Prevention Measures and Exploratory Pharmacological Treatments of Celiac Disease

Maud Pinier, Pharm D, MSc1,4, Gregor Fuhrmann, Pharm D3,4, Elena Verdu, MD, PhD3 and Jean-Christophe Leroux, B Pharm, PhD1,2

Increasing prevalence, protean clinical manifestations, and lack of pharmacological therapy make celiac disease (CD) a complex and highly relevant illness in gastroenterology. This chronic inflammatory disorder of the small intestine is caused by the ingestion of gluten containing cereals in genetically susceptible individuals, leading to a variety of gastrointestinal (GI) and non-GI manifestations. Awareness among physicians is growing due to accessible and highly accurate diagnostic and screening methods. Recent evidence suggests a possible rising incidence of CD. Environmental factors such as early life gluten exposure, intestinal infections, short duration of breast-feeding, and changes in intestinal microbiota have been proposed to have a role in CD pathogenesis. Thus, prevention approaches to diminish the rising prevalence of CD are currently being evaluated. Still, the cornerstone treatment of CD remains a strict gluten-free diet. This nutritional regime is demanding, and non-adherence is common because of social isolation, financial issues, or restriction of food diversity. Allowing patients to occasionally consume small amounts of gluten would greatly improve their quality of life. Owing to recent advances in the understanding of the pathogenesis of CD, different targets have been identified and have motivated the development of several experimental therapeutic strategies. The main goal of this review is to discuss the mechanisms that can be exploited therapeutically to prevent or delay CD, disease associations and its complications. Current treatments for complications of CD, including refractory CD and malignancy, are beyond the scope of this review.

INTRODUCTION

Ingestion of gluten-containing dietary cereals (Figure 1) triggers celiac disease (CD) in genetically susceptible individuals. Genetic predisposition is conferred by human leukocyte antigens (HLAs), as 90–95% of affected people exhibit HLA-DQ2 molecules and the remainder, HLA-DQ8 (1). CD is a common disorder worldwide. Its prevalence is as high as 0.5–1% (2), but recent findings indicate that it has increased substantially in American and Finnish populations in the recent years (3,4). The increment in prevalence cannot only be explained by improved diagnostic approaches, and the involvement of environmental factors such as breast-feeding, time of gluten introduction, and infections, in CD pathogenesis have been suggested (5–8).

Both the pathology and the clinical spectrum of CD can vary considerably from severe to subtle, and the clinical expression is not necessarily restricted to the presence of intestinal atrophy (2,9). Classical gastrointestinal (GI) manifestations include diarrhea, abdominal bloating, and discomfort (2,10). However, many patients have unrecognized CD (11,12) due, in part, to the absence of symptoms (silent CD) or extra-intestinal clinical presentations (13,14). Complications of CD include refractory CD, a complex disorder with severe and recurrent symptoms that is unresponsive to at least 6 months of strict adherence to a gluten-free diet (GFD) (12). Few patients with non-responsive CD develop enteropathy-associated T-cell lymphoma (15,16), a complication of CD that requires drug-based therapies (12). The prevalence of this rare disorder is about 0.5–1/1,000,000 (17). CD is also often associated with other autoimmune disorders such as autoimmune thyroiditis and type 1 diabetes (13,18). The prevalence of CD among siblings of children with type 1 diabetes has been shown to correlate with the prevalence of CD-associated HLA-DQB1 alleles (19). Moreover, the risk of CD in children with type 1 diabetes is significantly modified by the presence of HLA-DQB1*02–DQA1*05 (20). A recent genotyping study that enrolled 8,064 type 1 diabetes patients and 9,339 control subjects showed that patients with type 1 diabetes and CD express seven common alleles that regulate autoimmune responses (21).

