Review

Celiac Disease—New Insights in Pathogenesis, Diagnosis and Management

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Review

Celiac Disease—New Insights in Pathogenesis, Diagnosis and Management

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Abstract: Celiac disease is defined as a systemic immunological disorder caused by gluten in genetically predisposed individuals, who have a variety of gluten-dependent symptoms, specific antibodies, HLA DQ2 and DQ8 histocompatibility antigen presence and enteropathy. Celiac disease is considered one of the most common autoimmune diseases. Its prevalence, depending on the studied population and methodology, is estimated at 0.75-1.6% of the general population.Celiac disease is an autoimmune disease that develops as a result of the interaction of genetic, immunological, and environmental factors. During the complex immune reaction, most of the cells involved in inflammatory processes are activated, which leads to the gradual atrophy of intestinal villi and the proliferation of enterocytes within the intestinal crypts. The pathogenesis of celiac disease is extremely complicated and is still being studied.According to the current guidelines, in order to correctly diagnose celiac disease, the following criteria should be taken into account: clinical symptoms (intestinal and extraintestinal), presence of antibodies against tissue transglutaminase in the IgA class, taking into account the level of total IgA and presence of typical histological changes in a duodenal bioptates.The important clinical challenge is the diet-resistant celiac disease, causing the severe complications,Currently, the basic method of treating celiac disease is an elimination diet (i.e. exclusion of products that may contain gluten from the diet), however, new therapeutic strategies are still being sought, mainly based on supplementation of exogenous endopeptidases, modification of the immune response, the use of zonulin inhibitors and transglutaminase 2 inhibitors. Clinical trials of new drugs are ongoing. The gradually expanding knowledge of the pathogenesis of celiac disease may allow the development of new therapeutic strategies both for patients with a mild course of the disease as well as those diet-resistant.

Keywords: celiac disease; diet-resistant celic disease,; pecific antibodies; HLA DQ2 and DQ8; enteropathy

Introduction

Celiac disease (CD) is defined as a systemic autoimmune disorder induced by gluten and prolamin derivatives in genetically predisposed individuals who experience a variety of gluten-dependent symptoms, specific antibodies, HLA-DQ2 and DQ8 histocompatibility antigens presence and enteropathy [1,2]. Characteristic intestinal symptoms of celiac disease include: diarrhea (13-96%), abdominal pain (8-90%), vomiting (26-33%), flatulence (5-10%) and fatty stools [3–6]. There are also extraintestinal symptoms that are related to gastrointestinal dysfunction, mainly in the course of malabsorption, ultimately leading to numerous disorders affecting most body systems. The most frequent are weight loss (44-60%), failure to thrive in children (19-31%), anemia (3-30%), iron deficiency anemia (40%), folic acid deficiency (20%) and vitamin B12 deficiency (17%). Among celiac patients deficiencies of fat-soluble vitamins A, D, K and E and elevated levels of aminotransferases are observed more frequently than in the general population [7]. Osteopenia (54%) and osteoporosis (12%), mainly due to the vitamin D deficiency (34%), and hypocalcemia leading to tetany are among most common complications of celiac disease and are sometimes its first symptoms [8–11].

Celiac disease, untreated for years, can also lead to the development of malignant tumors, such as esophageal and small intestine cancer, lymphoma, especially T-cell lymphoma, which occurs

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mainly in the small intestine. These cancers are rare, but they occur significantly more often in patients with celiac disease than in the general population [12].

Patients with celiac disease also suffer from other comorbidities – the most prevalent are autoimmune diseases: type 1 diabetes (7%), Duhring's disease (3%) and thyroid diseases (5-21%) [13,14] but also neuropsychiatric disorders [15].

Epidemiology

Celiac disease is considered one of the most common autoimmune diseases. Its prevalence, depending on the investigated population and methodology, is estimated at 0.75–1.6% of all screened people [16–19]. A detailed analysis conducted among the inhabitants of the United States of America showed that celiac disease occurs significantly more frequently in Caucasians (1.01%) than in African Americans (0.2%) or Latinos (0.3%) [20].

