm gluten as the upper limit for a food to be labeled gluten-free.

Gluten detection strategies

To have value, reliable gluten detection methods must be in place. In practice, the detection of gluten in food products is challenging [72]. Wheat gluten is made up of high- and low-molecular-weight glutenins and α-, γ-, and ω-gliadins [73, 74]. While α-gliadin contains multiple T cell-reactive peptides, other reactive peptides are found in the glutenins and γ- and ω-gliadins. Barley hordeins and rye secalins contain similar immunogenic sequences. Brush border membrane enzymes in the small intestine generally break down peptides remaining from gastric and pancreatic digestion into single, dipeptides, or tripeptides; however, the high proline content of gluten results in longer peptides. Studies using gluten-reactive T cells have identified more than 200 immunogenic peptides, including an immunodominant α-gliadin 17-mer p57-73 peptide [75]. Quantitative mapping of T cell epitopes has revealed that the majority of gluten-reactive T cells recognized just three peptides generated from celiac patients ingesting a wide variety of gluten-containing grains [76]. Deamidation of certain gluten-derived peptides results in peptides with higher affinities for the celiac-associated HLA class II proteins, as demonstrated by their ability to react with a large number of gluten-specific T cell clones [77]. A 33-amino acid peptide (33mer) from gluten that is deamidated by TTG contains multiple epitopes recognized by T cells from patients with celiac disease [8]. Another α-gliadin peptide, p31–43 peptide, has been reported to trigger innate immune responses [78].

Differences in gluten detection strategies may impact consumers since the Codex Alimentarius Commission recommends different detection methods than the Association of Analytical Communities (AOAC), and both organizations have international influence. The ω-gliadin ELISA recommended by the AOAC is a sandwich ELISA that utilizes a

monoclonal antibody to detect ω-gliadin, which is heat and processing stable. In practice, this assay underestimates hordein from barley and may not accurately measure gluten content since the ω-gliadin fraction in wheat can vary from 6 to 20 % [79]. The R5 ELISA recommended by the Codex Alimentarius, and being considered by the FDA, uses the R5 monoclonal antibody that detects sequences present in all wheat gliadins, barley hordeins, and rye secalins [80]. The major drawback of the assay is that it overestimates barley hordeins. Both the ω-gliadin and the R5 ELISAs detect native and heat-treated gluten, but neither assay accurately detects hydrolyzed gluten [72].

Advantages of the GFD

The GFD has many advantages as a therapeutic. Most importantly, the GFD leads to clinical improvement in a majority of celiacs within a month [81]. The GFD may even reduce the risk of certain malignancies to that of the general population [82]. The diet is safe and effective in most celiac disease patients, and although initially it may be difficult for patients to adopt a GFD, over the past decade, there has been an exponential increase in the availability of gluten-free processed foods in specialty and general supermarkets. This has been accompanied by better and more detailed labeling of those products and an increase in information on cooking and eating gluten-free. Today, there is an abundance of healthy, naturally gluten-free foods and processed gluten-free foods available for the consumer, albeit at an increased cost for processed gluten-free goods compared to the gluten-containing equivalents [83].

Problems with the GFD

What then is the motivation for developing new therapies for celiac disease? Celiac disease is a lifelong disease. The GFD is a chronic therapy that must be maintained throughout life and requires diligent compliance. Moreover, social pressures such as those found in teens and college students, at business lunches and dinners, and during travel may decrease dietary compliance [84]. As most processed foods contain gluten derived from wheat or the analogous proteins present in rye and barley, adherence to the GFD and patient acceptance of the GFD are quite variable among celiac disease patients, with studies reporting strict compliance rates that vary from under 50 to over 90 % [83, 84].

Complications of celiac disease arise secondary to poor compliance or partial adherence to the GFD, and the intestinal mucosa may not completely return to normal in over 80 % of adults, with villous-to-crypt ratios being abnormal even in asymptomatic patients [4, 85]. Even these asymptomatic individuals may be at increased risk of serious health complications [86]. There are no data presently available on exactly how much gluten is present in the average individual's GFD, and the effectiveness of blood tests for measuring dietary compliance is unclear [87, 88]. The level of gluten cross-contamination may vary from country to country as it appears that the reversion of the damaged mucosa to normal among adults may be more complete in some countries than others (e.g., USA) [4]. Moreover, there is a marked variability in the sensitivity of different individuals with celiac disease to gluten, and it is not clear exactly how much gluten it takes to activate celiac disease in any particular patient.

Furthermore, there are conflicting data on what happens to the quality of life of individuals on a GFD. While quality of life generally improves after diagnosis, some studies have found it to be similar to and others lower than the general population [89, 90]. Even on a GFD, adult celiac disease patients have a higher occurrence of gastrointestinal symptoms than the general population [91]. Celiac disease patients on a GFD may also develop nutritional deficiencies [92]. Additionally, a small fraction of patients do not respond or respond poorly to the GFD, and they need other options to manage symptoms and resolve pathology [60].