Therefore, early prevention of CD may represent a cost-effective strategy, as the disease is highly prevalent. Indeed, mortality rates in celiac patients fluctuate between studies and European countries, but overall mortality is higher than in the general population (22). Recent data also confirm an elevated risk of mortality in individuals

1Faculty of Pharmacy, University of Montreal, Montreal, Quebec, Canada; 2Department of Chemistry and Applied Biosciences, Institute of Pharmaceutical Sciences, ETH Zurich, Zurich, Switzerland; 3Farncombe Family Digestive Health Research Institute, McMaster University, Hamilton, Ontario, Canada;
4These authors contributed equally to this work. Correspondence: Jean-Christophe Leroux, B Pharm, PhD, Department of Chemistry and Applied Biosciences, Institute of Pharmaceutical Sciences, ETH Zürich, Wolfgang-Pauli-Street 10, HCI H 301, Zürich 8093, Switzerland. E-mail: jleroux@ethz.ch

Received 15 June 2010; accepted 17 August 2010
with mild gluten-induced inflammation without villous atrophy (9). The impact of preventing CD on refractory CD and enteropathy-associated T-cell lymphoma remains to be determined.

GLUTEN PEPTIDES TRIGGER CD
CD is the only known autoimmune disease in which the trigger, gluten, has been identified. Gluten is the major storage protein fraction in wheat and one of the most common ingredients in human nutrition. The average gluten intake from a western diet is typically about 20 g/day. Generally, gluten can be separated into polymeric (glutenin) and monomeric (gliadin) fractions. Although glutenins are frequently recognized as ex vivo inflammatory triggers in celiac biopsies (11), gliadin fractions have a predominant role. Their uniquely high proline and glutamine content makes them resistant to digestive enzymes (23) (Figure 2). Incomplete gliadin cleavage in the upper small intestine generates peptides that are transported through the mucosal barrier, inducing inflammation in the lamina propria. Specific deamination of gliadin peptides by tissue transglutaminase 2 (tTG2) (24) introduces negative charges that increase the peptide’s affinity for disease-associated HLA-DQ2/8 molecules (25). Subsequently, activated CD4+ T-lymphocytes contribute to the inflammation through secretion of pro-inflammatory cytokines such as IFN-γ, interleukin (IL)-18, tumor necrosis factor (TNF)-α, and IL-21 (26–28) (Figure 2). Many specific peptides have been reported to trigger the inflammatory reaction in CD. Among them, the 33mer (p57–89 of α2-gliadin), contains at least three immunogenic epitopes (e.g., 13mer p62-75), recognized by most, if not all, T-cells from CD patients (23) (Figure 2). Certainly, the 33mer may be highly immunogenic, but it is only one of many harmful structures in gliadin that have been identified so far. For example, the non-immunodominant p31-43, found in α-gliadin, has been shown to stimulate the innate immune response (29) (Figure 2).

PREVENTION OF CD
As pharmacological treatments of CD are not yet available, preventive measures (Box 1) are regarded as potential options to reduce the incidence of the disease. Early infant nutritional regimes were first examined to determine the impact of diet on the breakdown of oral tolerance to gluten. A meta-analysis of observational studies carried out from 1966 to 2004, concluded that breast-feeding was beneficial in preventing the development of CD (5). Children with CD are usually breast-fed less or for a shorter period of time than healthy children (30). Ongoing breast-feeding, at the time of dietary gluten introduction, has been associated with a decreased risk of CD (5). However, it is unclear whether breast-feeding delays the onset of CD and whether the protective effect is long-lasting throughout life (5). Secretry immunoglobulin A, epithelial growth factors, and immune cells secreted into breast
Figure 2. Current concept of the pathophysiology of celiac disease. Gluten fragments are highly resistant to digestion by gastropancreatic and brush border membrane proteases and peptidases (23). Smaller peptides are produced upon incomplete processing in the upper part of the small intestine. The paracelluar route can be impaired by zonulin-induced opening of tight junctions (92). There is also evidence that gliadin-fragment transport can occur by direct transcytosis (98) and by a transferrin receptor-mediated transcytosis mechanism (i.e., retrotranscytosis) (93). Gluten peptides can gain access to the mucosa via these pathways. In the lamina propria, the peptides are recognized by tissue transglutaminase type 2 (tTG2) followed by regioselective deamidation, converting its glutamine residues into glutamate. CD4+ T-lymphocytes (LTCD4+) recognize deamidated peptides in the context of human leukocyte antigen (HLA)-DQ2 and DQ8 receptors. Subsequently, the activation of CD4+ T-cells leads to the release of pro-inflammatory cytokines such as interferon-γ, tumor necrosis factor-α (TNF-α), interleukin (IL)-21, IL-18, IL-6 that cause severe inflammation (26,27) (i). IL-15 released by enterocytes promotes epithelial cell apoptosis triggered by migration of inflammatory cells (CD8+ T-lymphocytes (LTCD8+) and natural killer cells (NK)) into the epithelium (27) (ii). In addition, intestinal wall injury occurs due to the activity of metalloproteases (MP) secreted by fibroblasts and lamina propria mononuclear cells (LPMC) (iii). Finally, the activation of B-lymphocytes (LB) by IFN-γ leads to the secretion of antibodies against gliadin (anti-gliadin antibodies, AGA) and against tTG2 (anti-tTG2) (27) (iv).

