

# Emerging Therapeutic Options for Celiac Disease: Potential Alternatives to a Gluten-Free Diet

Anita Bakshi, MD, Sindu Stephen, MD, Marie L. Borum, MD, and David B. Doman, MD

Dr. Bakshi is a Gastroenterology Fellow in the Division of Gastroenterology and Liver Diseases at George Washington University Medical Center and is affiliated with Medical Faculty Associates, both in Washington, DC. Dr. Stephen is a Gastroenterologist in Silver Spring, Maryland. Dr. Borum is a Professor of Medicine and the Director of the Division of Gastroenterology and Liver Diseases at George Washington University Medical Center; she is also affiliated with Medical Faculty Associates.

Dr. Doman is a Clinical Professor of Medicine at George Washington University School of Medicine in Washington, DC.

Address correspondence to:

Dr. David B. Doman  
12012 Veirs Mill Road  
Silver Spring, MD 20906;  
Tel: 301-942-3550;  
E-mail: drdbd@aol.com

## Keywords

Celiac disease, gluten-sensitive enteropathy, malabsorption, zonulin inhibitor, therapeutic vaccine, probiotics, transglutaminase inhibitors

**Abstract:** Celiac disease is an autoimmune disorder of the small intestine that is more common than was previously thought. This disease is caused by an inappropriate immune response to wheat gluten, barley, and rye. Three main pathways cause celiac disease: the environmental trigger (gluten), genetic susceptibility, and unusual gut permeability. The only treatment currently available is a strict gluten-free diet. Unfortunately, a majority of patients have difficulty complying with this diet, and the response to therapy is poor. Therefore, alternative treatments are being developed, and new insights into the pathophysiology of celiac disease have led to research into novel therapies. New treatments include engineering gluten-free grains, decreasing intestinal permeability by blockage of the epithelial zonulin receptor, inducing oral tolerance to gluten with a therapeutic vaccine, and degrading immunodominant gliadin peptides using probiotics with endopeptidases or transglutaminase inhibitors. These nondiet-based therapies provide hope for enhanced, lifelong celiac disease management with improved patient compliance and better quality of life.

Celiac disease (CD), also known as gluten sensitivity enteropathy or celiac sprue, is an immune-mediated enteropathy that is triggered in genetically susceptible individuals by the ingestion of gluten—the major storage protein of wheat, barley, and rye.<sup>1</sup> Globally, CD is one of the most common autoimmune disorders. The clinical presentations of the disease vary, with either typical intestinal symptoms or a spectrum of atypical extraintestinal symptoms. Clinically silent forms can also occur, which are often difficult to diagnose. Given the wide spectrum of presentations, the diagnosis is often missed.<sup>2</sup> CD can occur at any age, is more prevalent than was previously thought, and can affect a variety of organ systems.<sup>3</sup>

Early recognition and treatment of CD are important to prevent complications such as malnutrition, osteoporosis, infertility, and gastrointestinal malignancies. The only currently approved treatment for CD is dietary exclusion of foods containing gluten; unfortunately, a majority of patients have difficulty complying with this diet, and the response to therapy is poor in up to 30% of patients, resulting in persistent or recurrent symptoms, inadequate cure, and/or refractory disease.<sup>4</sup>

CD is more common than was previously thought, and recent studies have shown a much higher global prevalence rate.<sup>3</sup> In the past, CD was considered to be a rare disorder in North America, mostly affecting individuals of northern European descent, and the disease was usually diagnosed in childhood. At that time, diagnosis was made in patients with typical gastrointestinal symptoms and classic symptoms of malabsorption, with confirmation by small intestinal biopsy.<sup>5</sup> The discovery of highly sensitive and specific serologic markers—including antigliadin, antiendomysium, and antitransglutaminase antibodies—has allowed clinicians to evaluate the true prevalence of CD and identify patients with clinically mild, atypical, or even silent forms of CD.<sup>6</sup>