Celiac disease develops in genetically predisposed subjects. It has been shown to occur more frequently in relatives of celiac patients than in the general population. The risk of developing celiac disease was 4.5-7.5% among first-degree relatives and 2.3-2.6% among second-degree relatives. It was diagnosed more often in women than in men who were first-degree relatives of celiac patients (8.4% vs. 5.2%) [21,22].

Studies conducted around the world observed considerable geographic variation in the prevalence of celiac disease. It occurs mainly in Europe (2% in Finland, 1.2% in Italy, 0.9% in Northern Ireland and 0.3% in Germany), North Africa (5.6%), the Middle East, North America (1-1.4%) and India. It is much less frequent in South America (0.4-0.5%) and Australia (0.3%). It is equally rare in Asia, excluding India, as well as in Central and South Africa (0.4-0.6%) [23,24].

Etiopathogenesis

Celiac disease develops due to an abnormal immune response to ingested gluten and prolamin derivatives (contained in cereals such as rye, wheat, barley and oats which are respectively gliadin, secalin, hordein or avenin) in genetically predisposed individuals with specific histocompatibility antigens (HLA-DQ2 or HLA-DQ8) [25]. After ingesting gluten-rich cereals, gluten is partially broken down in the stomach by pepsin. The long-chain protein fragments obtained this way, after contact with G.I. mucosa, show significant immunogenicity, causing an increase in the plasma level of numerous inflammatory interleukins [26].

Prolamin degradation products are further digested in the duodenum and small intestine with prolyl oligopeptidase [27]. Gluten treated by endopeptidases is broken down into short glutamine and proline-rich peptide chains. Gluten proteins are a heterogeneous group consisting of high molecular weight (HMW), low molecular weight (LMW) subunits and gliadins (α-, β-, γ-, and ω). The level of degradation resulting from the digestive process depends on the type of the contained protein. Protein digestion results in the formation of oligopeptides which are then deamidated by tissue transglutaminase and stimulate the activity of T lymphocytes [28–30].

The intestinal microbiota plays an active role in the process of gluten protein digestion. In celiac patients, especially those not observing the gluten-free diet, the presence of a greater number of fermenting bacteria in the gut was demonstrated, which may explain accompanying flatulence [31]. It should also be noted that the introduction of a gluten-free diet leads to an increase in the number of *Enterobacteriaceae* and *Escherichia coli* with simultaneous reduction in the number of *Bifidobacter sp,* both in celiac patients and in healthy individuals [32–35]. Both oral and intestinal bacteria produce their own endopeptidases and hydrolases that contribute to the digestion of protein chains resulting from the degradation of gliadin [36–41]. Fragments of undigested proline-rich proteins in contact with the intestinal mucosa increase in IL-15, which in turn stimulates activated T and NK cells to produce chemokines and proinflammatory cytokines [42–44]. Moreover, IL-15 promotes NK cell apoptosis [45,46]. This process is additionally intensified by a direct contact reaction between gliadin fraction (p31-43) and intestinal mucosa, leading to the enterocyte cytoskeleton reconstruction and, subsequently, to apoptosis [47–49]. Furthermore, fragments of partially digested gliadin were

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shown to bind to CD95/FAS receptors belonging to the group of TNF-alpha (Tumor Necrosis Factor alpha) family receptors leading to apoptosis by activating caspase 8 and 10 [50].