milk might increase oral tolerance and protect infants from gastroenteritis, which has been known to favor immunogenic reactions (31). Other factors such as intestinal microbiota differences between formula- and breast-fed newborns may also have a role (32). It is well known that the intestinal microbiota impacts the integrity and maturation of the gut immune system (33) and may therefore modulate immune host responses to dietary antigens (34,35). Finally, the observed protective effect of breast-feeding on the reduced incidence of CD may be a consequence of a lower amount of gluten exposure due to a delay in the introduction of formula in breast-fed neonates (36). The timing of gluten introduction in the diet seems also important in the pathogenesis of CD. An optimal window (between 4 and 7 months) for dietary gluten introduction, when tolerogenic responses may be promoted, has been suggested (6,37). Moreover, breast-feeding during this “tolerance window” may protect genetically predisposed individuals from developing CD (38).

Despite exposure to gluten and genetic predisposition in 30% of the population, not all susceptible individuals will develop CD. Additional factors, such as gastroenteritis episodes in childhood, have been suggested to be involved in its pathogenesis (Box 1).

A prospective study reported a relationship between the frequency of rotavirus-positive serology and the occurrence of CD (7). Molecular mimicry was proposed to explain the rotavirus and CD linkage (8). Moreover, inflammatory cytokines released during viral intestinal infections, may favor antigen penetration into the mucosa (39). Inflammation also triggers the release and the activation of tTG (40,41), which is critical in CD pathogenesis. The viral hypothesis is supported by epidemiological observations, suggesting a seasonal influence on CD development (42). Other pathogens may also increase the risk of CD (43–45). Therefore, the impact of prevention of early life infection through vaccination strategies or probiotics (46) in the development of CD should be investigated.

The intestinal microbiota has been implicated in the pathogenesis of inflammatory disorders of the GI tract, such as inflammatory bowel disease. The hypothesis has been raised that intestinal colonization by potentially harmful bacteria may have a role in the pathogenesis of CD as well (47). CD patients have been shown to have an imbalanced intestinal microbiota compared with healthy individuals (48–50). Although the role of the intestinal microbiota in CD pathogenesis remains to be determined, preventive
approaches based on the administration of specific probiotic strains that regulate immune function and permeability (51) should be considered (39,52).

THE EFFECTS OF GFD

Once the diagnosis of CD is established, the current therapy dictates a strict, lifelong eviction of gluten and associated immunogenic proteins from related grains (Figure 1). Avoidance of gluten maintains CD in remission. Such a diet is, however, difficult to follow and demanding for patients (53). Family education and support by a dietitian are necessary to avoid misunderstanding and frustration (54). Strict adherence to a GFD and its success depend on the severity of symptoms, psychosocial factors (55), and patients’ knowledge of long-term benefits (56). Because of individual variability, it is difficult to determine and evaluate the exact gluten threshold that can be ingested safely by celiac patients. Nevertheless, a double-blind study has established that gluten intake should be kept under 50 mg/day (57) and, more recently, 10 mg/day was reported to be a safe limit (58).