A recently published, large, international, multicenter study investigated a wide population sample and found that the overall prevalence of CD is 1%, on average, with large variations among countries.<sup>7</sup> The prevalence of CD in the general population of the United States is approximately 1:133 (0.75%). Most cases of CD remain undiagnosed until later in life, with an average age at diagnosis of 45 years. The average time to diagnosis is 10–12 years, as many patients do not present with classic symptoms of diarrhea, weight loss, and abdominal pain.<sup>8</sup> The National Institutes of Health estimates that about 3 million people in the United States have CD and that more than 95% of people with the condition remain undiagnosed. Physicians should be aware that CD has a wide spectrum of presentations and that this condition may occur at any age, in both sexes, and in a wide variety of clinical circumstances.<sup>9,10</sup>

CD is a chronic, inflammatory, small intestinal disorder that can lead to severe villous atrophy, malabsorption, and malignancy. Susceptibility to CD, its activation, and the ensuing inflammatory cascade involve a combination of environmental and genetic factors that trigger immunologic mechanisms.<sup>11</sup> CD is the only autoimmune disease with a known trigger, which is the ingestion of the gluten proteins found in wheat, barley, and rye. All patients must express the antigen-presenting molecules human leukocyte antigen (HLA)-DQ2 and/or HLA-DQ8, the presence of which is the single most important predisposing genetic factor for CD.<sup>12</sup> HLA-DQ2 and HLA-DQ8 then bind gluten peptides

that have undergone deamidation by transglutaminase 2 (TG2), an enzyme tissue transglutaminase; this deamidation increases the gluten peptides' affinity for HLA-DQ2 and HLA-DQ8 and results in a more destructive intestinal CD4<sup>+</sup> T-cell response.<sup>13</sup> Once activated, gluten-reactive CD4<sup>+</sup> T cells produce cytokines and induce an inflammatory cascade that results in intestinal inflammation—characterized by villous atrophy, crypt hyperplasia, and infiltration of inflammatory cells—which leads to malabsorption.<sup>12</sup> Based on this mechanism of action, the current standard treatment of choice is strict, lifelong adherence to a gluten-free diet that eliminates wheat, rye, and barley.

A gluten-free diet is presently the therapy of choice for CD, as it improves gastrointestinal symptoms within a few weeks and has been shown to cause a histologic and serologic response within 1–2 years. If patients strictly adhere to this diet, vitamin deficiencies resolve, and the risk of concomitant autoimmune disease and CD-associated malignancies is reduced.<sup>14</sup> However, many patients fail to comply with this lifelong restrictive diet, as gluten is a common ingredient in diets throughout the world, and gluten-free foods are not widely available. Even if patients make every effort to avoid gluten in their diets, small levels of contamination frequently occur in food products, and many people inadvertently consume gluten-containing foods. Gluten-free foods are also more expensive than their gluten-containing counterparts. In addition, health-related quality of life has been shown to be lower in people with CD while they are on a gluten-free diet.<sup>15</sup> Therefore, maintaining this diet for life is challenging, and poor adherence often leads to incomplete resolution of symptoms.

Even in fully compliant patients, a gluten-free diet fails to induce clinical or histologic improvement in 7–30% of patients. After secondary causes of nonresponse have been investigated—including alternative diagnoses or complications of CD—persistent symptoms are attributed to refractory CD, in which symptoms persist despite strict adherence to a gluten-free diet. Refractory CD may be classified as type 1, in which there is a normal intraepithelial lymphocyte phenotype, or type 2, in which there is a clonal expansion of an aberrant intraepithelial lymphocyte population.<sup>16</sup> Intraepithelial T lymphocytes are considered to be aberrant when they express cytoplasmic CD3 but lack surface expression of the T-cell markers CD3, CD4, CD8, and the T-cell receptor.<sup>17</sup> The intraepithelial lymphocyte expansion may be driven by overexpression of interleukin-15 by the epithelium.<sup>18</sup> Type 1 refractory CD usually responds to steroid therapy, but type 2 refractory disease carries a more dismal prognosis, as it is usually steroid-refractory and is associated with an

increased risk of lymphoma. Current research is focused on nondietary therapies and treatment of refractory and diet-unresponsive CD.

