INTRODUCTION

Gluten intolerance is an umbrella term for gluten-related disorders. According to the new classification of gluten-related disorders, it can be distinguished into three major groups: autoimmune (mainly, Celiac Disease, CD, also known as Celiac Sprue, dermatitis herpetiformis, or gluten-sensitive ataxia), allergic (wheat allergy, WA), and non-autoimmune non-allergic (non-celiac gluten sensitivity, NCGS, or gluten sensitivity, GS). Pathogenesis and diagnostics of CD and WA are well established in contrast to NCGS, pathogenicity of which is still poorly understood and its symptoms are frequently misdiagnosed since most of the NCGS cases are currently identified via the process of CD and WA exclusion. By now, the only proven effective way for CD treatment is gluten-free diet (GFD). However, such an increasingly gaining popularity diet is apparently unsuitable for NCGS treatment because in this case gluten does not always arise as the major or exclusive culprit of gastrointestinal disorder. Furthermore, it is some physicians’ opinion that GFD can be deficient in fiber and in other vitamins and minerals. In many cases, GFD is commercially inaccessible for the most needy, whereas strict adherence to the diet is complicated by the presence of small amounts of the gluten components in some foods and even medicines. In this regard, a number of research groups and pharmaceutical companies are extensively developing alternative medicinal approaches to GFD for effective gluten intolerance treatment. This review summarizes our understanding of gluten-related disorders, possible mechanisms of gluten intolerance activation and advantages of gluten intolerance treatment using novel drug candidates obtained with a proper pharmaceutical design.

Keywords: Celiac sprue, toxic peptides, inflammation, glutenase, TG2, cytokines, zonulin, peptide vaccination.

CD is an autoimmune enteropathy caused by innate and adapted immune responses to dietary gluten peptides in HLA-DQ2/DQ8 haplotype-carrying individuals and affected approximately 1% of European and American population, whereas in the developing countries the occurrence of CD is ambiguous due to low availability of diagnostics and scant knowledge about the disease [5]. The digestion of gluten by endogenous gastrointestinal and pancreatic proteases leads to immunotoxic gluten peptides formation resulting in disruption of the mucosal structure with flattening of the villi and crypt hyperplasia of the small intestine following by nutrient malabsorption and various clinical manifestations via innate and adaptive immune response triggers [1].

WA is a negative immunologic reaction to wheat components, which includes several disorders with different mechanisms and clinical manifestations: food allergy and wheat dependent exercise induced anaphylaxis (as result of sensitization to ingested wheat via the alimentary canal), baker’s asthma (as result of sensitization to inhaled wheat flour), and contact dermatitis. The allergenic responses are categorized into IgE-mediated and non-IgE-mediated reactions. IgE antibodies play a central role in the pathogenesis of previously mentioned diseases [1, 2]. The prevalence of sensitization to wheat is assessed by IgE and varies from 0.4 to 9% (relying mostly on the available data from the USA, Germany and the UK) depending on age and countries reports [6].

In recent times, the most probable is the existence of gluten-related pathology, in which neither autoimmune nor allergic mechanisms can be identified - so-called non-celiac gluten sensitivity. NCGS is likely responsible for eczema [7], irritable bowel syndrome [8] and neurologic diseases such as schizophrenia and autism spectrum disorders [9, 10] in the cases when the gluten avoidance leads to improved clinical manifestations. However, the mechanism of action, by which gluten might induce these changes in NCGS, has insufficient evidence (in the case of psychiatric illness, it is
thought that there is perhaps impaired absorption of tryptophan, a precursor to serotonin, leading to serotonin deficiency and the presentation of mental illness) and considered as unknown [2]. In contrast to CD, for NCGS the extraintestinal symptoms are prevalent, such as behavioral changes, bone or joint pain, muscle cramps, leg numbness, weight loss and chronic fatigue (comparative data on gluten intolerance disorders are summarized in Table 1). The adequate estimation of NCGS prevalence is still impossible due to the deceitful self-diagnostics based on the lack of disease’s distinct symptoms and/or specific biomarkers.

Gluten-free diet (GFD) is considered all over the world as the only approved medical nutrition therapy for patients with CD [1]. Furthermore, GFD has gained popularity with the public but health practitioners are beginning to question its real health benefits now. However, varying degrees of symptomatic improvement with the GFD show some patients with no CD [2]. Nonetheless, patients with CD can experience comorbid nutrition deficiencies and are at a higher risk for the development of cancers and other autoimmune conditions that still was not shown for other GFD patients. A number of novel medicinal therapeutic strategies were suggested recently for the gluten intolerance strategies were suggested recently for the gluten intolerance treatment as alternatives to strict GFD or for their use in the combination with gluten-balanced diet [11, 12]. We summarize here our knowledge of gluten-related disorders, possible mechanisms of gluten intolerance activation and advantages of developing gluten intolerance medicinal treatment strategies.

