Chapter 14
Novel and Experimental Therapies on the Horizon

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Introduction

Celiac disease (CD) is a chronic, systemic, autoimmune disorder in genetically predisposed individuals in response to ingestion of toxic gluten. It affects approximately 1 % of the population in Europe and the United States. Gluten proteins belong to the superfamily of prolamins that have diverged among cereals and are unique to the subfamily of Pooideae that include wheat, barley, and rye. They can trigger an autoimmune injury to the gut, skin, liver, joints, uterus, and other organs [1]. Of the individuals with CD, 5–10 % may be sensitive to oats because some people have small-intestinal T cells that react to oat avenins [2]. The resultant lesion in the mucosa of the small intestine is villous atrophy with crypt hyperplasia and intraepithelial lymphocytosis. Villous atrophy is identified on small bowel biopsy, which is considered the gold standard for diagnosing this condition. False negative small bowel histology can be expected due to patchy small bowel mucosal changes [1]. Untreated CD is associated with significant mortality. Undiagnosed CD is associated with a nearly fourfold increased risk of death [3].

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Novel Therapies for Celiac Disease

The mainstay of treatment for CD is lifelong avoidance of foods containing gluten. CD, like type 1 diabetes, rheumatoid arthritis, and multiple sclerosis, has a chronic nature where particular HLA alleles are overrepresented among the patients [4]. Most patients go into complete remission when they are put on a gluten-free diet, and they relapse when gluten is reintroduced into the diet. CD is in this respect unique among the chronic inflammatory HLA-associated diseases in that a critical environmental factor has been identified [5].

Although GFD is an effective treatment for CD, it does have its limitations. This is due to its cost, side effects including constipation and weight gain, and the difficulty to maintain a strict GFD. This results in poor dietary compliance. In addition, patients with high-level gluten sensitivity are affected by trace amounts of gluten in foods that are declared gluten-free [6]. These limitations and the insight in the pathogenesis have lead to development of new diagnostics and encouraged investigating into possible novel treatments [7]. Potential therapies involve manipulating the dietary gluten, rendering it less toxic, degrading the enzymes that process gluten, decreasing intestinal permeability, blocking the gluten by inhibiting tissue transglutaminase 2, inhibiting binding of gluten to HLA-DQ with the use of inhibitory peptides, shifting the Th1 to Th2 inflammatory response, proinflammatory cytokine inhibitors, enhancing the immune system, inducing gluten tolerance, gluten vaccines, or preventing or reversing mucosal damage in response to inflammation [8]. Despite the potential treatments that show positive results in theory or ex vivo, the effectiveness, safety, drug delivery, and cost effectiveness of the treatment in vivo need to be taken into account.

See Table 14.1 for a list of experimental therapy, mechanisms of actions, and results.

Avoidance of Toxic Dietary Gluten

Consumption of Gluten with Low Immunogenicity

Selecting products that lack toxic gluten but remain palatable and retains the baking qualities of wheat could be seen as an alternative to the commercially available gluten-free products. Wheat and grains with low immunogenicity have been studied in the management of CD. Certain wheat accessions contain low levels of T-cell stimulatory molecules. By breeding wheat species with low or absent levels of harmful gluten proteins, grains with low or no immunogenicity in celiac patients can be produced. However, a major challenge is the alpha-gliadin gene family, which varies in copy number among wheat cultivars and those that are expressed at different levels (manuscript in preparation). Previously, it has been reported that the immunodominant 33mer encoded by alpha-gliadin genes on wheat chromosome 6D

