Coeliac Disease – New Pathophysiological Findings and Their Implications for Therapy

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Summary

Coeliac disease (CD) is one of the most common diseases worldwide, resulting from a combination of environmental (gluten) and genetic (human leucocyte antigen (HLA) and non-HLA genes) factors. Depending on the geographical location, the prevalence of CD has been estimated to approximate 0.5–1%. The only treatment currently available for CD is a gluten-free diet (GFD) excluding gluten-containing cereals such as wheat, rye, and barley, and other foodstuffs with natural or added gluten. However, adherence rates and patient acceptance are often poor. Moreover, even in fully adherent patients, the diet may fail to induce clinical or histological improvement. Hence, it is unsurprising that studies show CD patients to be highly interested in non-dietary alternatives. The following review focuses on current pathophysiological concepts of CD, spotlighting those pathways which may serve as new possible, non-dietary therapeutic targets in the treatment of CD.

Zusammenfassung

Die Zöliakie ist weltweit eine der häufigsten Erkrankungen, die aus einer Kombination von Umwelt- (Gluten) und genetischen (humanes Leukozyten-Antigen (HLA) und Nicht-HLA-Gene) Faktoren resultiert. Abhängig von der geographischen Lage, wird die Prävalenz der Zöliakie auf etwa 0,5–1% der Bevölkerung geschätzt. Die einzige Behandlung, die derzeit bei Zöliakie verfügbar ist, besteht in einer glutenfreien Diät (GFD), die glutenhaltige Getreide wie Weizen, Roggen und Gerste sowie andere Lebensmittel mit natürlichem oder zugesetztem Gluten ausschließt. Die Complianceraten und die Akzeptanz durch die Patienten sind jedoch oft schlecht. Weiterhin kann die Diät selbst bei Patienten, die diese vollständig einhalten, möglicherweise nicht zu einer klinischen oder histologischen Verbesserung führen. Daher ist es nicht verwunderlich, dass Studien zeigen, dass Zöliakie-Patienten sehr an nichtdiätetischen Alternativen interessiert sind. Die folgende Übersicht konzentriert sich auf aktuelle pathophysiologische Konzepte der Zöliakie, bei denen jene Signalwege herausgestellt werden, die als mögliche neue, nichtdiätetische therapeutische Ansatzpunkte in der Behandlung der Zöliakie dienen könnten.

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Introduction

Coeliac disease (CD) is defined as chronic small intestinal immune-mediated enteropathy elicited by gluten and related prolamines in genetically predisposed individuals [1], and characterised by the presence of a variable combination of gluten-dependent clinical manifestations, CD-specific antibodies, HLA(human leucocyte antigen)-DQ2 or HLA-DQ8 haplotypes; a characteristic histomorphological picture of the proximal small intestinal mucosa (inflammatory infiltrate, crypt hyperplasia, villous atrophy); and remission of clinical and serologic findings on keeping a gluten-free diet (GFD).

CD occurs in genetically susceptible populations in many regions worldwide, with a prevalence of 0.5–1% in Americans and Europeans, as well as in the populations of Australia, North Africa, the Middle East, India, and probably also northern China (depending on the prevalence of HLA-DQ2 and HLA-DQ8). In some populations, including those of Finland and Mexico, and among the Sahrawi children of North Africa, the prevalence lies between 2 and 5% [2].

CD can manifest itself clinically at any age. The disease aetiology is multifactorial, with a strong genetic susceptibility, as documented in studies of twins and in studies demonstrating a strong dependence on HLA-DQ2 and HLA-DQ8 haplotypes [3].

The clinical manifestations of CD can vary considerably, from none at all (asymptomatic CD) to a wide spectrum of intestinal symptoms such as diarrhoea, steatorrhoea and malabsorption (classical CD), and atypical symptoms (e.g. neurologic findings including depression and gluten ataxia; reproductive disease, including abnormalities in menarche and menopause; and oral/cutaneous disease, including dermatitis herpetiformis). Refractory CD (RCD), based on the expression of a clonal TCR-γ chain gene by intraepithelial lymphocytes (IELs) and divided into two types, RCD I and RCD II, is characterised by persistent or recurrent malabsorptive symptoms and signs with villous atrophy (VA) despite adherence to a strict GFD for more than 12 months (for review see [4–6]). While RCD I is associated with a slight increase of mortality, RCD II is a far more serious form of the disease, with a 5-year mortality rate of up to 50% following diagnosis. Patients with RCD II have also been shown to be at the highest risk of enteropathy-associated T cell lymphoma (EATL) [7, 8].