GFD as the therapeutic standard

While a GFD is generally effective for clinical improvement in most patients, the issues highlighted above support the ongoing development of new therapies that replace, or more likely, augment it. An overall guiding principal for new therapies, irrespective of whether used as a replacement therapy for or as an adjunct to the GFD, is that such therapies ideally should be as safe, as effective, and as affordable over the long term as the GFD. Of course, cost and side effect considerations may be different for therapeutics developed for celiacs who do not respond adequately to the GFD.

Strategies to decrease immune system exposure to gluten

Reduced-gluten grains

Various strategies attempt to reduce or remove disease-activating proteins from gluten-containing grains. Selective breeding and genetic manipulation of the disease-activating grains have been proposed to reach this goal (Table 1). However, significant modification of wheat cultivars to remove these regions is likely to result in a loss of the characteristics that make gluten-containing flours desirable for baking as they are influenced by glutenin and gliadin proteins [93]. Current wheat cultivars mostly are hexaploid resulting from the breeding of tetraploid species with genotype AABB with diploid species with the DD genotype

thousands of years ago [94]. Selective breeding over the past hundred years has resulted in wheat varieties with better crop yield and improved characteristics for food production that have a greater gluten content. The genetic analysis of wheat accessions for reduced immunogenic sequences followed by analysis of their reactivity using T cell clones has led to the identification of certain accessions with reduced levels of harmful gluten sequences [94, 95]. Natural mutations in gluten-encoding genes combined with an absence of certain of those genes may render certain wheat accessions useful in selective breeding programs.

Genetic manipulation of wheat varieties to remove or reduce antigenic gluten sequences is also possible. When α-gliadin encoding sequences were genetically removed from the hexaploid bread wheat, *Triticum aestivum* genome, some T cell-stimulating epitopes disappeared. Unfortunately, this was accompanied by a decrease in desirable technical properties of the wheat, including reduced dough strength and elasticity [93]. These technical properties were retained when γ- and ω-gliadin and low-molecular-weight glutenins were removed, but numerous T cell stimulatory epitopes remained intact. RNA interference technology has also been used to knock down the expression of α-, γ-, and ω-gliadins [96]. The knockdown strategy resulted in the reduced expression of the immunogenic gliadins and the significantly reduced immune responses of gliadin-specific T cell clones. In general, the various transgenic lines maintained technical properties consistent with moderate quality wheat, probably because they retained the high-molecular-weight glutenins. The tolerance of celiac disease patients to these genetically modified grains remains to be determined.

Another gluten-reducing strategy involves enzymatic treatment of gluten-containing grains. Sourdough bread made with wheat pretreated with lactobacilli and their cytoplasmic extracts did not increase intestinal permeability in celiac patients, unlike bread made using baker's yeast [97]. The lactobacilli contain proline-specific enzymes that were able to hydrolyze immunogenic gluten peptides including the immunogenic 33mer. A similar strategy using lactobacilli and fungal proteases reduced sourdough bread gluten content to <10 ppm, and in a 60-day challenge with the equivalent of 10 g of native gluten, the bread did not change gluten or TTG antibody levels or intestinal permeability in a cohort of pediatric celiac disease patients [98].

A different approach treated wheat flour with a microbial transglutaminase in the presence of lysine methyl ester to cross-link glutamine residues that would otherwise act as TTG deamidation substrates [99]. This microbial enzyme is commercially used to improve dough quality by increasing its stability and elasticity, and it does not deamidate glutamine residues like human TTG. Gliadin extracted from wheat flour treated in this manner induced significantly lower levels of IFN-γ than untreated flour in gluten-specific T cell lines.

Oral enzyme therapeutics

Oral enzyme therapeutics offer another avenue to reduce the amount of immunogenic gluten peptides that reach the small intestine when an individual with celiac disease ingests gluten. Because the human digestive tract lacks prolyl endopeptidases (PEPs) that are capable of hydrolyzing proline-rich peptides found in gluten, large and potentially immunogenic peptides are produced. A number of bacterial species produce PEPs that have been evaluated for their ability to digest gluten. PEPs derived from *Flavobacterium meningosepticum* (FM) were effective at reducing the levels of the immunogenic 33mer in vitro and in vivo in rats [8]. Subsequent studies of FM-PEP revealed that high amounts of enzyme would be required to detoxify a normal daily gluten intake of 20 g, and the instability of the enzyme in the stomach decreased efficiency [100]. Clinical studies of FM-PEP revealed a reduction in fat and xylose malabsorption in celiacs given gluten pretreated with FM-PEP compared with gluten alone, although there was no difference in symptoms [101].