Moreover, increased intestinal mucosal permeability is caused by loosening of tight junctions between enterocytes [51]. The pathomechanism of this phenomenon was described by Fasano et al. They showed that gliadin fragments (111–130 and 151–170) in the small intestine bind to the CXCR3 receptor [52]. This leads to the release of zonulin (a protein similar to the toxin produced by cholera bacillus) from enterocytes of the small intestinal mucosa [53–55]. This protein binds to the protease activating receptor (PAR2). As a result, tight junctions between enterocytes are disrupted and transepithelial electrical resistance is reduced, leading to increased intestinal wall permeability [56–59]. Both described processes are responsible for the increased penetration of undigested short-chain proline-rich proteins into the submucosa. At this stage, the intestinal bacterial flora may also play an important role. In non-diet celiac patients *B. lactis* and *L. fermentum* were shown to increase intestinal mucosal permeability by reducing transepithelial resistance and increasing zonulin expression [60].

In vitro studies demonstrated that gliadin fragments (mainly p62-75, p57-68) undergo tissue transglutaminase - mediated deamidation. The deamination process has been shown to be necessary to trigger further immune response [61–63]. In the next step, deamidated gliadin peptides bind with DQ2 or DQ8 receptors expressed on antigen presenting cells (APC)[64–66]. Two heterodimers, DQ2 (DQ2.2 and DQ 2.5) and DQ8, participate in the antigen presentation process [67–70]. As a result of the reaction, HLA-DQ2 and DQ8 are complexed with deamidated α-gliadin fragments [71]. The protein fragments are then presented to B lymphocytes by APCs, initiating further humoral response.

The existence of molecular mimicry resulting from similar 3-dimensional appearance of proteins, mainly tissue transglutaminase and specific gliadin fragments, has been proven [72]. As a result, B cells produce antibodies against both tissue transglutaminase and gliadin and deamidated gliadin peptides [73–76]. It should be noted that under the influence of a number of inflammatory cytokines produced by monocytes and T cells, such as INFα, IL-15, IL-18 and IL-21, the inflammatory response in the course of celiac disease is directed towards the Th1-dependent inflammatory pathway [77–81]. At the same time, INF γ and TGF α stimulate monocytes and myofibroblasts damaging the enterocyte stroma by producing matrix metalloproteinases (MMP-1, MMP-3, MMP-12). This leads directly to the intensification of the apoptosis process and to increased mucosal permeability [82–84].

The above-mentioned CXCR3 receptor present on enterocytes also interacts with fragments of gliadin (p261–277 and p270-286), leading to an increase in the concentration of inflammatory interleukins, especially IL-8, a neutrophil chemokine. This reaction was observed only in celiac patients and persisted despite a gluten-free diet [85].

On the other hand, contact of gliadin fragments with enterocytes leads to increased production of EGF (epidermal growth factor), which stimulates the crypt enterocyte proliferation leading to their hyperplasia [86,87].

As it results from the above description, during a complex immune reaction, most cells involved in inflammatory processes are activated, both in terms of cellular and humoral responses. Gliadin peptides in a complex mechanism lead to direct apoptosis of enterocytes which leads to the gradual villous atrophy. On the other hand, gliadin induces increased production of EGF, which leads to the cell hyperproliferation within the intestinal crypts. The pathogenesis of celiac disease is extremely complex and still not fully elucidated yet.

Celiac Disease Diagnosis

Pathomorphological Changes in the Duodenum

Chronic inflammation leads to gradual changes in the duodenal mucosa. The histopathological picture of the small intestinal mucosa was described and systematized initially by Marsh et al. and then modified by Oberhuber et al. According to current classification, the histopathological changes of the small intestinal mucosa are divided into five types. In type 0 (preinfiltrative) the mucosal picture is normal and the ratio of intraepithelial lymphocytes (IEL) to enterocytes is less than 30. In type 1 (infiltrative stage), an increase in the number of intraepithelial lymphocytes (IEL) above 30 per

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100 enterocytes is observed, without other changes. In type 2 (infiltrative-hyperplastic), in addition to the increased number of IELs, intestinal crypt hyperplasia and elevated mitotic index are observed, with normal villi structure. Within type 3 (flat destructive), three subtypes are distinguished. In subtype 3a (progressive villous atrophy), in addition to the changes characteristic of type 2, there comes to mild villous atrophy. In subtype 3b (almost complete shortening of the villi), villous atrophy is clearly marked, with crypt hyperplasia and the number of IEl>30/100 enterocytes, and in 3c (complete villous atrophy) - the villi are flat, with crypt hyperplasia and the number of IEL>30/100 enterocytes. Type 4, hypoplastic-atrophic, involves complete villous atrophy with normal crypt structure and normal number of IELs [88–92].