Table 14.1 Experimental therapy, mechanisms of actions, results, and citation

Experimental therapy	Mechanisms of action	Results	Citation
1. Avoidance of toxic dietary gluten			
1. Consumption of gluten with low immunogenicity	Breeding wheat species with low or absent levels of harmful gluten proteins *Triticum monoccum* and tetraploid *Triticum turgidum* pasta wheat Wheat-free sorghum products	Can achieve products with low toxicity levels Alpha-gliadin gene copy is variable which makes avoiding toxic peptides	[9–11]
2. Modified pretreated gluten	Gluten hydrolysis by lactobacilli	Good but slow fermentation and altered baking outcome	[12]
	Removing alpha-gliadin from *Triticum aestivum*	Reduced T-cell response. Compromised baking quality	[13]
	Removing omega- and gamma-gliadin and LMW-GS foci	Lower immune response. Good baking qualities	
	Genetically altering alpha2-gliadin	Eliminated T-cell response	[14]
2. Gluten detoxification			
3. Oral enzyme therapy			
1. Prolyl endopeptidases (PEP)	Degrade gluten ex vivo	Requires prolonged incubation. An immune response cannot be avoided	[15, 16]
2. Cystatins	Degradation of immunogenic T cells by cystatins in germinating wheat seeds	Good results but poor baking quality	
3. ALV003-treated wheat	Combines both germinating barley and PEP	Reduced IFN-gamma ELISpot to gliadin. No change in symptoms. Phase IIa trial	[17]
4. Probiotics	VSL#3 predigested gliadins	Reduction in zonulin release from intestinal epithelial cells	[18]
	Orally ingested IgG	Potentially good results. Phase 1 clinical trials expected	[19]

Table 14.1 (continued)

Experimental therapy	Mechanisms of action	Results	Citation
3. Inhibition of intestinal permeability			
5. Larazotide (AT-1001)	Tight-junction regulatory peptide that inhibits the opening of tight junctions in epithelial cells in the small intestine	Well tolerated. Intestinal epithelial damage may still occur. Phase II study	[20–22]
4. Tissue transglutaminase blockade			
1. Cystamine 2. 2-[(2-oxopropyl) thio]imidazolium inhibitors (L682777 or R283)	Prevents T-cell activation by inhibition of tissue transglutaminase 2 (TG2) and subsequently interfering with gliadin binding to HLA-DQ2/DQ8	In vitro studies promising Effects in vivo are unknown	Pasternack R, Hils M, Zedira Company, Darmstadt, Germany, personal communication, September 2009
5. Th1 to Th2 shift			
1. Decapeptide from durum wheat (sequence QQPQDAVQPF)	The 10mer can inhibit the abnormal immune response triggered by gliadin	Downregulation of IFN-gamma and upregulation of IL-10 (immunomodulator) and a shift from Th1 to Th2 response	[23–26]
6. HLA-DQ groove blockade	Blocks immune activation Amino acid substitution of gliadin rendering it unable to lie within HLA molecule		[14, 27–30]
7. Proinflammatory cytokines inhibition			
1. IL-10	Suppresses Th1 cells IL-10	Works ex vivo. No effect in Crohn's patients. The advances in the field of celiac disease are limited due to the low acceptability of side effects from these drugs	[31–33]
10. Anti-IFN-gamma	IFN-gamma blockade	Prevents histologic damage to healthy mucosa in celiac patients. Disappointing results in Crohn's disease phase I/II trials	[34–37]

(continued)

Table 14.1 (continued)