Although it is now some 60 years since Willem Dicke [9], a Dutch paediatrician, demonstrated a 'wheat factor' as the causative agent of CD, a lifelong GFD remains to this day the only effective treatment [10]. While daily gluten consumption in the general population has been calculated to be approximately 15–20 g [11], several studies have demonstrated that daily doses of as little as <1 g are sufficient to induce mucosal lesions in CD patients. For CD patients, 10–50 mg gluten per day has been shown to be a safe threshold [12–14].
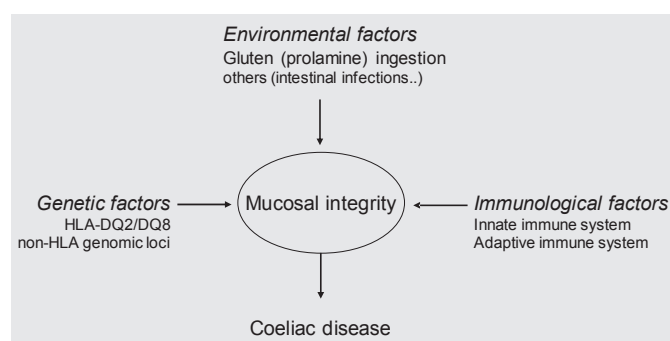
Fig. 1. The pathogenesis of CD is multi-faceted, involving environmental (gluten, intestinal infections), genetic, and immunological factors.

Although more than 95% of patients with CD have an uncomplicated disease that resolves under a GFD, the adherence rates and patient acceptance vary [15, 16]. Furthermore, even in fully adherent patients, the diet fails to induce clinical or histological improvement in 7–30% of the patients [17–19], and 2–5% of the patients develop RCD [20, 21].

Furthermore, while quality of life (QOL) has been shown to improve after diagnosis and subsequent to GFD introduction, some studies found reduced health-related QOL [22, 23]. CD patients on a GFD may also develop nutritional deficiencies [24, 25].

Unsurprisingly, a recent study demonstrated that a large proportion of patients with CD is dissatisfied with the GFD and therefore seeks therapeutic alternatives to it [26].

Based on an improved understanding of the pathogenic pathways underlying CD, several types of therapeutic approach with the potential to augment or supplant the GFD have been generated. This article aims to review novel non-dietary approaches based on new pathophysiological findings and their implications for therapy.

Pathogenesis of Coeliac Disease

The pathogenesis of CD is multifaceted, involving environmental (gluten, intestinal infections), genetic, and immunological factors (fig. 1).