Criteria for the Diagnosis of Celiac Disease

Knowledge on the pathogenesis of celiac disease has deepened over the last decades. As a result, the criteria for diagnosing celiac disease have changed. According to the 2012 ESPGHAN (European Society for Pediatric Gastroenterology, Hepatology and Nutrition) criteria, the diagnosis was based on the clinical picture (both typical and atypical symptoms) and intestinal villus atrophy type 2 or 3 according to the Marsh scale. Biopsy is recommended at upper G.I. endoscopy according to the following scheme - 2 samples from the duodenal bulb and 4 from the descending duodenum. In addition, detection of characteristic anti-endomysial antibodies, anti-tissue transglutaminase 2 (anti-tTG) or anti-deamidated gliadin peptides (anti-DGP) at a concentration exceeding the norm at least three times and the presence of specific histocompatibility antigens HLA-DQ2 or DQ8. However, the 2020 modification of the above guidelines places the main emphasis on the determination of IgA anti-tTG antibodies and on the determination of total IgA level. The determination of total IgA is necessary due to its frequent deficiency in CD patients. As a result, low levels of IgA anti-tTG antibodies may result from total IgA deficiency and produce a false-negative result. In the case of children and adolescents, simultaneous finding of a ten times increase in the upper limit of IgA anti-tTG antibodies with a normal level of total IgA and a typical clinical picture, allows for the diagnosis of celiac disease without the need for duodenal histology. In adults, the presence of typical histological changes is necessary for the CD diagnosis. The genetic tests assessing the presence of HLA-DQ2 and DQ8 antigens are used to exclude celiac disease in subjects observing the gluten-free diet. Neverheless, HLA-DQ2/DQ8 has a limited role in the diagnosis of CD. It's role is based on the negative predictive value in order to rule out CD in patients who are seronegative with typical histologic changes, in patients seronegative at the time of diagnosis, and in those patients with previously diagnosed CD before the introduction of celiac-specific serology.

This diagnostic criteria are also recommended by the American College of Gastroenterology [1,2].

New Diagnostic Techniques for Celiac Disease

New diagnostic techniques are still being sought to enable the diagnosis with greater precision. Recent studies indicate a higher reliability of the determination of antibodies against neo-epitope tTG complexed to gliadin (98–100% sensitivity and 93–96% specificity) in comparison with the assessment of anti-tissue transglutaminase antibodies (sensitivity: 74–100%, specificity: 78–100%) [93–97].

An additional test confirming celiac disease in the future may be the determination of the presence of T cells response to HLA-DQ2-α-gliadin complexes. The positive correlation was demonstrated between the number of gluten-reactive T cells in duodenal biopsy and the histological damage in the course of celiac disease as well as the concentration of anti-tissue transglutaminase antibodies [98]. It has been shown that three days of ingestion of gluten-containing food renders the memory T lymphocytes to be reactive against gliadin from gut-associated lymphoid tissue (GALT) and be detected in the peripheral blood of CD patients. Those antigen-specific T-cells can be detected with the enzyme-linked immunospot (ELISPOT) assays or by flow cytometry tetramer technology. Moreover, studies were conducted in T cells collected from the peripheral blood of patients for presence of the histocompatibility antigen HLA-DQ2 [99–101]. In the future, this test may become not only a new diagnostic method for celiac disease detection, but also a test to confirm the diagnosis of