Experimental therapy	Mechanisms of action	Results	Citation
11. IL-15 inhibitor	Inhibiting the inflammatory response to IL-15	Promising in celiac disease. HuMax-IL-15 has acceptable side effects in rheumatoid arthritis	[38, 39]
12. Anti-TNF-alpha	Management of refractory celiac disease	Small study group. Slow mucosal recovery	[40, 41]
8. Induction of gluten tolerance			
1. Intranasal administration of gliadin peptides in transgenic DQ8 mice	Induction of immune tolerance to gluten and prevents the immune-mediated response to gluten	Lowering T-cell proliferation and the immune response to gliadin. The response may be variable in individual patients	[42–44]
9. Gluten peptide vaccine	Gluten vaccination containing three select immuno-genic 16mer peptides derived from alpha-glia-din, omega-glia-din, and hordein and injected subcutaneously in transgenic mice	Suppression of CD4 T-cell proliferation and IL-2 and IFN-gamma produc-tion and increased the expression of T-reg by splenic CD4 cells in response to a gluten challenge	[45]
10. Pathogenic CD4+ Th cells inhibition	Gliadin-specific Tr1 cell clones suppressed proliferation of pathogenic Th0 cells	Numbers of Tr1 not enough to offer a therapeutic option	[46]
11. Anti-adhesion therapy			
1. Integrin-a4 antagonist: Natalizumab Alemtuzumab AJM300 15. Integrin-a4b7 Vedolizumab (MLN02) Etrolizumab	Inhibit leukocyte adhesion to intestinal mucosa and prevent the migration of leukocytes into inflamed tissue	Not studied in celiac disease. Some have conflicting efficacy data. Large cohort studies are required to conclude the potential safety and efficacy of these drugs in celiac disease	[47–56]
Intestino-trophic mitogens	R-spondin 1 (Rspo1) stimulates the growth of small and large bowel mucosa	Stimulate growth of crypt cells in mouse models of colitis and resort intestinal architecture. No human studies	

is absent in the diploid einkorn, also known as *Triticum monoccum* (gene AA), and the tetraploid *Triticum turgidum* (gene AABB) pasta wheat [9]. On the other hand, hexaploid wheat is needed for bread making. Still, there is a reduction in toxicity observed in vitro with two varieties of bread wheat, one poor in alpha- and beta-gliadins and the other poor in alpha-, beta-, gamma-, and omega-gliadins, which have been tested [10]. Sorghum is a grain that is closely related to maize. Wheat-free sorghum products are safe and palatable in individuals with CD [11].

Modified or Pretreated Gluten

Certain lactobacilli added to sourdough for fermentation hydrolyze the gluten peptide and render them less immunotoxic. This process requires prolonged fermentation, resulting in alteration in the size of the dough, so less fermentation time and mixing with 30 % fermented wheat flour was necessary for better baking results as demonstrated in one study [12]. Patients were challenged for 2 days in this pilot study so long-term safety of this method remains unknown.

Removing alpha-gliadin from *Triticum aestivum* (Chinese Spring) present in chromosome 6 of D-genome (6DS) led to a significant reduction in T-cell stimulatory epitopes but compromised the baking quality of bread. Genetically deleting omega-gliadin, gamma-gliadin, and LMW-GS loci from the short arm of chromosome 1 of the D-genome (1DS) produced a lowering of the immune response to exposure to the wheat with the added benefit of retaining the baking qualities [13]. Another method of detoxifying gluten involves genetically altering the alpha2-gliadin residue by replacing antigenic amino acids with alanine residue, leading to elimination of the T-cell activity [14].

Gluten Detoxification

Oral Enzyme Therapy

Proline residues in some gliadin peptides are resistant to enzymatic degradations in the digestive system leaving them available for abnormal immune response in celiac patients. Enzymatic degradation of this gluten with prolyl endopeptidases (PEP) prevents these peptides from reaching the lamina propria and allows the smaller substrates to be processed by the intestinal brush border enzymes. Microorganisms such as *Flavobacterium meningosepticum*, *Sphingomonas capsulata*, and *Myxococcus xanthus* are able to cleave the immunodominant proline-rich regions [6]. Pyle et al. demonstrated the benefit of PEP in a study when a 2-week gluten challenge with PEP showed no evidence of malabsorption of celiac antibodies [15]. In another study, PEP was reported to require 3 h incubation with the protein in

order to degrade it and prevent gluten-related toxicity. This concludes that the ingestion of PEP with diet is unlikely to avoid immune response to gluten [15].

In germinating wheat seeds, gliadin is under the control of intrinsic cystatins. This protease can degrade immunogenic T cell, making it possible to create flour based on germinating wheat safe for celiac individuals but with compromised baking quality.