Environmental Factors – the Role of Dietary Proteins

Proteins in the dietary cereal grains wheat, rye, and barley – collectively termed 'gluten' – are known to be the environmental factors which cause disease exacerbation. Strictly speaking, however, gluten is the scientific name only for wheat proteins, while the related proteins in barley and rye capable of activating CD are known as hordeins and secalins, respectively. Whereas wheat, rye, and barley have a common ancestral origin in the grass family, oats, which only rarely (if at all) trigger CD, are more distantly related, thus lacking
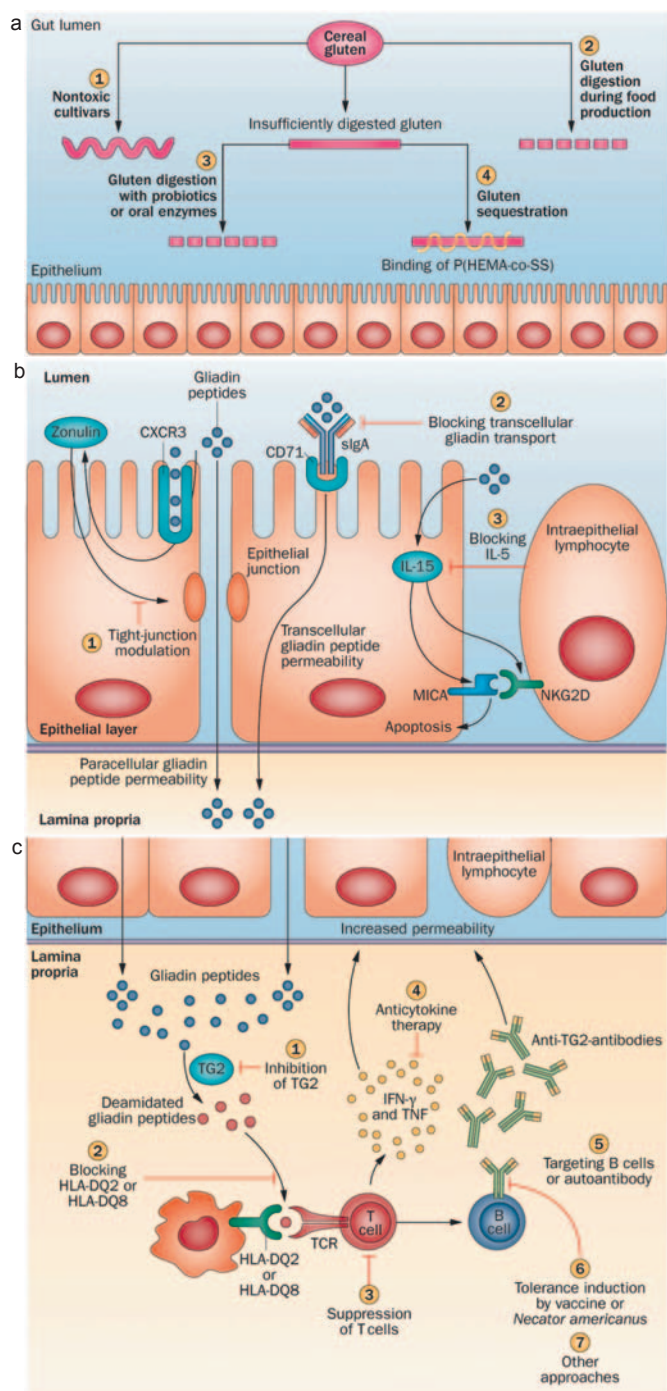
Fig. 2. Novel therapeutic approaches. **a** Therapeutic approaches acting in the lumen of the small intestine, either binding or degrading ingested gluten peptides in the intestine (glutenases, gluten binders, neutralising antibodies). **b** Suggested treatment options that prevent gluten-induced effects in the intestinal epithelium: Blocking the ZOT receptor with the octapeptide AT-1001 to decrease intestinal permeability is another option. Furthermore, since deamidation of gluten peptides by tTG2 and subsequent presentation by HLA-DQ2/8 initiates the adaptive immune responses, tTG2 inhibitors and DQ2-blocking peptides seem an attractive possibility to prevent inflammation. **c** Therapeutic options based on prevention of immunological cascades in cells of the lamina propria (especially in patients with RCD), either by lymphocyte blocking (anti-IL-15, anti-CCR9, anti-α4, -β7) or by tolerance induction (reprinted by permission from Macmillan Publishers Ltd from [95]).

many of the proteins found in wheat. In contrast, the proteins found in rice, maize, sorghum, and millet, which are even more distantly related, do not activate CD.

Gluten contains more than a hundred proteins, present either as monomers or as oligomers and polymers, and linked by interchain disulphide bonds characterised by high content of glutamine and proline (in the form of prolamines), and by low content of charged amino acids. Based on the availability of complete amino acid sequences, gluten can be divided into three broad groups: sulphur-rich (S-rich), sulphur-poor (S-poor), and high-molecular-weight (HMW) prolamines. Traditionally, gluten proteins have been classified according to their solubility in alcohol-water solutions (e.g. 60% ethanol) as soluble gliadins and insoluble glutenins. The alcohol-soluble gliadin fraction consists mainly of monomeric proteins, which either lack cysteine (ω-gliadins) or have only intrachain disulphide bonds (α-type and γ-type gliadins). ω-gliadins are characterised by the highest content of glutamine, proline, and phenylalanine, accounting for around 80% of the total composition [27].

The exceptionally high glutamine and proline content in the gliadins and glutenins of wheat, and also in hordeins and secalins, plays a key role in the pathogenesis of CD:

i) Lacking sufficient prolyl endopeptidases in the human intestine, the high proline content renders these proteins relatively resistant to proteolytic digestion, resulting in the accumulation of relatively large toxic peptides (mainly 50 amino acids in length);

ii) Due to their high content of glutamine and hydrophobic amino acid residues, gluten proteins, especially the alcohol-soluble fraction (e.g. gliadins of wheat, secalins of barley, and hordeins of rye) but also the glutenins, are preferred substrates for the ubiquitous cellular enzyme tissue transglutaminase 2 (tTG2). TG2 can either crosslink certain glutamines in one protein chain with a lysine residue on another chain by forming a covalent isopeptide bond, or merely deamidate this glutamine to an acidic glutamic acid residue, which is of major importance for the pathogenesis of CD.