Gass et al. [102] demonstrated that a combination of a PEP from *Sphingomonas capsulata* (SC) and a glutamine-specific endoprotease EP-B2 from germinating barley seeds substantially reduced the stimulation level of immunogenic peptides in whole-wheat bread in vitro. In an in vivo rat model, however, EP-B2 increased the level of immune stimulation, which was reduced to the level of untreated bread by the addition of SC-PEP. The activity of SC-PEP was subsequently enhanced using an iterative mutagenesis strategy [103]. This resulted in an enhanced specific activity of the enzyme at low pH and stability in pepsin when combined with EP-B2. This enzyme combination is the therapeutic product ALV003 produced by Alvine Pharmaceuticals that is currently undergoing clinical trials [104]. In a clinical study, a meal containing 16 g gluten was pretreated with ALV003 prior to being fed to individuals with celiac disease; immune responses were measured by IFN-γ ELISpot assay using peripheral blood T cells stimulated with gliadin or immunogenic 33mer [105]. Of the ten patients treated with ALV003, none showed significant ELISpot responses compared to six of ten in the control group; however, as with FM-PEP, no differences in symptoms were observed. Phase 1 safety data confirmed that ALV003 was well tolerated in patients, and using gastric aspirates, the highest dose degraded almost 90 % of the 1 g of wheat gluten administered [104]. In a 6-week randomized phase 2a clinical study that included a daily 2-g gluten challenge, ALV003 significantly reduced changes in villous height-to-crypt depth ratios and IEL numbers compared to placebo according to a conference abstract [106]. Another PEP in clinical trials whose results have been communicated in abstract form is AN-PEP from *Aperigillus niger* [107].

While AN-PEP was safe and well tolerated, the gluten challenge was not sufficiently long to cause mucosal damage and serum antibodies to appear in enough of the placebo group to allow for a meaningful evaluation of its efficacy.

Numerous digestive enzymes with claims to digest gluten are commercially available, including many that use dipeptidyl peptidase IV (DPPIV) from *Aspergillus oryzae*, but their clinical effectiveness has not been established in celiac disease patients. The lack of specificity of these enzymes is problematic, especially when gluten is ingested with other protein-rich foods like meat and dairy. Stan1, developed using commercially available food grade proteases including DPPIV, was able to reduce immunogenic gluten peptides present in wheat bread and whole gluten powder, although with low specificity [108]. Stan1 is currently in clinical trials with celiac disease patients (see clinicaltrials.gov).

Minimizing gluten absorption

An alternative to prevent the interaction of immunogenic gluten peptides with the intestinal mucosa involves using high-molecular-weight polymers that selectively bind gluten and prevent its break down and absorption. Styrene solfonate (SS) is a monomer capable of forming hydrophobic and electrostatic interactions with gluten. When copolymerized with hydroxyethylmethacrylate (HEMA) to form poly (HEMA-*co*-SS), it selectively bound to gliadin in simulated gastric and duodenal conditions and abolished the deleterious effects of gliadin on cells in culture [109]. When given orally with whole wheat and other food, this polymer reduced mucosal damage and pro-inflammatory cytokine production in gliadin-immunized HLA-DQ8/HCD4 transgenic mice, and it reduced TNF-α production in response to gliadin in celiac intestinal biopsies [110].

Decreasing intestinal permeability

The movement of water, solutes, and cells from the intestinal lumen to the lamina propria is limited by tight junctions [111]. These tight junctions are composed of protein complexes that create strong interactions between IECs and the underlying cytoskeleton. Tight junctions may restrict the paracellular transport of antigenic gluten-derived peptides, limiting the activation of antigen-presenting dendritic cells in the lamina propria. Patients with active celiac disease have increased intestinal permeability as measured by sugar permeability tests and tight junction structural analysis [112, 113]. It has not yet been determined whether the increased permeability is a cause or consequence of celiac disease since it is likely to result from significant alterations to the intestinal mucosa that are often seen in celiacs with active disease. Although certain intestinal barrier function genes are associated with celiac disease, they are not associated with increased anti-gliadin antibodies and therefore may not be important in the development of an immune response to gluten [114].

Tight junction permeability can be regulated by zona occludens toxin (Zot) from *Vibrio cholera*, which leads to increased paracellular permeability through alterations in the cytoskeleton [115]. A human protein, zonulin, was discovered to have a similar effect and is upregulated in celiac intestinal tissues [116, 117]. AT-1001 (larazotide acetate) is an octapeptide corresponding to the amino acid sequence of the receptor-binding motif of human fetal intestinal zonulin and acts as an inhibitor of intestinal permeability [116, 118]. The drug, developed by Alba Therapeutics, was well tolerated at doses tested in phase 1 and phase 2 clinical trials, and it decreased intestinal permeability, IFN-γ production, and intestinal symptoms following a single gluten challenge in celiac disease patients [118].