ALV003 is a mixture of two glutenases, an endoprotease that has the advantage of combining both germinating barley and PEP. Tye-Din et al. tested ALV003-treated wheat flour on celiac patients [17]. The group found no change in symptoms experienced by patients but a reduced IFN-g ELISpot to gliadin in patients consuming the treated flour compared to placebo controls. Further work will help to evaluate the value of this, and a phase IIa trial is currently recruiting in Finland to assess the safety and efficiency of ALV003 in a larger cohort of celiac patients (NCT1255696).

Probiotics

De Angelis et al. showed that VSL#3 predigested gliadins caused a less pronounced reorganization of the intracellular F-actin, which was mirrored by an attenuated effect on intestinal mucosa permeability. The release of zonulin from intestinal epithelial cells treated with gliadins was considerably lower when digested with VSL#3 [18].

Orally ingested IgG is highly resistant to gastric acidity, and approximately 50 % of neutralizing activity survives when reaching the terminal ileum [19]. In view of the low cost and ease of production of cow's milk antibodies, large-scale production of neutralizing gluten antibodies is potentially easy, safe to use, and cost effective. A clinical phase I trial in the USA is expected [6].

Inhibition of Intestinal Permeability

An important factor contributing to the influx of gluten to the lamina propria is increased intestinal permeability through open epithelial tight junctions. Gluten activates zonulin signaling in tight junctions between epithelial cells of patients with CD, leading to increased intestinal permeability to macromolecules. Larazotide (AT-1001) is an oral tight-junction regulatory peptide that acts locally to inhibit the opening of tight junctions in epithelial cells in the small intestine. The treatment appears to be well tolerated, but it does not prevent small-intestinal epithelial damage upon exposure to gluten [20, 21]. The primary end point of the phase II study on AT1001 has not been reached, and conclusions from other phase I and II studies on the clinical trial register have been performed and are not yet available (NCT362856, NCT492960, NCT 620451, NCT 889473) [22].

Tissue Transglutaminase Blockade

Tissue transglutaminase 2 (TG2) stimulates the process of gliadin binding to HLA-DQ2/DQ8 leading to T-cell activation. TG2 inhibition could possibly prevent the selective deamidation of gluten peptides and blocking the binding to the HLA molecules and preventing or reversing the process of T-cell activation and cell damage [5, 6].

Preclinical tests in vitro on small-intestinal samples from a celiac patient demonstrated inhibition of TG2 by cystamine, a competitive inhibitor, and 2-[(2-oxopropyl) thio]imidazolium inhibitors (L682777 or R283). The consequences of TG2 inhibitors in vivo and the effect of inhibiting all transglutaminase action are unknown [6]. Pasternak et al. demonstrated that TG inhibitors based on a high-affinity thiol binding group displayed a very high specificity for TG2 in vitro, which is very promising [6] (Pasternack R, Hils M, Zedira Company, Darmstadt, Germany, personal communication, September 2009).

Th1 to Th2 Shift

In CD, dietary gluten triggers Th1-type immune response leading to enteropathy. A decapeptide (sequence QQPQDAVQPF) isolated from durum wheat prevents the activation of peripheral lymphocytes in CD. This 10mer is isolated by affinity chromatography and gel filtration from alcohol-soluble protein fraction of durum wheat [23, 24]. Silano et al. [25] demonstrated the antagonist effect of this decamer and its ability to inhibit the abnormal immune response triggered by gliadin. The intestinal T lymphocytes derived from eight children with CD were incubated with deamidated gliadin peptide alone and simultaneously with the 10mer. The results revealed that the incubation of celiac intestinal T cells with deamidated gliadin peptides resulted in a significant increase in cell proliferation and IFN-gamma release. The 10mer caused a downregulation of IFN-gamma and upregulation of IL-10, which has an immunomodulatory role, leading to a shift from Th1 to Th2 lymphocyte response [26].