More than 50 distinct (deamidated) gluten peptides exerting cytotoxic, immunomodulatory, and gut-permeating activities have been described [28]. These activities have been partially mapped to specific domains in α-gliadin (fig. 2): the cytotoxic peptides 31–43 and 31–49, the immunomodulatory peptide 57–89 (33-mer), the CXCR3-binding, zonulin-releasing (gut-permeating) peptides 111–130 and 151–170, and the interleukin(IL)-8-releasing peptide 261–277. The 33mer peptide from α2-gliadin, which contains 6 partly overlapping HLA-DQ2-binding amino acid sequences and is also regarded as a coeliac 'superantigen' [28, 29], has been shown to be resistant to degradation by gastrointestinal peptidases reaching the submucosal immune system in an intact peptide [30].

After reaching the lamina propria, either by epithelial transcytosis or through increased epithelial tight junctional

Fig. 3. Posttranslational modification of gluten peptides. **a, b** Gluten peptides that are highly resistant to intestinal proteases reach the lamina propria, either via epithelial transcytosis or due to increased epithelial tight junctional permeability. Here, they are deamidated through the activity of tissue tTG2(transglutaminase 2)-generating peptides with negatively charged amino acid residues that bind with higher affinity to the disease-associated HLA-DQ2 or HLA-DQ8 molecules. P4, P6, and P7 pockets in HLA-DQ2 and P1 and P9 pockets in HLA-DQ8 have a preference for negatively charged anchor residues (modified from [3]).
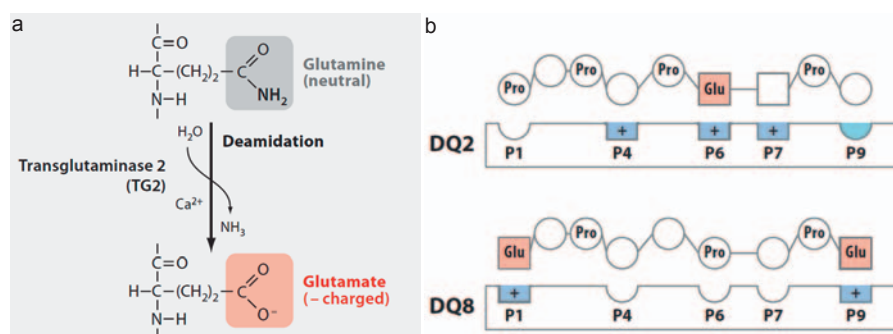
permeability, gluten peptides are deamidated through the activity of tTG2, and (except the 33mer peptide, which does not need further processing) [31, 32] presented in the lamina propria by dendritic cells to activate CD4+ T cells [3, 33].

In contrast, the cytotoxic α-gliadin peptides p31–49 and p31–49, which are thought not to bind to HLA-DQ2 and -DQ8, have been shown to upregulate IL-15 production in epithelial cells, macrophages, and dendritic cells, thus increasing IEL infiltration and epithelial cell apoptosis via NKGD2D und MICA receptors, respectively cumulating in cytotoxic damage to the epithelium [29, 33, 34].

Genetic Factors

Evidence for genetically-based susceptibility for CD has been gained from epidemiological studies showing that up to 20% of first-degree kin are equally affected by CD, with concordance rates >75% in monozygotic twins [3, 35]. The leucocyte antigen (HLA) class II genes HLA-DQ2 and -DQ8 have been demonstrated to be the strongest genetic susceptibility factors by far: Whereas more than 95% of CD patients carry HLA-DQ2 and -DQ8, these genes are found in only 25–30% of the Caucasian population, of whom only 4% develop CD, indicating that additional factors also play a role. Using genome-wide association study (GWAS), at least 115 genes harbouring non-HLA susceptibility factors associated with CD have been described, of which 28 have been shown to be immune-related [3, 36]. However, these genes do not contribute more than 4–5% to the overall genetic risk, which is dominated by HLA-DQ2 or -DQ8.

Immunological Factors

HLA-DQ2 and -DQ8 play a plausible key role in CD due to their unique ability to bind the proline-rich gluten peptides, especially those with a negative charge due to TG2-mediated deamidation, resulting in a more rigorous CD+ Th1 T cell activation. Both HLA-DQ2 and -DQ8, such as the lysine positioned at β71 and at positions P4, P6, and P7 of DQ2, contain positively-charged pockets which promote the binding of negatively-charged glutamic acid residues generated by the autoantigen TG2 (fig. 3) [3, 29].