There is also evidence describing the movement of gluten-derived peptides through a transcellular as opposed to a paracellular route [119]. In this putative retrotranscytosis pathway, the movement of gluten peptides bound to secretory IgA through the cell is initiated by binding to the secretory IgA receptor, CD71, on the surface of IECs. The impact of zonulin or AT1001 on this pathway has not been explored.

Preventing T cell activation by gluten

Blocking deamidation of gluten-derived peptides

The deamidation of gluten is not an absolute requirement to activate celiac disease in children [120], but is important for increasing T cell reactivity to gluten by increasing peptide affinity to HLA-DQ2 and DQ8 molecules [121, 122]. Whether gluten deamidation is a requisite to activate celiac disease in adults is not known.

TTG inhibitors may improve disease severity by reducing the affinity of gluten-derived peptides for the HLA molecules associated with celiac disease. Therapeutic approaches to inhibit TTG include competitive amine inhibitors, reversible inhibitors, and irreversible inhibitors [123]. The most widely available TTG inhibitors are the competitive amine inhibitors, which are generally primary amines attached to saturated hydrocarbon chains and function solely by competing with natural enzyme substrates. Cystamine is a competitive TTG inhibitor with neuroprotective effects in neurodegeneration models, which highlights the potential utility of this class of therapeutics for diseases other than celiac disease [124]. In a study of T cell responses to deamidated gliadin, cystamine inhibited endogenous TTG in biopsy samples, resulting in decreased T cell responses [122]. Reversible TTG inhibitors block substrate access to the active site, but do not covalently

Table 1 Potential and approved therapies for celiac disease and RCD patients

Therapy	Strategies	Products stage of development	Reference(s)
Genetically modified wheat	Selective breeding	In vitro[a,b]	[94, 95]
	Genetic deletions	In vitro[b]	[93]
	RNA interference	In vitro[a,b]	[96]
Enzymatic treatment of wheat flour	Microbial extracts	Clinical study	[97, 98]
	Microbial transglutaminase	In vitro[a]	[99]
Oral enzyme therapeutics	Prolyl endopeptidase (PEP)	FM-PEP—Clinical study[c]	[101]
		AN-PEP—Clinical phase 2a	[107]
	Glutamine endoprotease (EP)	ALV003(PEP+EP)—Clinical phase 2a	[104, 106]
	food grade proteases	Stan1—Clinical[d]/DPPIV commercial	[108]
Minimizing absorption	Bind gluten peptides	Poly(HEMA-*co*-SS)-in vitro[e]/in vivo[f]	[110]
Reducing intestinal permeability	Block zonulin receptor	AT-1001—Clinical phase 2	[118]
TTG inhibitors	Competitive inhibitors	In vitro[a,e]	[122]
	Reversible inhibitors		
	Irreversible inhibitors	In vitro[e]/in vivo[g]	[126, 128, 129]
DQ2 blockers	DQ peptide competitors	In vitro	[131, 132, 134]
	DQ mimics	In vitro[a]	[135]
IFN- γ/ROCK inhibitors	Antibody to IFN-γ	Clinical for other diseases	[137]
	ROCK inhibitors	Clinical for other diseases	[139]
MMP inhibitors	General MMP inhibitors	Clinical for other diseases	[142]
	Specific MMP inhibitors	In vitro other diseases	[143]
T cell migration inhibitors	CCR9/CCL25	CCX282-B—in vivo[h]/Clinical phase 2[d]	[147]
	α4	Clinical for other diseases	[148, 149]
	α4β7	Clinical for IBD	[151]
Modulating T cell activity	Immunization	Nexvax—Clinical phase 2	[153]
	Bacterial antigen expression	In vivo[f]	[156]
	Anti-CD3 therapy	Clinical for other diseases	[157, 158, 161]
Restoring intestinal homeostasis	Probiotics	In vitro[i]/In vivo[f]	[166–168]
	Helminth infection	Clinical phase 2	[170]
Immune suppressors	Systemic steriods	Prednisone—Approved for RCD patients	[60]
	Locally active steriods	Budesonide—Clinical for RCD patients	[171, 172]
	Anti-inflammatory	Mesalamine—Clinical for RCD patients	[173]
	Purine analogs	Azathioprine—Approved for RCD	[60]
		Cladrabine—Clinical for RCD patients	[174]
IL-15 signaling inhibitors	IL-2/IL-15Rβ antibodies	Clinical for other diseases	[176–178]
	NKG2D	In vivo[h]	[179]
Stem cell transplantation		Clinical for RCD patients	[183]
Mucosal repair	Increase proliferation	R-spondin1—In vivo[h]	[184]

Examples of specific classes of therapies are provided along with their stage of development.