HLA-DQ Groove Blockade

Inhibitors of HLA-DQ2 that present gliadin peptides have been studied to prevent the activation of the inflammatory cascade in CD following exposure to toxic gluten. Attempts to block immune activation were investigated in other immune-mediated conditions such as rheumatoid arthritis, multiple sclerosis, and type 1 diabetes mellitus. Part of the reason for the lack of success in these experiments was achieving effective drug delivery [27, 28]. However, in CD the DQ2 inhibitor will

need to reach the small intestine directly via the oral route either before or with gluten ingestion.

Amino acid substitution of gliadin can convert the epitope to an agonist or antagonist and affects in turn the inflammatory process [29]. Alanine amino acid substitution at key positions (3, 8, and 10) in the immunodominant peptide in residues 62–75 of a-2-gliadin in wheat abolishes the immunogenicity of the peptide when tested against T-cell clones [14]. The neutral alanine amino acid present in the peptide affected the capability of the peptide itself to lie within the cleft of the HLA-DQ molecules. Anderson et al. substituted an alanine or lysine amino acid in the immunodominant a-gliadin peptide sequence p57–73 QE65. The substitution to the gliadin peptides could abolish their capacity to stimulate IFN-g production from CD4 T cells and also have anti-inflammatory or protective effects in HLA-DQ2+ CD [30].

Proinflammatory Cytokines Inhibition

Various cytokine therapies are being developed for chronic inflammatory conditions. The advances in the field of CD are limited due to the low acceptability of side effects from these drugs.

IL-10 from regulatory T cells suppresses Th1 cells and likely acts as a mildly counter-regulatory cytokine [31]. IL-10 ex vivo suppresses gluten-dependent T-cell activation in cultured celiac small-intestinal mucosa [32]. But another study tested recombinant IL-10 in Crohn's disease was discontinued due to lack of effect [33].

In CD, the main cytokine produced by the gliadin-specific T-cell clones is IFN-gamma [34]. IFN-g blocking antibody can prevent histologic damage to healthy mucosa in celiac patients [35]. Studies in Crohn's disease revealed disappointing results of phase I/II trials. The drop in Crohn's disease activity index (CDAI) in a small cohort did not reach statistical significance due to an unusually high drop in CDAI in the placebo group [36]. Whereas Reinisch et al. found in a larger cohort of Crohn's disease patients, there was a significant decrease in CRP levels. However, this failed to translate into a clinical response [37]. Such studies may encourage research on testing anti-TNF in CD based on the studies undertaken so far in other inflammatory conditions.

Interleukin-15 (IL-15) is a key proinflammatory, innate response cytokine that plays an important role in several autoimmune diseases. IL-15 inhibitors could be used as a promising therapeutic strategy in CD. Baslund et al. demonstrated that HuMax-IL-15, which is a human IgG1 anti-IL-15 monoclonal antibody, had acceptable side effects in rheumatoid arthritis [38]. Another study investigated the effects of treatment with an IL-15 antagonist (CRB-15) that decreased the incidence and severity of collagen-induced arthritis [39]. More studies need to be conducted to observe the effects in CD in clinical practice.

Anti-TNF-a treatment has been instigated in studies on treatment of refractory CD [40]. Reports demonstrate slow mucosal recovery following treatment with

regular anti-TNF-a infusions at 8 weekly intervals [41], or a single dose TNF-a followed by azathioprine maintenance [40]. These studies were on a small group with selection bias so more studies need to be undertaken for accurate conclusions to be drawn.

Induction of Gluten Tolerance

CD is an immune-mediated response to ingested gluten. Induction of immune tolerance to gluten, if successful, could prevent this process from occurring. Intranasal administration of gliadin peptides in transgenic DQ8 mice resulted in lowering the T-cell proliferative response to gliadin and dampening of the inflammatory cascade [42–44]. However, there could be enormous variation in the response by individual patients, making this approach less robust.