Recently, studies have found that, in addition to environmental and genetic predispositions, abnormalities in the structure of the small intestine play a major role in the pathogenesis of CD. In most people, links known as tight junctions help keep enterocytes connected. In patients with CD, however, the junctions come apart, allowing a large number of indigestible gluten fragments to escape into the underlying tissue and incite immune system cells. New research has identified a protein that is secreted by intestinal epithelial cells, called zonulin, which induces tight junction disassembly; increased expression of this protein results in increased intestinal permeability.<sup>19</sup> In CD, gliadin (the toxic component of gluten) binds to the intestinal receptor CXCR3, which then initiates exaggerated zonulin secretion, resulting in abnormally high levels of zonulin.<sup>20</sup> Zonulin makes the intestine more permeable and allows gluten to seep out of the gut, where it then interacts freely with the genetically sensitized elements of the immune system that cause intestinal damage. Therefore, CD treatments are being directed toward reducing this permeability.<sup>20,21</sup>

Recent advances and insights have improved understanding of the molecular basis for CD; with further knowledge of the pathophysiology of this disease, many new targets for therapy have been identified and are currently being developed (Table 1). Alternative therapeutic strategies are directed at disrupting 1 of the 3 major pathways that cause the disease, including the environmental trigger (gluten), genetic susceptibility, and unusual gut permeability.<sup>21</sup>

## Novel Therapies

### *Genetically Modified Gluten*

Because removing gluten from the diet results in clinical, serologic, and histologic improvement in most patients, this approach is currently the recommended treatment of choice for CD, and many patients have been forced to eat bread made with gluten-free flour. Bread is one of the most commonly consumed foods in the Western diet, and it is typically made from grains that contain gluten, such as wheat. Wheat gluten is the protein that strengthens and binds dough in baking; thus, gluten is an important component of bread. However, gluten-free flour is now available as a substitute; this flour is made from a variety of materials, such as almonds, rice, sorghum, corn, and legumes. Flourless breads made with gluten-free grains have also been created; they use amaranth flour, arrowroot flour, brown rice flour,

**Table 1.** New Therapeutic Agents for Celiac Disease and Their Mechanisms of Action

Therapeutic agent	Mechanism of action
Genetically modified gluten	Decreases gluten exposure by transamidation of gliadin
Zonulin inhibitor	Decreases zonulin secretion and inhibits intestinal permeability
Therapeutic vaccine	Creates immune tolerance to gluten fragments and desensitizes celiac disease patients to the toxic effects of gluten
Probiotics	Detoxify gliadin and promote intestinal healing
Tissue transglutaminase inhibitors	Stop tissue transglutaminases from modifying gluten fragments, a process that otherwise triggers the immune response

buckwheat flour, chia flour, chickpea flour, corn flour, cornmeal hemp flour, maize flour, millet flour, potato flour, quinoa flour, soya flour, tapioca flour, teff flour, and white rice flour. Although proteins found in these alternatives are a source of complex carbohydrates, they lack B vitamins, and vitamin deficiencies may occur. These alternatives also lack many of the essential nutrients and flavor of wheat flour.<sup>4</sup>

Therefore, genetically modified gluten with reduced immunogenicity is being developed as a potential future option to decrease the toxicity that occurs in CD. CD is caused by T-cell responses to peptides derived from the gluten proteins found in wheat, which bind to DQ2 or DQ8 molecules and cause an inflammatory response mediated by interferon. In particular, deamidation of certain glutamine residues to glutamate by the transglutaminase enzyme results in peptides that are more acidic and thus have greater affinity for DQ2 and DQ8, resulting in a heightened T-cell-mediated inflammatory response.<sup>22</sup> Gianfrani and colleagues found that blocking these glutamine residues with lysine methyl ester (Lys-CH<sub>3</sub>) strongly inhibited the immune response to immunotoxic peptides in T cells from patients with CD.<sup>23</sup> In their study, a transamidation reaction attached Lys or Lys-CH<sub>3</sub> to a glutamine residue of  $\alpha$ -gliadin p56–68, an immunotoxic derivative of gluten. The altered peptide had a reduced affinity for the DQ2 molecule, compared with deamidated peptides, and consequently reduced interferon- $\gamma$  release from T cells of patients with CD. Treating wheat flour with microbial transglutaminase in the presence of Lys-CH<sub>3</sub> neutralized the immunotoxicity of the digested products. Therefore, transamidation of wheat flour with an appropriate amine group donor

can be used to block T-cell–mediated gliadin activity and gliadin immunotoxicity.<sup>23</sup>