[a] Assayed by T cell clones from celiac disease patients

[b] Epitopes detected by gliadin-specific monoclonal antibodies

[c] Gluten pretreated with FM-PEP

[d] See clinicaltrails.gov

[e] Biopsies from celiac disease patients

[f] DQ8 mouse model

[g] dsRNA mouse model

[h] IBD mouse model

[i] Cell culture

modify TTG, while irreversible inhibitors prevent substrate binding through a covalent modification of the enzyme. The viability of the TTG knockout mouse [125] suggests that irreversible inhibition of the enzyme may be a therapeutic option that does not result in serious health consequences. Importantly, the irreversible TTG inhibitor R283 (L682777) significantly reduced T cell activation in celiac biopsies in response to a gliadin peptide [126]. Numerous reversible and irreversible inhibitors exist; however, they are all in the discovery phase [127]. A mouse model for small intestinal damage induced by long double-stranded RNA results in the activation of TTG and has shown promise in the study of intestinal TTG inhibitors [128, 129].

DQ2 blockers

An alternative to preventing deamidated gluten peptides from interacting with antigen-presenting cells is to block the binding of these peptides to celiac disease-specific HLA proteins. High-affinity blockers are required because only a limited number of HLA–peptide complexes are likely required to activate T cells [130]. Recent attempts to generate a DQ2-blocking peptide have led to the generation of peptides that can outcompete gluten peptides due to greater binding affinities for DQ2 [131, 132]. Attempts to determine the optimal binding sequences have utilized HLA binding parameters determined by sequencing natural ligands and studying the binding of synthetic peptides. Juse et al. [131] generated blocking peptides using a positional scanning nonapeptide library, resulting in peptides with 50-fold higher binding to DQ2 than the immunodominant α-gliadin. An in silico approach [133] led to the generation of a nonapeptide flanked by an N-terminal and C-terminal sequence that enhanced binding [132]. Additionally, the addition of a non-proteinogenic amino acid increased binding, resulting in affinities 100- to 200-fold higher than the highest affinity gluten-derived peptide. A recent strategy screened peptide libraries for binding to HLA-DQ2.5 followed by mass spectrometry sequencing of promising peptides [134].

An alternative strategy to prevent T cell activation used a recombinant HLA-DQ2.5 mimic tethered to a gliadin peptide as a means to induce antigen-specific tolerance in T cells [135]. The recombinant T cell ligand significantly reduced proliferation and pro-inflammatory cytokine production in celiac disease biopsy-derived T cells in response to deamidated gluten and gluten-specific peptides.

Inhibitors of pro-inflammatory signals and their effects

Targeting IFN-γ

IFN-γ is produced in high amounts by gluten-reactive T cells upon stimulation with antigen and is associated with increased epithelial permeability [136]. A therapeutic IFN-γ antibody, fontolizumab, induced a clinical response in some Crohn's disease patients, but the response was not significant and some adverse events were associated with the treatment [137]. The mechanism by which IFN-γ increases intestinal permeability was demonstrated to be through the Rho-associate kinase (ROCK) in the T84 intestinal epithelial cell line [138]. ROCK inhibitors are being studied for the treatment of other diseases, but due to the lack of specificity of many of these inhibitors [139], their chronic use in celiac disease patients may not be desirable.

MMP inhibitors

Matrix metalloproteinases (MMPs) are endopeptidases responsible for tissue remodeling and are upregulated in celiac disease [140]. These proteins are capable of tissue destruction and have been shown to be important mediators of T cell-mediated damage in the small intestine [141]. Broad-spectrum MMP inhibitors have not shown success in the clinic and have serious side effects [142]. Selective MMP inhibitors have been developed that may have fewer side effects, but their success in the clinic has not yet been established [143].

Inhibitors of T cell migration

CCR9/CCL25

Since celiac disease is a T cell-mediated disease, preventing or limiting T cell migration to the small intestine may limit local T cell activation and reduce intestinal damage. The migration of T cells to the small intestine is controlled by their expression of the cell surface chemokine receptor CCR9 and the integrin α4β7 [144]. Expression of CCL25, the ligand for CCR9, in the small intestine results in the selective migration of CCR9-positive lymphocytes [145]. Antibodies targeting CCR9 and CCL25 have shown efficacy in an animal model of intestinal inflammation [146]. Also, a selective small-molecule human CCR9 antagonist, CCX282-B, reduced the severity of histopathology in a Crohn's disease mouse model [147]. Two clinical studies were completed with CCX282-B in Crohn's disease patients and a phase 2 clinical trial was conducted in celiac disease patients in 2008, but the results have not yet been published.