PATHOGENESIS OF GLUTEN INTOLERANCE

Celiac Disease (CD)

The CD presently is a complex autoimmune disorder integrating both strong genetic (exhibiting human leukocyte antigen (HLA) Class II DQ2 and/or -DQ8 molecules on antigen-presenting cells) and extraneous (gluten consumption) indicators result in the various clinical manifestations. Certain gluten peptides reach the lamina propria by transcellular or paracellular transport and then are presented by the disease-associated HLA-DQ2/DQ8 molecules leading to stimulation of gluten-specific T cells. The important factor contributing to the CD pathogenesis is considered a human tissue

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<th>Table 1. Comparative data of Gluten Intolerance.</th>
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<td>Immune response</td>
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transglutaminase (transglutaminase-2, TG or TG2) as the highly specific endomysial autoantigen, modifying gluten peptides and enhancing their affinity to HLA molecules [15]. Gluten peptide-HLA-DQ2/DQ8 complexes induce gluten-specific T cells adaptive response with a concurrent abundant production of interferon gamma (IFN-γ), which results in immune-mediated enteropathy, characterized by villous atrophy, crypt hyperplasia, and increased infiltration by intraepithelial lymphocytes (IEL) [14, 15].

Innate immune system plays an important role in the pathogenesis of CD. In particular, some toxic gluten peptide-stimulated overproduction of interleukin (IL)-15 by epithelial cells and lamina propria dendritic cells affects the epithelial barrier by increasing the permeability through disruption of the tight junctions (TJ) [16]. IL-15 seems to be involved in inducing enterocytes apoptosis after IEL converts into natural killer-like cells, increases the expression of certain epithelial cells surface ligands, which contributes to pathological processes associated with CD, e.g. refractory celiac disease Type 2 and enteropathy associated T-cell lymphoma [17, 18]. In addition, in CD pathogenesis may be involved other proinflammatory cytokines (IL-18, IL-21, IL-27) [19]. The molecular mechanisms of proinflammatory response to gluten are currently extensively studied.

Wheat Allergy (WA)

WA is a particular case of food allergy based on the development of sensitization to wheat protein allergens, which involves the interaction of specific antibodies or sensitized T-lymphocytes with the wheat-containing food components. The factors such as genetic predisposition (atopy), abnormality of immune tolerance to allergens entering the gastro-intestinal tract, and the changes in the functional state of digestive apparatus (especially digestive barrier disorder) are closely associated with the pathogenesis of food allergy. Entering into bloodstream food (wheat) antigens can induce pathogenic mechanisms including IgE-mediated and cell-mediated allergy, which are characterized by the timing of reactions during oral challenges.

The time interval between food ingestion and the appearance of symptoms, distinguishes immediate (occur within a few hours of food ingestion) and non-immediate reactions (occurring from several hours to 1 or 2 days after food intake, and are characterized mainly by eczematous manifestations) [20].

As opposed to CD, wheat allergy is an IgE-mediated reaction to the gliadins, particularly α-5 gliadin - a major allergen of wheat-dependent exercised induced anaphylaxis [21, 22]. Wheat allergy does not cause permanent gastrointestinal or other organ damage after the acute phase passing. The main age group at risk, affected by wheat allergy is children during the early infancy or toddler years. These groups of the children usually have other food allergies, but unlike lifelong CD, WA is usually outgrown between the ages of 3 and 5 years. It is assumed that the allergenicity of wheat intensifies by TG2 and increased absorption of allergens through the gastrointestinal tract in these patients [6, 23]. The WA symptoms can be precluded with wheat avoidance and antihistamines/corticosteroids treatment. The highly allergic individuals always need to have available epinephrine to avoid anaphylactic reaction. The latter can be potentially life threatening in contrast to CD, which distinguishes the severe immunological reactions, but does not cause life-threatening anaphylaxis.

Non-Celiac Gluten Sensitivity (NCGS)

At present, the pathogenesis of NCGS is poorly recognizable but multiple mechanisms are suspected including innate immune reactions [24]. NCGS can be defined as a gluten reaction, in which both allergic and autoimmune mechanisms have been ruled out. This condition is characterized by gastrointestinal and/or extraintestinal (predominantly) symptoms that largely overlap with those of the irritable bowel syndrome (right up to the assumption is that these conditions can be synonyms). To date, it is known that NCGS is a disorder, for which the strong hereditary, malabsorption or nutritional deficiencies and increased risk for autoimmune disorders or intestinal malignancy are uncharacteristic [25, 26]. The definition of the symptoms and specific biochemical markers for NCGS is an important investigational area, since this is essential for patients’ treatment. In 2014, the 3rd International Expert Meeting on Gluten Related Disorders was held, where formulation of diagnostic protocol for the confirmation of NCGS was suggested as a result of a consensus of leading experts in the field [27].