Gluten-responsive activated T cells produce proinflammatory cytokines, predominantly interferon-γ (IFN-γ). IFN-γ activates macrophages, which in turn secrete tumour necrosis factor α (TNF-α) and proteolytic matrix metalloproteinases (MMPs). Both cytokines trigger the expression of proteolytic MMPs in intestinal myofibroblasts, resulting in matrix proteolysis alteration, which in turn leads to mucosal injury and villous atrophy. In addition, via a phosphatidylinositol-3-kinase-dependent pathway and mediated by thioredoxin (TRX), IFN-γ triggers the activation of tTG2, thereby establishing an autoamplificatory loop for gluten-induced inflammation [37]. In duodenal biopsies of CD patients, neutralisation of IFN-γ has been shown to ameliorate gluten-induced mucosal damage [38].

Chronic exposure of CD patients to dietary gluten is invariably accompanied by the production of autoantibodies against tTG2. Anti-tTG2 antibodies are preferentially localised in the subepithelial layer, where they adhere to extracellular tTG2 on fibroblasts and on the basement membrane of the small intestine. Anti-tTG2 have been shown to induce enterocyte proliferation and inhibit enterocyte differentiation, and are able to modulate epithelial barrier function, thereby promoting intestinal crypt hyperplasia and villous blunting (reviewed in [37]).

Non-Dietary Therapies of Coeliac Disease

As already mentioned, a lifelong GFD is not only burdensome, but difficult to maintain and frequently unsuccessful. Thus, there is a need for effective, inexpensive, and safe alternative treatment options for CD and new approaches in adjunctive therapy. Although, at the present time, it is unrealistic for such novel therapies to counteract effects of gluten at the levels typically contained in the Western diet (15–20 g daily), their initial aim should be to neutralise at least small

amounts of up to 3 g gluten per day. Since few therapeutic alternatives are currently available to treat RCD, more costly compounds with less favourable side-effect profiles may be acceptable in these patients. In the second part of this paper, we discuss new treatment strategies for the future which have shown potential in early studies in CD, with encouraging results in vivo and in vitro.

Novel non-dietary treatment options can be classified by their targets during different phases in the pathogenesis of CD (e.g. candidates targeting the disease-inducing prolamines, endogenous molecules involved in the pathogenesis) or by their ability to induce tolerance to gluten (fig. 2).

Targeting Disease-Inducing Gluten and Other Prolamines

Preventing Intestinal Gluten (Prolamine) Absorption

Another strategy to prevent the interaction of immunogenic gluten peptides with submucosal immune cells is the use of HMW polymers to selectively bind gluten, thereby preventing its breakdown and absorption [39].

Poly(hydroxyethylmethacrylate-co-styrene sulfonate (P(HEMA-co-SS))) is a synthetic polymeric compound that sequesters food-derived gluten in the gastrointestinal lumen by forming high-affinity complexes with α-gliadin, thus preventing enzymatic digestion of gluten and other prolamines into smaller, medium-length immunogenic peptides [39, 40].

In a first preclinical study, Pinier et al. [41] assessed the capacity of P(HEMA-co-SS), a copolymer of hydroxyethyl methacrylate and styrene sulfonate, to reduce paracellular permeability, and demonstrated its ability to normalise antigliadin immunoglobulin A in intestinal washes and reduce gliadin-invoked TNF-α secretion in duodenal biopsies of CD patients.

Oral Enzyme Therapy

Because the human digestive tract has an insufficient supply of prolyl endopeptidases (PEPs), the enzymes capable of hydrolysing the immunogenic proline-rich peptides found in gluten and related prolamines, oral PEP therapy may offer another strategy to reduce the amounts of immunogenic gluten peptides reaching the small intestine. This approach is analogous to exogenous lactase supplementation in the treatment of lactose intolerance. Enzymatically active PEPs are expressed in several microbial species, including *Aspergillus niger, Sphingomonas capsulata, Flavobacterium meningosepticum*, and *Myxococcus* [42].

A. niger PEP (AN-PEP) is enzymatically active in a pH ranging from 2 to 8, and is therefore active both in the stomach and in the intestine. It has also been shown to be resistant to gastric pepsin. In vitro, AN-PEP is able to break down gluten and gluten peptides into non-immunogenic fragments within a few minutes [43, 44]. A recent pilot study of 16 subjects demonstrated AN-PEP to be well-tolerated, but no efficacy data have been presented [45].

PEPs derived from *F. meningosepticum* (PEP-FM) have been shown to effectively reduce levels of the immunogenic 33mer in vitro and in vivo in rats. However, subsequent studies revealed that large quantities of the enzyme would be required to detoxify a normal daily gluten intake, and that PEP-FM activity decreased due to its instability in the presence of gastric enzymes [42, 46].