Gluten Peptide Vaccine

Another strategy used a gluten vaccination containing three select immunogenic 16mer peptides derived from alpha-gliadin, omega-gliadin, and hordein that account for 60 % of the overall gluten T-cell response. The vaccination was given subcutaneously to gliadin-specific TCR/DQ2 transgenic mice. The result was a suppression of CD4 T-cell proliferation and IL-2 and IFN-g production and increased the expression of T-reg by splenic CD4 cells in response to a gluten challenge. Tye-Din et al. [45] demonstrated that the same 16mer peptides are recognized by the majority of HLA-DQ2-positive, gluten-positive peripheral blood T cells. A patented vaccine continuing the 16mer has finished recruiting for a phase I clinical trial. HLA-DQ8, on the other hand, has a different immunodominant epitope and will not respond to the vaccine. In addition, as the innate immune system plays a role in activating the immune system, celiac patients have different response to the same antigen stimulus [45].

Pathogenic CD4+ Th Cells Inhibition

Gliadin-specific type 1 regulatory T (Tr1) cells are found in the intestinal mucosa of individuals with CD. Gianfrani et al. [46] reported that gliadin-specific Tr1 cell clones suppressed proliferation of pathogenic Th0 cells. Methods to boost the numbers of Tr1 to offer a therapeutic measure need to be sought.

Anti-adhesion Therapy

Chronic inflammatory diseases exhibit leukocyte migration and retention. Adhesion molecules regulate the influx of leukocytes in normal and inflamed gut, local lymphocyte stimulation, and antigen presentation in intestinal mucosal cells. MadCAM-1 is an adhesion molecule specific to the gut. In inflammatory bowel disease (IBD), most of the adhesion molecules are upregulated in inflammatory bowel disease [47]. Inhibiting leukocyte adhesion will prevent the migration of leukocytes into inflamed tissue, which could be a promising treatment for CD. These inhibitory molecules are being studied in IBD so far. There are two humanized antibodies under evaluation for IBD. The first is INTEGRIN-a4 antagonist and includes natalizumab, which is an antibody used in multiple sclerosis and IBD [48, 49], and alemtuzumab, which has been studied in the treatment of refractory CD with conflicting efficacy data [50]. A trail in IBD patients failing anti-TNF-a treatment reported improvement with natalizumab infusion (clinical trial NCT00801125). However, Ananthakrishnan et al. [51] demonstrated that in patients with moderate to severe CD failing two TNF-antagonists, using a third TNF-antagonist therapy appears to be a cost-effective strategy compared to using natalizumab as a third-line therapy without significantly compromising treatment efficacy. Natalizumab is associated with 0.1 % risk of developing progressive multifocal leukoencephalopathy (PML) [52].

AJM300, which is an orally active small molecule, also antagonizes INTEGRIN-a4. Studies in a small cohort of Crohn's disease patients demonstrated reduction in CDAI but no difference compared to placebo [53].

The second humanized antibody targets the adhesion molecule INTEGRIN-a4b7 expressed by gut T cells. Molecules in this group include vedolizumab (MLN02), which demonstrated in phase II trials the capacity to induce remission in ulcerative colitis [54, 55], and etrolizumab, which appears to be well tolerated in moderate to severe ulcerative colitis, but phase II studies are warranted to observe clinical improvement [56].

Overall, large cohort studies are required to conclude the potential safety and efficacy of these drugs in CD.

Intestinotrophic Mitogens

Intestinotrophic mitogens prevent intestinal damage. R-spondin 1 (Rspo1) is a novel epithelial mitogen that stimulates the growth of small and large bowel mucosa. Zhao et al. [57] demonstrated in mouse models of colitis that Rspo1 is able to stimulate the growth of crypt cells, which will hasten mucosal regeneration. This in turn restores the intestinal architecture. The effect of Rspo1 in humans is unknown and studies are yet to be conducted.

Conclusion

While there have been a number of new approaches developed over the years to prevent the onset of the disease in CD patients, they do not have reached a level that would permit abolishing a gluten-free diet for them. Furthermore, most studies rely on the use of synthetic peptides to analyze the immune response rather than intact proteins to predict what is toxic or not. A new approach is therefore needed where intact proteins can be tested in the future.

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