GLUTEN AS THE CULPRIT OF GLUTEN INTOLERANCE

It is well established that gluten is the major and in many cases exclusive culprit of CD and WA. Presumably, gluten possesses a specific role in the pathogenicity of NCGS as well [1, 2].

Gluten is a complex heterogeneous mixture of storage proteins found in wheat, barley and rye, which can be divided into two main fractions based on their solubility in aqueous alcohols: the soluble gliadins (multiple α-, γ-, and ω-types according to their different primary structures) and insoluble glutenins (low- and high-molecular weight according to glutenin subunits primary structures). Some gluten protein types contain unique repetitive sequences rich in glutamine and proline [28]. Due to high glutamine content (~30%), gluten enrich in nitrogen, an essential factor for seed germination. The high proline content (~15%) causes highly resistance of gluten to degradation by gastrointestinal enzymes, making it possible for large immunogenic gluten peptides to reach the mucosal surface [29, 30]. It is generally accepted that glutenins have a prominent role in strengthening wheat dough by conferring elasticity, while gliadins contribute to the viscous properties of dough by conferring extensibility. Due to the unique visco-elastic properties, gluten is widely used in the food industry in products that are readily associated with wheat, like bread, cookies and pasta, but also as a hidden ingredient in sauces, instant soups, and even medication [31, 32]. Thus, the ubiquity of gluten makes adherence to a gluten-free diet difficult for CD patients.

Gliadin, a mixture of well-investigated Pro- and Gln-rich proteins, is a principal toxic component of wheat gluten. It undergoes incomplete enzymatic degradation during digestion, producing toxic and immunogenic peptides. The 33-mer peptide fragment of α2-gliadin (residues 57-89), which evolved most likely as a result of wheat polypliodization [33], is resistant to degradation in the gastrointestinal tract and contains six partly overlapping copies of three T cell stimulatory epitopes (PFPQPOLPY, three copies of PQPQLPYQPO and two copies of PQPQPQLPY) (Fig. 1). These immunodominant epitopes in CD are Pro- and Gln-rich sequences in gluten generating autoimmune-like disorder of the small intestine after deamidation of glutamine residues by endogenous TG2, promoting its recognition by DQ2/DQ8-HLA [29, 34, 35].

A LQLQPPFPQPOLPYQPQOLPYQPOQPQPF
B PFPQPOLPY PQPQLPYQPO QPQLPYQPO PYQPOQLPY

Fig. (1). α2-Gliadin-derived 33-mer peptide (residues 57 to 89) (A) contains six partially overlapping copies of DQ2-restricted T cell epitopes (B). Three glutamine residues, which presumably are deamidated by TG2 are underlined.
In addition to the aforementioned α-gliadin-derived 33-mer peptide and its major immunodominant epitopes, it was shown that in pathogenesis of gluten intolerance are involved several other α-, γ-, ο-gliadin and glutenin peptides containing Pro/Gln repeats [36, 37]. Some of them cause mucosal damage only, whereas the others stimulate specific HLA-restricted T-cell proliferation [38].

Specific Role of TG2 in Gluten Metabolism

TG2 is an 80-kDa highly complex multifunctional protein [39, 40]. TG2 is expressed in many different tissues and organs, and is found at intracellular as well as extracellular locations. In the extracellular environment, TG2 was shown to play a role in extracellular matrix assembly, cell adhesion and wound healing [41]. TG2 catalyzes Ca²⁺-dependent protein deamidation that results in conversion of glutamine residues of the reactive protein into glutamic acid residues. Such transformation in the protein primary structure may cause conformational and activity alterations of target proteins [42, 43], thereunto TG2 possesses substrate specificity [44].