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celiac disease in patients already observing a gluten-free diet, without exposing them to a long-term gluten challenge. The recent popularity of gluten-free diet self-administered without the clear indication represents the frequent challenge for clinicians, when comes to the CD confirmation or exclusion.Analysis of the presence of gluten-reactive T cells in peripheral blood can also be used to assess the adherence to the gluten-free diet [99–102]. Such a test could be helpful in diagnosing celiac disease, especially since current studies in healthy individuals with HLA-DQ 2.5+ did not show any reactivity of memory T cells specific for immunodominant gluten epitopes [103]. Although Özgenel et al., and Cecilio et al., showed an increased frequency of HLA-DQ2/DQ8 in first-degree relatives of celiac patients [104,105], their use in the primary diagnosis of celiac disease is not confirmed [106].

New genetic determinants of celiac disease are still being sought due to the significant genotypic-phenotypic divergence among individuals with HLA DQ2/DQ8 antigens. A GWAS (genome-wide association study) study conducted in 336 celiac patients from Poland demonstrated a significant association between the development of celiac disease and the presence of the MSH5 gene [107]. In the study evaluating single nucleotide polymorphisms (SNPs), 57 non-HLA variants predisposing to the development of CD were identified. In turn, within HLA, a significant predictive value was demonstrated for the presence of HLA-DQ 2.5 rs2187668, HLA-DQ7 rs4639334, DQ8 rs7454108 [108–110]. So far, few studies have been published examining the association of non-HLA genes with the risk of developing and the severity of CD.

In some patients with celiac disease, despite the gluten-free diet adherence, the intestinal villi do not recover and chronic symptoms do not subside. In order to monitor and detect a group of patients who may require more careful surveillance and introduction of additional management, it may be useful to detect patients' whole blood IL-2 release [111,112]. This relationship was confirmed by Tye-Din et al., in the study of 295 patients on a gluten-free diet who were challenged with gluten [113]. Gliadin-specific T cells found both in the G.I. tract [114–116] and selected from peripheral blood [117,118] can also be used to assess unconscious exposure to ingested gluten. Zühlke et al., demonstrated an increased expression of CD38 on gluten-specific CD4+ T cells in patients after gliadin exposure [119].

Types of Celiac Disease

According to current criteria, depending on the clinical picture and additional tests, several forms of celiac disease have been identified: classic, atypical, silent, latent (hidden), potential and refractory. Overt (classic) celiac disease is diagnosed based on the intestinal symptoms (such as chronic diarrhea, abdominal pain, vomiting, flatulence, steatorrhea), Marsh 3 villus atrophy, the presence of characteristic antibodies, and the presence of HLA-DQ 2 or DQ8. Atypical celiac disease differs from overt celiac disease by the presence of extraintestinal symptoms and the absence of classical symptoms. Silent celiac disease is asymptomatic, with the presence of anti-tTG, -EMA and -DPG antibodies and villous atrophy detected at endoscopy for other indications. Latent celiac disease is characterized by the presence of characteristic HLA, elevated levels of characteristic antibodies but without enteropathy, in individuals who had symptoms of gluten-sensitive enteropathy in the past. It should be noted that patients with latent celiac disease are at risk for villus atrophy. Potential CD affects asymptomatic subjects, without villous atrophy with high concentration of characteristic antibodies and in the presence of tissue antigens [2,120]. Refractory celiac disease (RCD) occurs when a patient, despite adherence to strict gluten-free diet for 12 months does not achieve villous regeneration [121–123]. Two forms of RCD are distinguished: type I in which in the histopathological examination activated T cells constitute up to 20% of all those visible in the preparation, and type II, when their presence is higher [124–126]. RCD accounts for 0.04–1.5% of all celiac disease cases and is mainly observed in patients diagnosed over the age of 50 [127,128]. In spite of research progress, RCD represents the important clinical challenge and it's management is difficult.