In Europe, the Codex alimentarius states that gluten content in gluten-free products should not exceed 20 p.p.m., whereas those that have been rendered gluten free should contain < 100 p.p.m. In contrast, in the United States, the Food and Drug Administration has proposed the “gluten-free” label only for food products containing < 20 p.p.m. Thus, labeling rules are different among countries, and this may contribute to increasing confusion and uncertainty among CD patients. Another setback relates to inadvertent dietary consumption. Non-adherence to a GFD is thus common because the use of gluten as an additive in processed foods is widespread (54). Finally, gluten-free products are generally more expensive than their gluten-containing counterparts, not widely available and not so diverse (59,60). It is possible to substitute common pastry ingredients from wheat (starch/flour) in gluten-free baking using potato/corn starch, or potato/tapioca/rice flour (61,62). However, gluten replacement is technologically challenging owing to its favorable effects on texture and palatability. Pseudo-cereals (i.e., grains not belonging to the botanical Poaceae family), such as amaranth, quinoa and buckwheat, are interesting alternatives because of their good baking properties in the formulation of high-quality gluten-free products (63).

Although a GFD is assumed to be safe, it is not completely devoid of unwanted effects, especially if not under careful medical surveillance. Immune (64) and nutritional imbalance (65) has been associated with a GFD. A recent study has reported a reduction in beneficial gut bacteria populations in healthy subjects on GFD and this may have unknown pathophysiological consequences in the host (64) that require further investigation. Moreover, in order to compensate for decreased flavor and acceptability, gluten-free foods tend to have high fat and calorie content making patients prone to gain weight (54). Patients on a GFD frequently exhibit nutrient and fiber deficiencies (65–67). Thus, dietary enrichment or supplementation is commonly advocated to correct such deficits, and some fiber sources are recommended to avoid constipation (54). Additional labeling consensus, regarding nutritional values, is desirable to balance recommended daily intake (65). Uncontaminated oats are now considered safe for the majority of CD patients (68–71) (Figure 1). A long-term study has revealed that moderate ingestion of uncontaminated oats (50–70 g/day for adults) was well tolerated according to histological and immunological parameters. However, some patients interrupted the trial due to the appearance of rash and flatulence (72).

The impact of a GFD on lifestyle cannot be ignored. As a consequence of such rigorous diet, patients are restricted in their common activities and suffer from social isolation (54,56). This may in return lead to insufficient compliance to a GFD and persistence of CD symptoms. Gluten is a major component of human nutrition, and it would be more acceptable to CD patients if their daily diet could include minimal amounts of gluten. The following section discusses the recent progress achieved in the search for supportive therapies of CD (Table 1; Figure 3).
Table 1. Pharmacological approaches to the treatment of celiac disease

<table>
<thead>
<tr>
<th>Therapeutic option</th>
<th>Compound</th>
<th>Level of development</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral enzyme supplementation</td>
<td>ALV003</td>
<td>Phase II</td>
<td>81</td>
</tr>
<tr>
<td>Polymereic binders</td>
<td>(HEMA-co-SS)</td>
<td>Phase II</td>
<td>86</td>
</tr>
<tr>
<td>Modulator of paracellular permeability</td>
<td>AT-1001</td>
<td>Phase II</td>
<td>97</td>
</tr>
<tr>
<td>Inhibitory analogues of gliadin T-cell epitoles</td>
<td>Decapeptide (Durum wheat)</td>
<td>Discovery</td>
<td>111</td>
</tr>
<tr>
<td>HLA-DQ2 inhibitor</td>
<td>Several compounds</td>
<td>Discovery</td>
<td>100-103, 107,137</td>
</tr>
<tr>
<td>Human hookworm inoculation</td>
<td><em>Necator americanus</em></td>
<td>Phase II</td>
<td>—</td>
</tr>
<tr>
<td>Peptide vaccination</td>
<td>Nexvax2</td>
<td>Phase I</td>
<td>113</td>
</tr>
<tr>
<td>Other therapeutics</td>
<td>Stimulator of intestine regeneration (R-spondin)</td>
<td>Discovery</td>
<td>134</td>
</tr>
<tr>
<td></td>
<td>NKG2D/MICA blockers</td>
<td>Discovery</td>
<td>132,133</td>
</tr>
</tbody>
</table>

CAN WE “TREAT” CD?

Celiac-safe wheat?