In addition, a recent study found a large genetic variation in wheat products and the amount of T-cell–stimulatory peptides present in the wheat accessions. The study found that it was possible to select and breed gluten proteins that lack 1 or more of the known T-cell–stimulatory sequences. This breeding could allow selection of wheat that contains low amounts of T-cell–stimulatory sequences and thus is suitable for consumption by CD patients.<sup>24</sup> The results of this study suggest that wheat products can be enzymatically engineered to eliminate their immunotoxic effects on individuals with CD. This finding demonstrates that there is a potential to reintroduce detoxified wheat into the diets of patients with CD and thus provide well-balanced meals and better quality of life.

### **Zonulin Inhibitor**

The discovery of Zot, an enterotoxin elaborated by *Vibrio cholerae* that reversibly opens tight junctions, has enhanced understanding of the complex mechanisms that regulate the intestinal epithelial paracellular pathway.<sup>25</sup> Zot regulates tight junctions in a rapid, reversible, and reproducible fashion; based on this knowledge, researchers were able to discover zonulin, which is a similar, endogenous modulator of epithelial tight junctions.<sup>26</sup> Gliadin is known to cause increased secretion of zonulin, which alters intestinal permeability, facilitates the transport of gluten, and triggers an inflammatory process. This mechanism has been the target of advanced research that focuses on blocking tight junction modulators.

Alessio Fasano, one of the lead investigators in this area, helped to develop a promising new zonulin inhibitor known as larazotide acetate (AT-1001, Alba Therapeutics). This drug inhibits the human protein zonulin, which regulates intestinal permeability. This agent is currently in phase IIb trials, following positive results in 2 randomized, placebo-controlled, human trials conducted in 2009. The first of these studies showed that larazotide acetate reduced gluten-induced intestinal barrier dysfunction, production of inflammatory molecules, and gastrointestinal symptoms in CD patients. The second, larger study showed that CD patients who received placebo produced antibodies against tissue transglutaminase, but the group treated with larazotide acetate did not.<sup>21</sup>

In the most recent phase of testing, 75% of CD patients who were treated with a placebo pill and exposed to gluten developed classic symptoms, but symptoms occurred in only 14% of those who were treated with larazotide acetate and exposed to gluten. This drug is designed to be taken prior to the consumption of a gluten-containing meal, and it effectively blocks the toxic

effect of zonulin. Phase III clinical trials are now being planned to evaluate the safety, efficacy, and maintenance of the effect of larazotide acetate.

This agent is a potentially effective drug that, if it continues to show promise, should be available within the next 5 years and could improve quality of life among patients with CD by allowing them to enjoy gluten-containing foods for the first time. Recently, Alba Therapeutics received approval from the US Food and Drug Administration (FDA) to expand studies of larazotide acetate to other autoimmune disorders, including type 1 diabetes and Crohn's disease, as these conditions are also associated with high levels of zonulin. If approved, larazotide acetate would stop the autoimmune process by blocking a specific trigger, as opposed to previous therapeutic options that have been directed at decreasing the body's overall immune response or producing a general anti-inflammatory effect.<sup>21</sup>

### **Therapeutic Vaccine**

A peptide-based therapeutic vaccine that is currently being developed is a promising treatment for patients with CD. In contrast to other nondietary therapies that have been proposed, a peptide-based therapeutic vaccine is being developed to specifically modify the pathogenic T-cell response rather than reduce the amount of gluten peptide presented to the T cell. Currently, the vaccine would be effective only in patients with genotype HLA-DQ2, which is about 90% of patients with CD.