Once a correct diagnosis has been made, in accordance with the guidelines for patients with celiac disease, serologic surveillance is recommended every 3-6 months for the first year after diagnosis and then every 1-2 years. It has been considered that the lack of normalization of antibody

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levels within a period of 12 months indicates gluten contamination of consumed food or RCD.[2,123,129–135].

Celiac Disease Treatment

According to the current guidelines, the basic method of treating celiac disease is an elimination diet (i.e. excluding products that may contain gluten) [1,2], i.e. foods produced using substrates derived from wheat, rye and barley [130]. Although oats contain prolamins that are toxic to some patients, pure oats are not contraindicated in patients with celiac disease. However, its use is associated with more careful patient monitoring [136–138]. Current studies indicate that the consumption of 10 mg of gluten daily in patients with celiac disease should not cause an exacerbation of the disease, although in some cases the daily dose may be several times higher [139–142]. The applicable certification standards allow for a gluten content of 20 ppm (20 mg per kilogram of product) in gluten-free products and 100 ppm in low-gluten products [143]. In the European Union, gluten-free products are marked with the crossed-out ear of wheat symbol and in accordance with "Commission Regulation (EU) 41/2009 on the composition and labelling of foodstuffs suitable for people intolerant to gluten". The gluten content in food products marked with this logo may not exceed 20 mg per kilogram of product. Similar legal regulation was also introduced in the United States [144]. It should be noted that even trace amounts of gluten can lead to chronic inflammation in the intestinal mucosa [145,146]. However, in most cases constant adherence to gluten-free diet, especially among young patients, leads to complete recovery of the villi and resolution of the inflammatory infiltrate, despite the presence of trace amounts of gluten contamination in food [147]. In adults, especially over the age of 60, histological changes may not undergo complete remission despite strict adherence to gluten-free diet [148,149].

New Treatment Strategies

The Use of Bacteria in the Treatment of Celiac Disease

The use of endopeptidases naturally produced by bacterial strains and fungi is one of the suggested treatment methods. *Flavobacterium meningosepticum* were the first strains in which the presence of endopeptidases capable of digesting prolamine-rich protein fragments was detected. As the result of gliadin degradation with endopeptidases, fragments are formed that are non-immunogenic for celiac patients [35]. Further studies showed the presence of similar endopeptidases also in other bacteria and fungi [33,34]. Proteases were also purified from probiotic bacteria of *Lactococcus* and *Lactobacillus* [150–152]. Alpha-gliadins were reduced by more than 50% by peptidases produced by *Lactobacillus spp*. [153].

Endopeptidases are also used in the fermentation process of flour, which can be used in further stages for bakery products. Flour processed in this way does not increase the permeability of the intestinal mucosa and thus does not cause it's injury [154–157]. Further studies showed a synergistic effect of simultaneous administration of the above-mentioned probiotics with endoproteases derived from yeasts used in baking on the both gliadin and glutenin hydrolysis [158–161].

Oral Supplementation of Endopeptidases

Clinical trials are currently underway on the oral administration of endopeptidase derived from *Aspergillus Niger* (AN-PEP). Although first reports indicate an effective reduction in the frequency of immune responses, the authors indicate that the dose of enzyme necessary to digest gluten contained in food strictly depends on the type of meal and the method of its preparation and therefore effective supplementation may prove difficult [162,163].

Oral preparations of endopeptidase mixture (ALV003) obtained from *Sphingomonas capsulata* (SC-PEP) and endopeptidase from barley seeds (EP-B2) are also used in clinical trials. Studies conducted so far indicate that subjecting gluten-containing products to enzymatic treatment with ALV003 before consumption significantly reduces the immune response to prolamins contained in the meal [164–166].