Wheat has been a traditional element of human nutrition, but evolutionary studies have revealed that certain celiac-toxic *Triticum* varieties were not cultivated at the beginning of wheat domestication and agriculture. Today’s most common wheat breeds are hexaploid and arose in the Fertile Crescent 9000 years ago. During domestication, tetraploid (AA BB) *Turgidum* was hybridized with the diploid (DD) *Aegilops* variety. Such a step most likely introduced D genomes in current wheat species encoding for enhanced cold hardness, but also for immunogenic α-gliadin sequences (Figure 1). The consumption of these relatively modern wheat varieties are believed to have had an impact on the incidence of CD. Wheat *cultivars* with a lower amount of T-cell epitopes may be better tolerated by CD patients or help reduce the overall prevalence of the illness. A very recent approach with genetic deletion lines of Chinese Spring wheat has demonstrated the possibility of decreasing the T-cell stimulatory ability of wheat flour while retaining analogous dough properties (73).

The enzymatic treatment of flour is being tested to reduce gluten immunogenicity. A microbial transglutaminase from *Streptomyces mobaraensis* has been found to decrease T-cell reactivity (74), after modifying toxic gliadin peptides with lysine or lysine methyl ester. Also, bacterial fermentation can diminish gluten intolerance. *Lactobacilli*-fermented wheat flour mixed with celiac-safe flour (i.e., buckwheat) has led to well-tolerated bread with short-term safety (75). A similar approach has been proposed for the preparation of fermented durum wheat pasta containing 80% less gluten than its untreated counterparts (76). These examples demonstrate that the generation of wheat flour with less T-cell stimulatory epitopes that retain technological properties is potentially feasible (Figure 3). However, it remains questionable whether such measures can in fact ensure the long-term safety of wheat in CD patients.

Exogenous enzymes can degrade gluten

The enzymatic approach is one of the most studied potential therapeutic strategies for CD (Figure 3; Table 1). Prolyl endopeptidases (PEPs) are proline-specific enzymes, which, in contrast to human luminal enzymes, effectively cleave and therefore detoxify gluten peptides. Earlier studies investigated PEPs from *Flavobacterium meningosepticum*, *Myxococcus xanthus*, and *Sphingomonas capsulata* (SC-PEP). PEPs from *Flavobacterium meningosepticum* and *Myxococcus xanthus* favorably cleave longer peptides, whereas SC-PEP is more specific for shorter sequences (77). These enzymes also are poorly stable at low pH (stomach) and in the presence of digestive enzymes (77,78). Moreover, they are most active at pH 6–9 and presumably less functional in the gastric environment. To alleviate the pH-dependent decomposition of SC-PEP, variants of the enzyme have been developed by protein engineering and showed up to 20% enhanced activity with improved resistance to pepsin (79).

SC-PEP is currently being tested in a combinatorial approach with EP-B2. The latter is a gastric-active, glutamine-specific endoproteinase from germinated barley seeds. The proenzyme of EP-B2 is highly resistant to low pH and pepsin. Furthermore, it rapidly self-activates under acidic conditions (80). The combinatorial approach with EP-B2/SC-PEP (ALV003, Alvine Pharma, San Carlos, CA) (81) is currently undergoing clinical phase IIa testing. ALV003-predi digested gluten has been shown to significantly reduce gluten-related T-cell responses in CD patients compared with placebo-digested gluten (82). However, these findings were only observed with *in vitro* predigested gluten, and GI symptoms typically induced by the gluten meal were not significantly reduced by ALV003-pretreatment (83). Another enzyme, *Aspergillus niger* (AN-PEP; DSM Food Specialties, Delft, The Netherlands), has reached clinical testing as well. AN-PEP is reported to be resistant to low pH in the presence of pepsin and significantly degrades peptid-tryptic gluten digests under simulated gastric conditions (83). Recently, AN-PEP was tested in a clinical phase II and was well tolerated by CD patients. However, the enzyme treatment was not superior to placebo in preventing gluten-induced GI deterioration as measured by serological, histological, and life-quality changes (84).

Although the enzymatic approach can effectively detoxify gluten peptides *in vitro* and seems to be well tolerated by CD patients, its relative sensitivity to the harsh conditions encountered along the GI tract may require specialized delivery systems such as enteric coatings (85).