In order to create an effective peptide-based therapy for CD, an important step is the identification of the gluten peptides that trigger intestinal T-cell responses when patients with CD consume wheat, rye, or barley. Recently, researchers from the Australian company Nexpep analyzed the gluten protein and broke it down into about 2,700 distinct fragments. These fragments were then added to the blood of 200 CD patients, and the immune responses to the fragments were compared to the responses seen after the same patients consumed wheat bread, rye muffins, and boiled barley. Three peptides—gliadin, hordein, and secalin—were found to trigger a heightened immune response.<sup>27</sup>

The company then designed Nexvax2, which combines the 3 key, gluten-derived peptides into a vaccine. This vaccine is administered in weekly injections in order to desensitize CD patients to the toxic effects of gluten. The vaccine recently underwent a phase I clinical study. The randomized, placebo-controlled, double-blind, phase I study compared weekly intradermal injections of Nexvax2 versus placebo in CD patients with the HLA-DQ2 genotype who were on a strict gluten-free diet. In the 3-week study, the safety and tolerability profile of Nexvax2 was similar

to that of placebo. Gastrointestinal symptoms were more common in patients given the highest dose of the vaccine, confirming the selection of the toxic peptides that can eventually induce tolerance.<sup>28</sup> The symptoms and mobilization of gluten-specific T cells observed after administration of Nexvax2 were similar to those triggered by acute, oral gluten exposure in HLA-DQ2 patients on a gluten-free diet. Similar to traditional desensitization therapy for allergies, the peptide-based vaccine is designed to be given through injections in multiple small doses over a period of time in order to create immune tolerance to the selected gluten fragments and to lower the toxicity of related gluten molecules.<sup>29</sup> This approach will ultimately prevent the T cells from initiating the immune cascade that damages the small bowel. The vaccine is expected to enter phase IIa clinical trials to evaluate its efficacy.

### **Probiotics with Enzymes**

CD is a T-cell-driven intolerance to wheat gluten. The gluten-derived T-cell epitopes are proline-rich and highly resistant to proteolytic degradation within the gastrointestinal tract. The abundance and location of proline residues contribute to the resistance of the 33-mer gliadin peptide to breakdown by endogenous proteases in the gastrointestinal tract. An additional advance in the alternative treatment of CD includes oral supplementation with prolyl oligopeptidases to help degrade toxic gliadin peptides before they reach the mucosa. Shan and coauthors identified a unique 33-amino acid peptide (from the 266-amino acid  $\alpha 2$ -gliadin) that is resistant to degradation in the gastrointestinal tract; this 33-amino acid peptide contains several T-cell stimulatory epitopes that initiate the inflammatory response to gluten in CD patients.<sup>30</sup> This peptide was able to be degraded and lost its antigenicity in both in vitro and in vivo assays when it was exposed to a bacterial prolyl endopeptidase (PEP) derived from *Flavobacterium meningosepticum*, suggesting an oral bacterial peptidase could be used to detoxify the immunodominant gliadin epitopes.<sup>31</sup> Researchers in Ireland tested the combination of bacterial-derived and barley-derived proteases—PEP and endoprotease B—isoform 2, respectively—and demonstrated that, when given orally to CD patients, they can break down gluten to nontoxic fragments; therefore, these proteases may be a beneficial treatment and might allow CD patients to include modest amounts of gluten in their diets.<sup>32</sup>

In addition, bacterial enzyme preparations and intact probiotic preparations have also been shown to directly alter the function of intestinal cells. Supplementation with a variety of bacterial strains can help inhibit gluten/gliadin-induced damage in the small intestine.