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After a successful trial using 1.2 g of gluten per day for 6 weeks, which showed a significant decrease in the inflammatory response and reduction of symptoms [167], further clinical trials with Latiglutenase were initiated in a large group of patients. Research has also been initiated on a computer-modified enzyme, Kuma 030 obtained from *Alicyclobacillus sendaiensis*, which is an endopeptidase that effectively degrades the linkage between proline and glutamine (TAK-062) [168], which in preliminary studies shows high efficiency in digesting a significant amount of gluten [169].

Modification of Immune Response

Apart from the application of bacterial and fungal endopeptidases, research is still ongoing on probiotic strains that reduce the inflammatory process. Strains of the genus *Bifidobacterium spp.* are mainly used in the studies. They have been shown to reduce the level of TNF α, the number of IELs and the level of antibodies in patients with celiac disease compared to the group of patients with celiac disease who did not receive probiotics [170–173].

Systemic steroids therapy has been used for years to modify the inflammatory response. Their possible use in celiac disease, especially if resistant to diet, has been considered for many years [174]. Studies using prednisolone at a dose of 1 mg/kg bw did not demonstrate any significant effect on the villous regeneration [175]. However, administration of 9 mg of budesonide in patients with refractory celiac disease for 3 months resulted in improvement and led to the villous regeneration [176]. Numerous studies indicate the effectiveness of budesonide in patients with celiac disease not responding to gluten-free diet (NRCD) as well as refractory celiac disease (RCD) [177–179]. Azathioprine is also used in RCD. Remissions have been demonstrated in small groups of patients with this type of CD [180]. In subsequent studies, the use of azathioprine was found to be effective in type I RCD, but in some cases of type II RCD it's effect seems to be unsatosfactory [181,182].

There are very few case reports of successful use of anti-TNFα antibodies in RCD. However, so far there has been no broader analysis of such treatment. Therefore, such management should be considered non-standard and limited to selected cases [183–185].

The monoclonal anti-IL-15 antibody (AMG 714; currently PRV-015) has been evaluated in CD [186–188]. In one study the authors did not demonstrate any significant difference between the use of the preparation and placebo in terms of pathological changes of intestinal mucosa in patients exposed to gluten challenge but they did demonstrate a significant reduction in symptoms [190]. A phase 2b clinical study (NCT04424927) is currently underway in adult patients with refractory celiac disease.

In vitro studies have shown that tofacitinib, a Janus kinase inhibitor, has the potential to regulate the activity of abnormal IEL cell population. In phase 2 open-label clinical study [(EudraCT): 2018-001678-10] in patients with RCD type 2, 12-week treatment with tofacitinib led to resolution of diarrhea/loose stools, disappearance of abdominal pain and weight gain, however, the primary immunologic end point of absolute decrease in total IELs was not met and mucosal improvement as a secondary end point was observed in four of six patients. In all patients, a rapid recurrence of symptoms, including weight loss, was observed after treatment discontinuation, while the reintroduction of therapy led to a rapid and complete improvement [189].

According to current knowledge, celiac disease is a Th1-mediated autoimmune process. Attempts are being made to modulate this response by redirecting patients' immune response to Th2-mediated pathway. For this purpose, CD patients received hookworm larvae (*Necator americanus*) transcutaneously. In the studies conducted so far, in small groups, patients undergoing the procedure developed gluten tolerance without other clinical implications [191–193]. Moreover, *N. americanus* infection in gluten-challenged patients leads to an increased microbial richness by improving homeostasis, which may normalize inflammatory parameters and increase gluten tolerance [194,195].