Complexing gliadins in the gut

Recently, it was proposed that polymeric binders could be implemented to sequester gliadins in the GI tract (86) (Figure 3;
Figure 3. Investigational treatments for celiac disease. (a) Ingestion of celiac-safe flour. This flour can either be obtained by genetic modification of cereals (73), enzymatic treatment (74), or bacterial fermentation (75,76). (b) Oral administration of exogenous enzymes to degrade proline- and glutamine-rich immunogenic peptides that trigger the disease (81,83,141). Depending on the enzyme, hydrolysis occurs in the stomach or in the upper small intestine. (c) Polymeric binders can sequester gliadins, forming complexes in the gastrointestinal lumen. These complexes hinder gliadin digestion, and diminishing the formation of immunogenic peptides (86). (d) Use of modulators of paracellular permeability to prevent the opening of tight junctions and restrict the passage of gluten peptides (i) (96,97). Inhibition of tissue TG2 (ii) (102) or blockage of human leukocyte antigen (HLA) molecules (iii) (103,111,137). (e) Modulation of immune responses by peptide vaccination (113), helminth inoculation (118), or interference with inflammatory cell recruitment (128).

Table 1). The synthetic polymer poly(hydroxyethylmethacrylate-co-styrene sulfonate) (P(HEMA-co-SS)) complexes gliadin, forming supra-molecular particles at gastric and intestinal pHs (87,88), and abrogates gliadin’s deleterious effect on epithelial cells (86). This complexation is relatively selective toward gliadin in vitro (86). In addition, the sequestrant decreases gliadin digestion by GI enzymes, minimizing the formation of immunogenic peptides (86). The co-administration of P(HEMA-co-SS) attenuates gliadin-induced changes in permeability and inflammatory parameters in gluten-sensitive HLA-HCD4/DQ8 mice (86). Rodents treated with the polymer do not exhibit side effects, even after massive and prolonged dosing. As with other polymer-based therapies (89), systemic absorption of P(HEMA-co-SS) should be poor. This, in conjunction with its low cost and possibility to be taken, occasionally, together with gluten-containing food, makes it an attractive option. Further investigation of the mechanisms of action of P(HEMA-co-SS) and its interaction with human tissues is required before its efficacy is investigated in human trials. A similar approach neutralizing gluten with anti-gluten antibodies from cow’s milk has been also proposed to treat CD (90). As IgG can survive in the GI tract and can be extracted from milk, this could also represent an efficient and safe supportive strategy for CD.

“Sealing” tight junctions

The intestinal epithelial uptake of gliadin has been extensively studied in the context of CD (91–93). Gliadin has been reported to increase intestinal permeability enabling non-specific paracellular diffusion of gliadin and its subsequent interaction with the mucosal immune system (91,92). Gliadin peptides can also translocate transepithelially by interacting with the transferrin receptor CD71 on the apical side of immature enterocytes (93). This translocation further increases the availability of poorly digested gliadin to the mucosa, leading to antigen presentation of HLA-DQ2/DQ8-bound gliadin peptides to CD4+ T-cells, and to the generation of effector cells (94) that cause autoimmune enteropathy. Thus, therapies based on the modulation of intestinal permeability aimed at reducing the uptake of immunogenic gliadin peptides into the lamina propria have been developed. The modulator of paracellular permeability, lazarotide acetate (AT-1001, Alba Therapeutics, Baltimore, MA), is a synthetic hexapeptide derived from Zonula Occludens toxin of Vibrio cholera (95) (Figure 3; Table 1).

In a pilot clinical trial that included CD patients, lazarotide acetate was well tolerated, and levels in plasma were below the limit of detection (<0.5 ng/ml) after oral administration of 12 mg of drug. Acute symptoms were less frequent upon treatment. Intestinal barrier function, as measured by the lactulose/mannitol assay, was maintained despite a gluten challenge of 2.5 g. However,
statistical significance between groups was not achieved regarding permeability, the differentiation of peripheral monocytes and IFN-γ production (96). In a 6-week phase IIb trial, encouraging results were obtained in terms of symptoms and antibody titers (97). Unfortunately, the primary end point, which was set for permeability improvement and a decrease in the lactulose/mannitol ratio with escalating doses, was not reached. This may relate to the difficulty of measuring accurately intestinal permeability in a clinical setting. Overall, the results show that larazotide acetate remains a promising drug candidate, and raises the possibility that paracellular passage through tight junctions may not be the only mechanism target by this drug. Indeed, as gliadin may gain access to the mucosa through transcellular pathways in addition to paracellular diffusion (93,98), this strategy might be best exploited in combination with other treatments.