Researchers in Finland added probiotic bacteria to cultures of intestinal epithelial cells to determine the bacteria's effect on gliadin-induced cellular damage. Two probiotic bacterial species were evaluated: *Lactobacillus fermentum* and *Bifidobacterium lactis*. In this study, *B. lactis* was able to inhibit permeability caused by gliadin. In addition, both *B. lactis* and *L. fermentum* were able to protect against cell ruffling and alterations in tight junctions. The bacteria alone, without gliadin, did not cause any significant changes to the intestinal epithelial cells. Inclusion of probiotics appears to be able to reduce the damage caused by eating gluten-contaminated foods and may even accelerate mucosal healing after the initiation of a gluten-free diet.<sup>33</sup> Therefore, the addition of probiotics with enzymes, which cause detoxification of gliadin and promotion of intestinal healing, could be a potentially useful treatment for CD patients.

### **Transglutaminase Inhibitors**

Human TG2, the enzyme that is involved in the pathogenesis of CD, catalyzes the transamidation and deamidation of glutamine residues in peptides. TG2 has a critical role in the pathogenesis of CD in that it deamidates glutamine residues from gluten peptides and converts them into glutamic acids, thus increasing their binding affinity to HLA-DQ2 and HLA-DQ8 receptors, which in turn mediates the patient's T-cell response.<sup>34</sup> Given that TG2 increases the pathologic effect of gluten peptides, TG2 inhibitors are potential therapeutic agents for the treatment of CD.

There are 3 classes of TG2 inhibitors that differ based on their mechanisms of action: competitive amine inhibitors, reversible inhibitors, and irreversible inhibitors. Competitive amine inhibitors are the most common glutaminase inhibitors; they inhibit TG2 activity by competing with natural amine substrates, such as protein-bound lysine residues, in the transamidation process. Therefore, TG2 is still enzymatically active, and transamidation continues to occur in the presence of competitive amine inhibitors. However, the resulting isopeptide crosslink is mainly formed between the natural glutamine substrate and the competitive amine inhibitor rather than between the natural glutamine substrate and natural amine substrate.<sup>35</sup> Reversible TG2 inhibitors prevent enzyme activity by blocking substrate access to the active site without covalently modifying the enzyme. TG2 cofactors, such as guanosine triphosphate and guanosine diphosphate, are examples of allosteric, reversible inhibitors of the enzyme.<sup>36</sup> Finally, irreversible TG2 inhibitors prevent enzyme activity by covalently modifying the enzyme, thereby preventing substrate binding.

Molberg and coworkers showed that culturing small intestinal biopsies from CD patients with either

TG2-treated (deamidated) or non-TG2-treated (non-deamidated) gluten digests resulted in the generation of patient T-cell lines that preferentially recognized deamidated gluten peptides rather than nondeamidated gluten peptides.<sup>37</sup> Also, by using cystamine to block the activity of endogenous TG2 in the CD patient biopsies, the authors demonstrated that more than half of the resultant T cells had reduced proliferative responses to deamidated gluten digests compared to non-cystamine-treated controls; they also showed that these cell lines still did not respond well to the nondeamidated digests.<sup>37</sup> In another study, Maiuri and coauthors showed that the 2-[(2-oxopropyl)thio]imidazolium inhibitor L682777 was able to prevent the in situ crosslinking of gluten peptides to endogenous proteins in tissue sections taken from both CD patients and controls.<sup>38</sup> Also, the authors showed that incubation of intact CD small intestinal biopsies with L682777 prevented T-cell activation induced by the nondeamidated form of an immunodominant gluten peptide.<sup>38</sup> These studies suggest that treatment of CD biopsies with TG2 inhibitors decreases the induced response of gluten-reactive T cells; these studies also suggest that irreversible inhibition of endogenous TG2 in CD patient biopsies can prevent gluten peptide deamidation and ultimately reduce T-cell activation.

## Conclusion

CD, a genetically driven, aberrant immune response to dietary gluten, is more common than was previously thought. CD can present with typical intestinal manifestations or atypical extraintestinal manifestations. CD has traditionally been treated using a gluten-free diet, which may be problematic from a standpoint of patient compliance. New insights into CD pathophysiology have led to research into novel therapeutic opportunities. Research approaches include engineering gluten-free grains, decreasing intestinal permeability by blockage of the epithelial zonulin receptor, inducing oral tolerance to gluten with a therapeutic vaccine, and degrading immunodominant gliadin peptides using probiotics with endopeptidases or transglutaminase inhibitors. These treatment options have shown encouraging preliminary results in phase II and phase III clinical trials. These non-diet-based therapies hold promise for enhanced, lifelong CD management with improved patient compliance. If successful, these novel approaches raise the possibility of reintroduction of gluten, in amounts to be determined, into the diets of CD patients. Nonetheless, a gluten-free diet is the mainstay of CD therapy for the immediate foreseeable future, pending FDA approval of any of these treatment options.