α4β7 integrin

Multiple strategies have attempted to limit the migration of pro-inflammatory lymphocytes expressing the α4β7 integrin by targeting the integrin and its target MAdCAM-1, which is expressed by endothelial cells in the small intestine

and colon [148]. Anti-α4 integrin antibodies and α4 small-molecule inhibitors are currently being tested in animal models and clinical trials for other inflammatory diseases [148, 149]. Because the α4 integrin subunit is commonly associated with the β1 subunit, which can bind fibronectin and VCAM-1, in addition to associating with the β7 integrin subunit, the potential for side effects with α4-inhibitors is higher than specific α4β7 antagonists [150]. Vedolizumab is a humanized anti-α4β7 integrin antibody that has shown promise in the treatment of ulcerative colitis [151]. Currently, there are no data from celiac-related studies on this class of therapeutics.

Reestablishing T cell tolerance to gluten peptides

Immunization with gluten antigens

While long-term administration of therapeutics may benefit some celiac disease patients, therapeutic strategies with long-term effects are desirable. Immunization strategies using peptide antigens to induce tolerance may help reestablish tolerance to self-proteins in autoimmune diseases [152]. The potential of these vaccines to delete or suppress gluten-specific T cells, possibly through the induction of regulatory T cells, could prevent the pathogenic T cell activation that occurs in celiac disease. For this type of strategy to be effective, a limited number of immunogenic peptides must induce tolerance. Although many immunogenic peptides have been recognized in celiac disease patients, a limited number appear to be sufficient to activate a majority of T cell responses [76]. Phase I clinical trials have been conducted with Nexvax2, a vaccine utilizing three 15- to 16-mer DQ2 immunodominant peptides to induce tolerance to gluten. A double-blind, randomized, placebo-controlled trial studied weekly vaccinations for 3 weeks in celiac disease patients on a GFD. Vaccine-specific T cells responses were observed along with some gastrointestinal symptoms at high dosage according to an abstract [153]. If this strategy is successful, a separate DQ8-specific vaccine may be required due to the differential affinity of DQ2 and DQ8 for gluten antigens [154].

Engineered bacteria to induce tolerance

Another method of reestablishing mucosal tolerance to gluten utilized an engineered bacteria strategy that was successful in generating oral tolerance to ovalbumin (OVA) in OVA TCR-expressing mice [155]. Huibregtse et al. [156] generated a *Lactococcus lactis* strain that produced and delivered a DQ8 gluten-specific antigen to antigen-immunized DQ8 mice bred in a NOD background to increase autoimmunity and pathogenesis. Importantly, *L. lactis* is a nonpathogenic bacterium that does not colonize the intestine. Mucosal delivery of antigen by *L. lactis* suppressed systemic gluten-specific T cell responses in a TGF-β- and IL-10-dependent manner. Additionally, splenocytes from *L. lactis*-treated animals had increased numbers of Tregs, suggesting that tolerance was at least partly induced by these cells.

Anti-CD3 therapy

Due to the central role of T cells in the development of celiac disease, a strategy that generally reduces T cell responses may have value. CD3, a co-receptor to the T cell receptor, is a target for an antibody-based therapy being explored as a treatment for newly diagnosed type I diabetes mellitus and patients with psoriatic arthritis [157, 158]. Of relevance to celiac disease, this therapy has the potential to induce Tregs [159] that play an important role in the induction of mucosal tolerance. An oral anti-CD3 therapy [160] may have more utility for the treatment of celiac disease. A trial in healthy humans demonstrated that oral anti-CD3, OKT3, reduced Th1 and Th17 responses and had other effects, suggesting that it effectively activated Tregs in the gut-associated lymphoid tissue [161].

Modulating intestinal homeostasis

Probiotics

The "hygiene hypothesis" attempts to explain the concomitant increased prevalence of immunological diseases like allergies and certain autoimmune diseases with a decreased infectious disease burden in developed countries [162]. It is possible that immune system homeostasis is altered in an environment mostly devoid of chronic infections, resulting in enhanced immune responses to nonpathogenic sources. An evolutionary relationship between certain genes, i.e., interleukins, associated with celiac disease and those coding for resistance to viruses and bacteria has been described [163]. While direct evidence of improved hygiene or altered pathogen burden as a cause for celiac disease is lacking, differences in microbiota between celiacs and controls exist. Short-chain fatty acid alterations are indicative of differential microbiota metabolic activity in celiac disease patients [164]. Interestingly, this was found to be independent of a GFD and inflammatory state. Another study examined the microbiota of pediatric celiac disease patients with active disease and following treatment with a GFD [165]. Active celiacs had a higher inter-individual similarity than controls and showed a higher diversity of duodenal mucosa-associated microbiota than controls. This diversity decreased with a GFD, suggesting that a higher bacterial diversity could have

a harmful impact on homeostasis and sensitivity to disease. It has not been established whether alterations in the microbiota in individuals with celiac disease are a cause or consequence of disease.