To enhance gluten degradation, combinations of complementary peptides have been introduced [47]. ALV003 is composed of two gluten-specific proteases: a modified recombinant version of a *Hordeum vulgare* (barley) cysteine endoprotease (EP-B2) and a modified recombinant version of a *S. capsulata* prolyl endopeptidase.

In a phase IIb trial involving 41 patients with stable CD, in which individuals were randomised to receive oral ALV003 or placebo daily for 6 weeks at the time of ingestion of 2 g gluten, ALV003 was reported to significantly attenuate gluten-induced intestinal mucosal injury. Importantly, no serious adverse events were reported.

Currently, a growing number of enzyme preparations claiming to aid gluten digestion are becoming commercially available, e.g. compounds containing dipeptidyl peptidase IV (DPPIV) from *Aspergillus oryzae* [48]. However, their effectiveness in CD patients has not been confirmed in clinical trials.

Decreasing Intestinal Permeability

Increased intestinal permeability (IP) in active CD has been measured both clinically by non-invasive sugar permeability tests (e.g. urinary lactose/mannitol (LAMA) fractional excretion ratio) [49] and by in vitro tight junction (TJ) analysis [50]. Although it is not yet finally determined whether increased IP is a primary cause or a consequence of CD, an increase of IP via opening epithelial TJs seems to be an important contributor to the influx of gluten peptides into the subepithelial adaptive immune system.

The *Vibrio cholera* zona occludens toxin (ZOT) is known to increase intestinal paracellular permeability by altering different TJ proteins via the 66 kD ZOT receptor (for review see [28]). Based on the observation that the inflamed intestinal epithelium of CD patients releases a paracrine protein (zonulin) [51], which acts similar to ZOT, an octapeptide (AT-1001) corresponding to the amino acid sequence of the receptor-binding motif of human zonulin was developed. By antagonising zonulin receptor activation, AT-1001 thus protects intestinal TJ integrity.

Based on encouraging data from a phase I trial showing

that AT-1001 (larazotide acetate) was not only well tolerated, but also decreased IP, IFN-γ production, and intestinal symptoms following a single gluten challenge in CD patients, phase II placebo-controlled randomised trials were performed. In the study of Leffler et al. [52], 86 patients with CD in diet-controlled remission were randomly assigned to larazotide acetate (0.25, 1, 4, or 8 mg) or placebo three times per day with or without gluten challenge (2.4 g/day) for 14 days. Although the primary efficacy endpoint (decrease of LAMA fractional excretion ratio) was not reached, larazotide acetate improved gluten-induced exacerbation of gastrointestinal symptom severity as measured by the Gastrointestinal Symptom Rating Scale (GSRS) at lower doses, but not at the higher dose [52]. No serious adverse events were observed. In a dose-escalation study (1.4 and 8 mg) in 184 CD patients in remission who were challenged with 0.9 g gluten three times daily over 42 days, Kelly et al. [53] demonstrated that, compared to placebo controls, patients treated with larazotide acetate showed a significantly improved symptom score and a less pronounced anti-tTG response. However, this study also failed to demonstrate significant improvement in IP as measured by the urinary LAMA ratio.

Results from the first multicentre trial conducted in 74 sites in North America, including 342 patients, were presented as a late breaker abstract at the 2014 Digestive Disease Week (DDW) in Chicago, reporting significant symptom reduction under the 0.5 mg dose of larazotide acetate. This study represents the largest therapeutic trial in CD to meet its primary endpoint of reducing signs and symptoms [54].

Preventing T Cell Activation by Gluten-Derived Peptides

Blocking Deamidation of Gluten-Derived Peptides: Transglutaminase Inhibitors

Although the deamidation of gluten by tTG may not be an absolute prerequisite for the initiation of CD, it does at least play an important role, increasing T cell reactivity by improving peptide affinity to HLA-DQ2 and -DQ8 molecules. Therefore, therapeutic approaches targeting the inhibition of tTG would seem logical.

Several competitive (mainly polyamines, e.g. putrescine, spermidine, or cystamine), reversible (mainly guanosine triphosphate analogues), and irreversible (e.g. iodoacetamide, 3-halo-4.5-dihydroisoxazoles) inhibitors have been developed [55–60], which have been demonstrated in vitro to be able to attenuate the toxic effects of gliadin in epithelial cell cultures. A few have also been tested successfully ex vivo in duodenal biopsy specimens of CD patients. More recently, a group of high-affinity tTG2 inhibitors (ZED 1098, ZED 1219, and Zedira) has been developed, shown to be stable and soluble in the GI tract and not cytotoxic over a wide dosing range [47].