## References

- Green PH, Jabri B. Coeliac disease. *Lancet*. 2003;362:383-391.
- Green PH. The many faces of celiac disease: clinical presentation of celiac disease in the adult population. *Gastroenterology*. 2005;128:S74-S78.
- Lohi S, Mustalahti K, Kaukinen K, et al. Increasing prevalence of coeliac disease over time. *Aliment Pharmacol Ther*. 2007;26:1217-1225.
- Green PH, Cellier C. Celiac disease. *N Engl J Med*. 2007;357:1731-1743.
- Lionetti E, Catassi C. New clues in celiac disease epidemiology, pathogenesis, clinical manifestations, and treatment. *Int Rev Immunol*. 2011;30:219-231.
- Murray JA, Van Dyke C, Plevak MF, Dierkhising RA, Zinsmeister AR, Melton LJ III. Trends in the identification and clinical features of celiac disease in a North American community, 1950–2001. *Clin Gastroenterol Hepatol*. 2003;1:19-27.
- Mustalahti K, Catassi C, Reunanen A. The prevalence of CD in Europe: results of a centralized, international mass screening project. *Ann Med*. 2010;42:587-595.
- Cranney A, Zarkadas M, Graham ID, et al. The Canadian celiac health survey. *Dig Dis Sci*. 2007;52:1087-1095.
- Fasano A, Berti I, Gerarduzzi T. Prevalence of CD in at-risk and non at-risk groups: a large, multicentre study. *Arch Intern Med*. 2003;163:286-292.
- AGA Institute. AGA Institute medical position statement on the diagnosis and management of celiac disease. *Gastroenterology*. 2006;131:1977-1980.
- Schuppan D, Junker Y, Barisani D. Celiac disease: from pathogenesis to novel therapies. *Gastroenterology*. 2009;137:1912-1933.
- Sollid LM. Coeliac disease: dissecting a complex inflammatory disorder. *Nat Rev Immunol*. 2002;2:647-655.
- Sollid LM, Lie BA. Celiac disease genetics: current concepts and practical applications. *Clin Gastroenterol Hepatol*. 2005;3:843-851.
- Cosnes J, Cellier C, Viola S, et al; Groupe D'Etude de Recherche Sur la Maladie Coeliaque. Incidence of autoimmune diseases in celiac disease: protective effect of the gluten-free diet. *Clin Gastroenterol Hepatol*. 2008;6:753-758.
- Häuser W, Stallmach A, Caspary WF, Stein J. Predictors of reduced health-related quality of life in adults with coeliac disease. *Aliment Pharmacol Ther*. 2007;25:569-578.
- Cellier C, Delabesse E, Helmer C, et al. Refractory sprue, coeliac disease, and enteropathy-associated T-cell lymphoma. *Lancet*. 2000;356:203-208.
- Verbeek WH, Goerres MS, von Blomberg BM, et al. Flow cytometric determination of aberrant intra-epithelial lymphocytes predicts T-cell lymphoma development more accurately than T-cell clonality analysis in refractory celiac disease. *Clin Immunol*. 2008;126:48-56.
- Mention JJ, Ben Ahmed M, Begue B, et al. Interleukin 15: a key to disrupted intraepithelial lymphocyte homeostasis and lymphomagenesis in celiac disease. *Gastroenterology*. 2003;125:730-745.
- Fasano A, Not T, Wang W, et al. Zonulin, a newly discovered modulator of intestinal permeability, and its expression in coeliac disease. *Lancet*. 2000;355:1518-1519.
- Lammers KM, Lu R, Brownley J, et al. Gliadin induces an increase in intestinal permeability and zonulin release by binding to the chemokine receptor CXCR3. *Gastroenterology*. 2008;135:194-204.
- Fasano A. Surprises from celiac disease. *Sci Am*. 2009;301:54-61.
- A treatment that abolishes the immunotoxicity of gluten in celiac disease. *Nat Clin Pract Gastroenterol Hepatol*. 2007;4:647.
- Gianfrani C, Siciliano RA, Facchiano AM, et al. Transamidation of wheat flour inhibits the response to gliadin of intestinal T cells in celiac disease. *Gastroenterology*. 2007;133:780-789.
- Spainij-Dekking L, Kooy-Winkelaar Y, van Veelen P, et al. Natural variation in toxicity of wheat: potential for selection of nontoxic varieties for celiac disease patients. *Gastroenterology*. 2005;129:797-806.
- Fasano A, Baudry B, Pumphlin DW, et al. *Vibrio cholerae* produces a second enterotoxin, which affects intestinal tight junctions. *Proc Natl Acad Sci U S A*. 1991;88:5242-5246.
- Wang W, Uzzau S, Goldblum SE, Fasano A. Human zonulin, a potential modulator of intestinal tight junctions. *J Cell Sci*. 2000;113:4435-4440.
- Walter and Eliza Hall Institute. Celiac disease vaccine shows promising results in phase I trial. *ScienceDaily*. May 9, 2011. <http://www.sciencedaily.com/releases/2011/05/110509091559.htm>.
- Brown GJ, Daveson J, Marjason JK, et al. A phase I study to determine the safety, tolerability and bioactivity of Nexvax2 in HLA DQ2+ volunteers with celiac disease following a long-term, strict gluten-free diet. Presented at Digestive Disease Week; May 7–10, 2011; Chicago, Illinois. Abstract Su1235.