Interfering with immune recognition
In humans, transglutaminases belong to a family of eight enzymes with diverse functions. They are part of several biological and pathological processes (99). tTG2 is one of the key enzymes in CD development (Figure 2), making it an attractive drug target. The two essential classes of tTG2 inhibitors are irreversible and reversible inhibitors (100). A number of irreversible inhibitors have been reported to form a stable covalent bond with this enzyme and prevent the deamidation of gliadin-related peptides (100,101). Given the ubiquitous nature of tTG, reversible inhibitors would be more desirable in order to minimize possible side effects. Cinnamoyl triazole derivatives (102), aldehyde-bearing tTG modulators (103), and a modified peptide targeting the active cysteine site of tTG2 (26) can be cited in this class. The latter displays up to 225-fold specificity for tTG2 over other transglutaminase subtypes. Nevertheless, this approach will not abolish the innate response (104) or the immune response induced by non-deamidated peptides (105). As all human transglutaminases share strong sequence identity and are important physiologically, specific inhibition of tTG2 remains critical in the design of a clinically viable drug.

It is now well established that gliadin peptide presentation via the HLA system is fundamental in CD pathogenesis (Figure 2) (106). Thus, the development of DQ2/DQ8 inhibitors is another attractive strategy that is currently being explored. Peptidomimetic HLA-DQ2 blockers have been designed by sequence modification of the inflammatory 33mer peptide as non-inflammatory derivatives. This approach has produced analogues retaining their strong affinity for DQ2, whereas not being recognized by fixed T-cells from CD patients. However, these blockers were effective only at high concentrations (107). Unlike other HLA proteins, DQ2-binding sites display a lysine residue whose positive charge has a role in the higher affinity of HLA-DQ2 for negatively charged deamidated gliadin peptides. Semi-synthetic peptides, bearing aldehyde groups that can reversibly react through Schiff’s base formation with the active lysine site in the HLA-DQ2-binding pocket, have been tested and found to decrease T-cell stimulation compared with their native parent (103). A recent study has described the design of peptides exhibiting 100- to 200-fold greater binding affinity for HLA-DQ2 than the best binding gluten peptide (108). For an optimal effect, these inhibitors would be ingested simultaneously or shortly before the gluten-containing food. Besides, the delivery of such molecules in CD is less challenging compared with other MHC-II-associated diseases (e.g., rheumatoid arthritis) (109), as they are aimed at acting directly in the intestine. The consequences of inhibiting DQ2-mediated antigen presentation on immune responses to pathogens need to be considered.

A naturally occurring decapeptide in *Durum cultiar* has recently been reported to inhibit the immunotoxicity of gliadin peptides (110). Co-incubation of peripheral blood mononuclear cells with this peptide and gliadin peptides decreased cell proliferation and release of the pro-inflammatory cytokines TNF-α and IFN-γ (111). Its activity was explained by a shift of the pathogenic T-cell immune response from the immunostimulant Th1 to the Th2 phenotype (111). These results were obtained ex vivo, and only in vivo data will validate their relevance.

Restoring immune tolerance toward gluten
Impairment of immunoregulatory mechanisms that control oral tolerance have been proposed as mechanisms that lead to autoimmune enteropathy in CD (112). An emerging strategy aiming at restoring immune tolerance of wheat peptides has been proposed after the systematic peptide mapping of T-cell reactive epitopes recognized by ~90% of CD patients. A clinical phase I study has been initiated with a vaccine-containing mixture of immunotoxic peptides from α- and ω-gliadins and hordeins (113). This immunization compiles an assortment of CD-associated peptides, and its success will depend on the remaining intolerance of patients to less frequently recognized gluten epitopes. In addition, tolerance has been successfully induced by oral administration of engineered *Lactococcus lactis* secreting a DQ8-restricted gliadin peptide (114) or nasal application of recombinant α-gliadin in a HLA-DQ8 transgenic mouse model (115).