A polymer conjugated to a deamidated gliadin peptide (KAN-101) has also been developed, which when administered intravenously, liver-targeted, is expected to induce immune tolerance to gluten [196]. The first-in-human study of KAN-101 demonstrated an acceptable safety profile in patients with celiac disease. Furthermore, KAN-101 showed the potential to induce gluten tolerance

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by blunting the inflammatory response of gliadin-specific CD4+ cells and intestinal CD8+ cells after gluten challenge (NCT04248855) [196]. KAN-101 is currently being evaluated in phase Ib/II and phase II studies (NCT05574010, NCT06001177). Another similar strategy is the use of nanoparticles being a copolymer of gluten particles and PLGA (TAK-101). Currently, the second phase of clinical studies has been completed, confirming the safety of the preparation and demonstrating the lack of immune reaction to 14-day gluten challenge - TAK-101 was well tolerated in celiac patients and no evidence of systemic immunosuppression was observed (NCT03486990 and NCT03738475) [197]. A phase II study is currently underway to investigate the efficacy and safety of TAK-101 in preventing gluten-specific T cell activation in celiac patients on a gluten-free diet (NCT04530123).

There has also been an attempt to create a vaccine (Nexvax2) designed to induce gluten tolerance by modifying the T cell response. Clinical trials have been initiated in this aspect. After vaccination, the immune response to gliadin is significantly lower than in unvaccinated patients. Studies have confirmed lower concentrations of IL-2 and INF-γ as well as significantly lower CD4+ T cells proliferation [198–201].

Zonulin Inhibitors

In celiac disease, according to the pathophysiology described above, contact of gluten with intestinal mucosa results in an increase in zonulin release. This leads to enterocyte tight junction dysfunction and increased mucosal permeability [55,202–204]. Knowledge of this pathomechanism was used to develop a protein substance (larazotide acetate) with properties that regulate tight junctions between enterocytes, modulate the intercellular tension (TEER) and inhibit the zonulin effect. This leads to a reduction in the permeability of partially digested gliadin fragments and thus reduction in the immune response. Additionally, larazotide promotes the repair of enterocyte structural defects resulting from direct reaction with gliadin [205–210]. Developed by 9 Meters Biopharma, it was investigated as an adjunctive treatment for celiac disease patients who continued to have symptoms despite adherence to gluten-free diet. The trial was discontinued in 2022 after an interim analysis explaining that additional number of patients needed to determine a significant clinical outcome between placebo and larazotide was too large to support trial continuation [210]. The therapeutic potential of larazotide acetate was assessed to be lower than expected due to the presence of both paracellular and transcellular gliadin transport pathways, whereas larazotide acetate is intended to block only the paracellular pathway.

Tissue Transglutaminase 2 Inhibitors

A number of substances have been developed as tissue transglutaminase 2 (tTG2) inhibitors [211–213]. However, studies in mice have shown that complete congenital deficiency of tissue transglutaminase 2 leads to numerous complications, such as glomerulonephritis, splenomegaly and impaired phagocytosis [214,215]. For this reason, it is impossible to introduce complete transglutaminase 2 inhibition in clinical practice. There are ongoing studies investigating the use of partial tTG2 inhibitors in patients with celiac disease [216,217]. Phase II clinical trial of a selective oral inhibitor of activated tissue transglutaminase 2, ZED 1227, has been completed. In the initial phases of the study, ZED 1227 was shown to be effective in preventing gliadin deamidation. The application of the preparation in a group of CD patients undergoing gluten challenge also brought good results, including a reduction in mucosal damage compared to the group receiving placebo [218,219]. A phase IIb trial is currently ongoing in CD patients experiencing symptoms despite the gluten-free diet (EudraCT 2020-004612-97).

Celiac disease results from a complex immune reaction to gluten. The gradually expanding knowledge about its pathogenesis enables the development of new therapeutic strategies both in patients with a mild course of the disease and in those who do not observe clinical improvement after the gluten-free diet. However, currently the only recognized treatment for celiac disease remains a gluten-free diet. The main clinical challenges are diet-refractory disease and the increased risk of small intestine neoplasia, which is particularly difficult to detect. Early small intestine cancers symptoms are not characteristic and the diagnostic methods, as MR enterography and enteroscopy

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not widely available and highly operator-dependent. Although it is possible that in the coming years new diagnostic and treatment methods will also find their application in clinical practice.

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