29. Keech CL, Dromey J, Chen ZJ, et al. Immune tolerance induced by peptide immunotherapy in an HLA Dq2-dependent mouse model of gluten immunity. *Gastroenterology*. 2009;136:A57.
30. Shan L, Molberg Ø, Parrot I, et al. Structural basis for gluten intolerance in celiac sprue. *Science*. 2002;297:2275-2279.
31. Pyle GG, Paaso B, Anderson BE, et al. Effect of pretreatment of food gluten with prolyl endopeptidase on gluten-induced malabsorption in celiac sprue. *Clin Gastroenterol Hepatol*. 2005;3:687-694.
32. Watson R, Ding A, McMillan SA, et al. Implications of enzymatic detoxification of food gluten in coeliac disease. *Gastroenterology*. 2008;134:A213.
33. Lindfors K, Blomqvist T, Juuti-Uusitalo KM, et al. Live probiotic *Bifidobacterium lactis* bacteria inhibit the toxic effects induced by wheat gliadin in epithelial cell culture. *Clin Exp Immunol*. 2008;152:552-558.
34. Quarsten H, Molberg O, Fugger L, McAdam SN, Sollid LM. HLA binding and T cell recognition of a tissue transglutaminase-modified gliadin epitope. *Eur J Immunol*. 1999;29:2506-2514.
35. Siegel M, Khosla C. Transglutaminase 2 inhibitors and their therapeutic role in disease states. *Pharmacol Ther*. 2007;115:232-245.
36. Lai TS, Slaughter TF, Peoples KA, Hettasch JM, Greenberg CS. Regulation of human tissue transglutaminase function by magnesium-nucleotide complexes. Identification of distinct binding sites for Mg-GTP and Mg-ATP. *J Biol Chem*. 1998;273:1776-1781.
37. Molberg O, McAdam S, Lundin KE, et al. T cells from celiac disease lesions recognize gliadin epitopes deamidated in situ by endogenous tissue transglutaminase. *Eur J Immunol*. 2001;31:1317-1323.
38. Maiuri L, Ciacci C, Ricciardelli I, et al. Unexpected role of surface transglutaminase type II in celiac disease. *Gastroenterology*. 2005;129:1400-1413.