Blocking the Binding of Deamidated Gluten Peptides to HLA Proteins: HLA-DQ2 Inhibitors

Alternatively, blocking the binding of deamidated gluten peptides to CD-specific HLA proteins from interacting with antigen-presenting cells could also be a promising approach. A similar strategy has already been tested in other autoimmune diseases (e.g. type 1 diabetes, rheumatoid arthritis) but results in terms of clinical efficacy have been disappointing, presumably due to inadequate drug delivery. In spite of new technological advancements (e.g. use of a positional scanning nonapeptide library or silico approach) [61–64] allowing the development of ultra-high affinity peptides, in light of specific requirements regarding nontoxicity and non-immunogenicity, this approach still requires a great deal of work before reaching clinical practice.

Therapy Targeted at Immune Cells

Immunosuppression by Topical Steroids

Budesonide, a topical glucocorticoid with low systemic bioavailability mainly used in inflammatory bowel disease (IBD) affecting the distal part of the intestine, was reported to be effective also in the treatment both of RCD (e.g. non-responsive to GFD) [65, 66] and non-RCD [67]. Presumably, changing the drug formulation to target the proximal intestine should further improve its efficacy for CD.

Inhibitors of T Cell Homing

As in other T cell-mediated disease (e.g. IBD), homing of effector/memory T cells to the small intestine (i.e. the intestinal segment affected by CD) is mainly controlled by their expression of the cell surface chemokine receptor CCR9 and the integrin α4β7. Antibodies targeting CCR9 and α4β7 to prevent or limit T cell migration (homing) to the small intestine, thereby limiting local T cell activation, may also reduce intestinal damage and are therefore a potentially promising therapy for (refractory) CD.

Vedolizumab, a humanised anti-α4β7 integrin antibody, has been approved by the Food and Drug Administration (FDA) and European Medicines Agency (EMA) in 2014 as the first anti-adhesion therapy for the treatment of IBD. Phase II trials have shown that etrolizumab, a humanised monoclonal IgG1 antibody targeting β7, is effective for the treatment of ulcerative colitis (reviewed in [68]). Currently, there are no data from CD trials.

Encouraging results for CCX282-B, a specific, orally-administered chemokine receptor CCR9 antagonist, in a clinical phase II trial in patients with Crohn's disease, have already led to the initiation of phase III clinical trials in CD [69]. Also,

Table 1. Treatments for CD beyond the GFD (status May 2014)

Underlying pathology	Therapeutic approach	Compound/organism	References
Immunodominant gluten peptides	wheat variants; less immunogenic ancient wheat species; genetically modified (gluten-depleted) wheat strains with maintained baking properties; gluten detoxification; enzymatic cleavage of gluten fragments; inactivation by polymeric binders	prolyl endopeptidases AN-PEP; oral enzyme supplementation; AVL003P (HEMA-co-SS)	preclinical preclinical preclinical phase II clinical trial phase II clinical trial phase I
Impaired mucosal barrier	permeability inhibition; zonulin/ZOT receptor blockade	AT-1001 (larazotide)	phase IIb clinical trial
Adaptive immunity	antigen presentation suppression; inhibitors of transglutaminase; blockers of HLA-DQ	ZED1227, Zedira	phase I preclinical
Inflammatory response	anti-TNF-alpha; anti-IL-15	infliximab AMG 714	preclinical preclinical
Lymphocyte recruitment ('homing')	adhesion blockade; anti-α4β7/MAdCAM-1; anti-CCR9/CCL25	natalizumab Ccx282-B, CCX025	preclinical phase II concluded
Immunomodulation	gluten vaccination; parasite infection	nexvax2 trial *Nectarus americanus* (hookworm)	phase IIa clinical trial phase II clinical trial

a phase II clinical study in CD (ClinicalTrials.gov identifier: NCT00540657) has already been completed, although publishing of the results is still pending.

Inhibitors of Proinflammatory Cytokines

The central role of IL-15 in the onset and the severity of CD is evidenced by both animal and human data [70–74]. Based on the potent immune-modulating effects of IL-15, blockage of the IL-15 pathway either via the inhibition of IL-15 or by blocking the IL-15 receptor, or inhibition of the downstream JAK3-kinase, may be especially useful approaches for the treatment of RCD. In a phase I/II clinical trial in rheumatoid arthritis (RA), HuMax-IL15, a human monoclonal antibody targeting IL-15 (HuMax-IL15; Genmab, Copenhagen, Denmark) which blocks the epitope of IL-15 binding to the c subunit of the IL-15 receptor, was well tolerated and effected substantial improvements in disease activity [74].