The use of probiotics as a therapy for celiac disease is also being explored. Intestinal cell culture demonstrated that a particular probiotic, *Bifidobacterium lactis*, inhibited membrane permeability and cytoskeletal rearrangements induced by gliadin, whereas *Lactobacillus fermentum* did not [166]. While this suggests that probiotics may improve disease, care must be taken in the selection of probiotics. *Lactobacillus casei*, another probiotic, actually enhanced IFN-γ and reduced IL-4 and IL-10 mRNA in a DQ8 mouse model of gluten sensitivity in gliadin-immunized mice, although there was no apparent change in the small intestinal mucosa structure [167]. These results, along with others [168], suggest that certain probiotics may have stimulatory as opposed to suppressive effects on inflammatory mediators.

Helminth infection

Helminth infections, now only common in developing nations where rates of allergic and autoimmune diseases are significantly lower than industrialized countries, have a potent ability to modulate the immune system. The ability of parasitic worms to induce IL-4, IL-10, TGF-β, and Tregs while reducing IFN-γ and IL-17 production makes them a potential candidate to treat a variety of inflammatory diseases [169]. The use of helminths in animal models and in the clinic for human trials has shown therapeutic potential for inflammatory bowel disease. It is possible then that this type of therapy might have utility in celiac disease patients. In a randomized, double-blind, placebo-controlled clinical trial, ten celiac disease patients were inoculated at two separate times with *Necator americanus*, resulting in the colonization of all patients [170]. *N. americanus* does not replicate in humans, and anti-helminth therapy can be used to effectively remove the parasite. These patients were then challenged at week 20 with 16 g gluten for 5 days. Unfortunately, *N. americanus* infection did not improve gluten tolerance in colonized celiac patients as measured by biopsy and IFN-γ ELISpot.

Refractory celiac disease therapeutics

Immune suppression

RCD is traditionally treated with corticosteroids combined with other immunosuppressive drugs including azathioprine, which can have serious systemic side effects. Budesonide is a synthetic glucocorticoid with potent local anti-inflammatory activity and low systemic bioavailability that improved symptoms in a study of patients with RCD [171]. Unfortunately, the use of budesonide in Crohn's disease patients did result in significant reductions in bone mineral density over a 2-year period, suggesting that systemic side effects may occur with long-term use [172]. Small intestinal release mesalamine, a 5-aminosalicylic acid anti-inflammatory treatment used in ulcerative colitis, has shown promise in a study of RCD I patients when used alone or in combination with budesonide [173]. Also, cladribine, a drug used to treat hairy cell leukemia and multiple sclerosis, increased survival in a cohort of RCD II patients who responded to therapy, although only half of patients were responders [174].

IL-15 signaling inhibitors

The role of IL-15 in the onset and severity of celiac disease is supported by animal and human biopsy studies [28, 29]. Due to the potent immune-modulating effects of IL-15, therapy directed inhibiting IL-15 or blocking the IL-15 receptor (IL-15R) may be useful for patients with RCD. Studies using RCD II IELs demonstrated that IL-15 promotes the survival of abnormal IELs that may lead to lymphoma [175]. In an IL-15-driven mouse model of intestinal inflammation, an antibody to the IL-2/IL-15Rβ reversed intestinal damage [176]. Clinical trials for other disease have shown that antibodies to IL-15 and IL-2/IL-15Rβ are active and are well tolerated [177, 178]. IL-15 signaling increases the surface expression of NKG2D on NK cells and its ligand MICA on IECs [14]. The interaction of NKG2D on natural killer cells and MICA on IECs leads to the killing of IECs, which is thought to contribute to the small intestinal damage observed in celiac disease [15]. While no studies have been done in the clinic, prophylactic targeting of NKG2D significantly reduced the development of colitis in an animal model [179].

Stem cell transplantation

Hematopoietic stem cell transplantation has shown mixed results in the treatment of celiac disease and RCD. Two instances in the literature describe allogeneic hematopoietic stem cell transplantation from a matched family member that resulted in the curing of celiac disease [180, 181]. Celiac disease has also been transferred by allogeneic bone marrow transplantation from an HLA-matched sister with celiac disease [182]. Autologous stem cell transplantation was found to be unsuccessful in four patients with EATL, although one patient was in complete remission almost 3 years after the procedure [183].

Enhancing intestinal healing

An adjunctive therapy for patients with RCD and celiac disease may include the introduction of agents that enhance intestinal healing. R-Spondin1 increases proliferation in the crypts and the number of goblet cells, which thicken the mucus lining [184]. This drug reduced colon inflammation and improved mucosal integrity in mouse colitis models.