Another original attempt to modulate the immune response to gluten consists of dermal inoculation of a “therapeutic helmihth” (the human hookworm *Necator americanus*). Epidemiological studies have revealed that the incidence of autoimmune inflammatory diseases has increased rapidly in the past 40 years. Today’s hygiene standards and anti-helminthic strategies have a strong stake in this progression (116,117). Many findings in this field are still based on animal models, but “therapeutic” infections with helminth parasites could diminish overshooting autoimmune reactions. Pilot studies have been undertaken by successfully inoculating Crohn’s disease patients with *Necator americanus* larvae (118). Very recently, a similar concept was completed in a phase II trial with CD patients (119). In this case, the hookworm infection is expected to reduce immune reactivity and gluten sensitivity.

Other avenues
In addition to hematopoietic stem cell transplantation (120), biomedical therapies aiming at hampering immune cell recruitment or modulating cytokine production are being evaluated for the treatment of several autoimmune diseases. Although their side effects
may be severe, after a careful benefit/risk evaluation they might be used in refractory CD treatment as combination therapies. Modulation of IL-15 and IL-10 has been suggested to influence the balance between tolerance and autoimmunity (121–126). Blocking IL-15 may contribute to the maintenance of epithelial integrity, limiting epithelial destruction, and subsequently decreasing the passage of dietary antigens.

Given the role of adhesion molecules, such as integrins, during inflammatory processes, inhibiting leukocyte adhesion and preventing their migration may also prove useful in CD. Such potential therapies have been investigated in inflammatory bowel disease and include anti-α4-integrin antibody (Natalizumab) (127), anti-α4/ß7-integrin humanized antibody (MLN-02) (128) and α4-integrin antagonist (SB-683699) (129).

Finally, NKG2D receptors have been reported to be important in epithelial destruction (130–132). NKG2D antagonists have been proposed as a possible therapeutic target in CD (130). The potential usefulness of anti-NKG2D antibodies is supported by their success in rodent colitis (133). Another possibility currently being investigated in inflammatory bowel disease relies on the stimulation of intestinal architectural regeneration by R-spondin, an intestinotrophic mitogen (134). Even though the efficacy of such approaches has not been validated in CD, they may be worth considering in the future development of strategies for refractory CD and other complications.

CONCLUSION
CD displays a complex clinical and pathological spectrum and is one of today’s most prevalent chronic GI disorders. Many aspects of CD pathophysiology have been unraveled, making the implementation of therapeutic and prevention strategies a feasible goal. To assess the efficacy of prevention methods and impact on overall lifetime risk, long-term epidemiological studies will be needed. Regarding pharmacological therapies, a strict GFD should, and probably will remain the method of choice, as it is an effective and safe strategy. Successful, supportive pharmacological therapy of CD should be correlated with disease severity. It should be affordable, almost completely devoid of side effects, efficient and easy to administer. For non-refractory cases, in our opinion, the oral route is the most appropriate approach. For treatments that are not intraluminal, bioavailability will be an important issue, especially with peptide-based approaches.

These therapies should be accompanied by strict medical surveillance and should not encourage indiscriminate gluten consumption. This is an exciting new era in the therapeutic approach of CD and a variety of promising strategies are being developed. This will be a boon for CD patients by possibly allowing occasional consumption of small amounts of gluten (135). Furthermore, it can be hypothesized that avenues such as re-induction of tolerance will allow much greater freedom in food consumption. It can be foreseen that, for ethical reasons, these treatments will find their place as measures supportive of a GFD. The development of therapies that allow occasional exposure to low levels of gluten in adults with CD is expected to decrease the burden of disease and improve patients’ quality of life.

CONFLICT OF INTEREST
Guarantors of the article: Maud Pinier, Pharm D, MSc; Gregor Fuhrmann, Pharm D; Elena Verdu, MD, PhD; and Jean-Christophe Leroux, B Pharm, PhD.

Specific author contributions: Primary authors: Maud Pinier and Gregor Fuhrmann; co-authors: Elena Verdu and Jean-Christophe Leroux.

Financial support: This work was supported by the Canadian Celiac Association and CAG/CIHR (E.V.), the Fonds Québécois de la Recherche sur la Nature et les Technologies (FQRNT), the Canadian Celiac Association, and IG Zöliakie der Deutschen Schweiz.

Potential competing interests: None.

REFERENCES