Tofacitinib, an oral inhibitor of the Janus kinases 1.2 and 3 which blocks signalling involving gamma chain-containing cytokines such as IL-15, has recently been demonstrated in phase II trials to be highly effective in the treatment of RA [75] and IBD [76, 77]. These studies suggest that manipulation of the IL-15 pathway might also have therapeutic possibilities in CD, especially for RCD and EATL, in which malignant T cell expansion is driven by IL-15. Although there are as yet no data from clinical studies in CD, recently published results from animal studies [78] would encourage further research of this strategy.

Targeting IFN-γ and TNF-α

IFN-γ, produced by submucosal gliadin-sensitive CD4+ T cells, triggers the activation of MMPs and is thus responsible for mucosal injury and villous atrophy. In addition, by increasing intestinal permeability, probably via a Rho-associated kinase-dependent pathway, it promotes the influx of gliadin peptides through the intestinal wall [79–81]. A small study using biopsies from the intestinal mucosa of patients with CD demonstrated that IFN-γ-blocking antibodies were able to prevent mucosal damage induced by proinflammatory cytokines released by gliadin-specific HLA-DQ2-restricted T lymphocyte clones [82]. However, at least in Crohn's disease, phase II clinical trials using IFN-γ monoclonal antibodies have not shown very promising results [83, 84].

A more promising approach seems to be to target TNF-α, which has been found to be more elevated in RCD [85]. The use of monoclonal antibodies against TNF-α, which has now been the mainstay in the treatment of Crohn's disease for more than 10 years, has been described in several case reports in the treatment of RCD [86–90].

CXCR3/CXCL10 Inhibitors

CXCL10, also referred to as IFN-γ-inducible protein-10 (IP-10), is another chemokine which plays an important role in the integrin activation and migration of activated T cells, monocytes, and natural killer cells. Stimulation of IP-10 by the chemokine receptor 3 (CXCR3) results in the generation and recruitment of proinflammatory cells responsible for in-

flammation and tissue destruction [91]. Recently, Lammers et al. [92] identified a novel immunomodulatory gliadin peptide that causes IL-8 release in a chemokine receptor CXCR3-dependent manner exclusively in patients with CD, supporting the CXCR3/CXCL10 axis as a future therapeutic target for CD.

In a most recently published phase II trial by Sandborn et al. [76], BMS-936557, a human monoclonal antibody targeting CXCL10, was well tolerated and produced substantial improvements in disease activity in patients with moderately-to-severely active ulcerative colitis. To this day, clinical data from CD patients are lacking.

Immune Modulation and Induction of Tolerance to Gluten Peptides

A phase Ib/IIa trial of infection with the nematode *Necator americanus* in patients with CD, aiming to effect a shift from a Th1 to a Th2 milieu, has recently been reported. However, despite promising safety data, the study failed to show obvious improvement in disease activity following gluten challenge [93].

Also currently under investigation is NexVax2, a therapeutic vaccine derived from a mixture of three 15- to 16mer peptides aiming to generate gluten tolerance. A phase I study of NexVax2 in 40 HLA-DQ2+ CD patients, using subcutaneous doses of up to 90 µg vaccine weekly for 3 weeks, showed no clinically relevant adverse events [94]. Based on these promising safety data, further trials to examine long-term efficacy are warranted.

Conclusion

Recent progress made in understanding the pathogenesis of CD has opened the doors for a variety of new non-dietary treatments which may be used at least as adjunctive therapy (table 1). To date, however, only a limited number of experimental therapies for CD have been assessed in phase I–II randomised, controlled clinical trials. Larazotide acetate (AT-1001), which is assumed to hinder the paracellular passage of gluten through the epithelial barrier into the lamina propria by inhibiting tight junctions, has been studied in almost 100 patients to date, but an effect on hard end points, such as protection of mucosal integrity, needs to be demonstrated. Though fewer patients were studied, evidence for the efficacy of the endopeptidases contained in ALV003 which break down gluten to less or non-immunogenic peptide fragments is more obvious. Other therapies like TG2 inhibition, preventing immunogenic potentiation of gluten, or vaccination to induce tolerance to ingested gluten are less advanced but have potential for high efficacy. It should be kept in mind that most therapies discussed do not have the potential to allow the ingestion of gluten at normal daily levels of >15 g, but offer only an adjunctive therapy, eliminating the detrimental effects of small amounts of up to a few grams of gluten in CD patients. However, in light of the relative ease at which the obvious sources of gluten can be avoided in contrast to the many nutritional sources of hidden gluten, even a 'neutralization' of a minor amount of gluten would take most of the dietary burden from patients with CD.

Disclosure Statement

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