Regulatory considerations

Patient selection

A review of the history of the development of the GFD as the standard of care for celiac patients clearly illustrates several challenges associated with measuring the success of therapeutic interventions, many of which are relevant today. Early studies lacked specific diagnostic criteria for patients with celiac disease, no doubt leading to the inclusion of non-celiacs and exclusion of celiacs from studies. Prior to the development of small intestinal mucosal biopsy for diagnosis, celiac patients were diagnosed by a set of common symptoms including gastrointestinal symptoms, weight loss, diarrhea, and steatorrhea [185]. Response to the GFD further supported diagnosis, but it was not until the development of serological tools that a more effective diagnosis of celiacs with a diverse set of symptoms occurred [186]. Current diagnostic methods more clearly identify celiac patients, although now investigators must decide whether to definitively diagnose patients based upon serological markers alone or in combination with biopsy and what grade biopsy abnormality is required. This type of selection may lead to the exclusion of individuals with latent celiac disease, where no mucosal damage is apparent.

Biomarkers

Another challenge that investigators face today is finding reliable biomarkers or end point measures of therapeutic effect. Studies attempting to show the therapeutic benefit of treatments in celiac disease patients must rely on effective and accepted biomarkers for evaluating the therapeutic response. It is also essential to select biomarkers that measure therapeutic effects over the course of a study, which may be too short of a period for traditional markers to change.

While early studies used fecal fat content as a biomarker of disease, currently, intestinal permeability is often measured by lactulose/mannitol absorption assay, but this assay lacks specificity for celiac disease [187]. Intestinal biopsy and blood tests provide the most accurate diagnosis of celiac disease and measures of disease state. Biopsy is the gold standard for celiac disease diagnosis and recovery, but it is not feasible or desirable to use it as the sole determinant of therapeutic success. In addition to being time-consuming, invasive, and costly, biopsy is not a useful measure of the effectiveness of some therapies, like glucocorticoids in RCD patients where improvements in other signs and symptoms of disease occur without an improved histological score. It is therefore important to carefully evaluate additional biomarkers and evaluation tools that are available. Video capsules can provide a snapshot of intestinal health in a less invasive manner than endoscopy and biopsy, and this technique has been successful in monitoring RCD [188, 189]. The detection of antibodies against TTG or deamidated gliadin peptide can be useful in the detection and monitoring of celiac disease, but they often require periods longer than those desired for clinical studies to change and may not detect low levels of gluten that are sufficient to cause disease [87]. Symptom scores and patient-reported outcomes may be useful assessments of therapeutic benefit, but the diversity of symptoms of patients with celiac disease and the coexistence of other conditions may complicate the interpretation of these assessments [190]. Ideally, one might develop a patient-reported outcome instrument that directly and accurately reflects celiac disease severity and histology scores, but judging by the large numbers of asymptomatic celiacs who also have mucosal damage, this seems unlikely. Currently, there are no universally accepted disease-specific biomarkers specific for assessing a therapeutic response in celiac disease clinical studies.

Ethical issues

It is important to recognize ethical considerations in clinical studies of new therapies for celiac disease patients. Often, patients in remission on a GFD are challenged with gluten, which can reactivate symptoms. Because most patients respond to a GFD, which is a safe and effective therapy, there is a need for preclinical and clinical safety evaluations and clinical study designs that minimize risks for patients. While some therapeutics may be evaluated without gluten challenge in patients who are not adequately responding to a GFD, other studies will rely on the ability of a new therapy to prevent disease activation by gluten in patients with proven celiac disease who have responded to a GFD. The amount of gluten used in the challenge should be in line with the level of the anticipated therapeutic effect. It must be high enough to activate signs and symptoms that serve as primary and secondary end points in the study, but not so high that it leads to significant patient withdrawal, especially in the placebo group. Small doses of 1 or 2 g, as used in trials of the oral enzyme therapeutics [104, 106], may be sufficient to observe effects on histology and other measures if the duration of the study is sufficient. On the other hand, RCD II patients with limited therapeutic options may be

more willing to be participate in studies with therapeutics that have unknown or possibly serious side effects.

Conclusions

Recent advances in our understanding of the immunopathogenesis of celiac disease have opened the doors for a variety of new treatments that may be used as stand-alone therapies or as adjuncts to the GFD. While the GFD is the only approved treatment of disease, some therapies are in human trials and may be available within the next 5–10 years. It is likely that the first approved therapeutics will be used along with the GFD to decrease reactions and symptoms to incidental or accidental gluten exposure. Future generations of therapy may be able to restore immunologic tolerance to gluten, and perhaps even effectively cure celiac disease.

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