In the pathogenesis of celiac disease TG2 attracts attention as an enzyme catalyzing deamidation of peptides derived from the wheat gluten with high content of glutamine residues, and therefore gluten components (especially gliadin and gliadin-derived peptides) are able to act as TG2 substrates [15, 45]. As mentioned above CD is strongly associated with HLA-DQ2 and HLA-DQ8 molecules [46-48], whose peptide-binding motifs prefer negative charges at anchor positions in the bound peptides [49-51]. Gluten molecules initially contain few negative charges and their additional deamidation by TG2 (Gln→Glu) enhances gliadin-specific T cell reactivity [52-55]. Thus, glutamine-rich gliadin peptides are excellent substrates for TG2. However, tissue transglutaminase selectively modifies gliadin peptides [42, 53], so the specificity of gluten deamidation by TG2 is a crucial factor in the generation of toxic gluten peptides. To verify that only particular glutamine residues are targeted by TG2, Vader et al. [56] tested a large panel of synthesized and treated with TG2 gliadin peptides. The results indicated that the sequences QXP, QXXF(Y, W, M, L, I, or V) and QXPF(Y, W, M, L, I, or V) are favorable for deamidation of Gln mediated by TG2 in contrast to QP and QXXP sequences [56]. It is assumed that the action of TG2 has a key role in the pathogenesis of celiac disease. In addition to deamidation enzymatic activity, it was demonstrated that TG2 can be found in CD patients as a target autoantigen in the immune response. Thus, a strong indication for CD is the presence of antibodies specific for the tissue transglutaminase in patients [57].

Gluten and NCGS: Pros and Cons

Despite the relative paucity of patients with precise diagnosis ‘celiac disease’ an increasing number of people with gastrointestinal problems, and/or without them (especially in the contemporary western world), become supporters of the gluten-free trend. Often NCGS was self-diagnosed based on symptoms improvement on a gluten-free diet. It was also prematurely confirmed in the double-blind, randomized, and placebo-controlled trials performed by Gibson and co-workers in 2011 [58], and this fact in some degree raised the prestige of GFD. According to the experts, a third of Americans would like to eat less gluten, and so sales of gluten-free products are estimated to hit $15 billion by 2016 (twice the rates of 2013) [59]. However, some gastroenterologists believe that not only and not so much gluten cause gastrointestinal disorders [58, 60-62]. The results of repeated trials in 2013 [61] with Gibson’s task group have shown no evidence of specific or dose-dependent effects of gluten in patients with NCGS-based diets that were low in FODMAPs (fermentable, oligo-, di-, monosaccharides, and polyols). The subjects with self-reported NCGS sequentially had a diet low in FODMAPs, a diet with added gluten (high- and low-gluten), or placebo. It transpired that each treatment diet (with or without gluten) aroused the patients similar symptoms of aggravation in gastrointestinal tract. The worsening symptoms have been observed even with placebo diet, without specific response to gluten. These data can be explained by the nocebo effect (antonym to placebo effect), i.e. patient’s gluten-free gastrointestinal distress can interpret likely psychological, non-physical cause [61]. It is well established that FODMAPs produce adverse gastrointestinal symptoms because they are not easy to digest and absorb in the small intestine resulting in discomfort in the gut [63]. In that way, even a small reduction in FODMAPs consumption can be more improved and less restrictive than GFD in some case of self-diagnosed NCGS. There is an assumption that the commercial interests, not scientific justifications, predominantly can stimulate the rise in the diagnosis of ‘non-celiac gluten sensitivity’ since current evidence of the existence of NCGS remains unconfirmed.

Notwithstanding the foregoing, it is irrational to reject the existence of NCGS as solitary disease. Large group of leading experts in the field recently developed criteria and accepted NCGS diagnostic protocol aimed to optimize clinical care avoiding self-diagnosis and clarifying the concept of NCGS [27]. Proper adherence of this protocol is also aimed to establish the role of gluten in NCGS in the future. Nonetheless, to date a few reports suggest that gluten ingestion restrictions affect the reduction of some symptoms of the disease for instance in the case of psychologic and dermatologic symptoms [64, 65].

DIAGNOSTIC OF GLUTEN INTOLERANCE

Due to improved understanding of the gluten intolerance pathomechanism, diagnostic tools become progressively advanced. These diagnostic tests can be divided into 3 stages: (i) HLA typing; (ii) specific serology; and (iii) biopsy. In the case of gluten-related disorders, it is extremely important to establish diagnosis and/or to exclude it. It is important to understand that GFD cannot be applied preventively (before diagnosis) or in doubtful cases.

First of all, CD diagnostic algorithm consists of identification of HLA-class II haplotype -DQ2 and -DQ8 [66]. Expression of these haplotypes is a necessary factor, but not sufficient to develop the enteropathy, and, therefore, measurement of antibody titers of anti-TG2 and anti-deamidated gliadin-derived peptides (IgA/IgG) is necessary. Indeed, DNA-profiles representing viable alternative to classical antibody-based techniques [67] were designed already for their use in sensitive gluten detection approaches [68].

To confirm CD diagnosis small intestinal biopsy is required [69]. Most patients with suspected CD are characterized by histological changes including an increased number of IELs, elongation of the intestine crypts, partial to total villous atrophy and a decreased villous : crypt ratio [70]. The most recent European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESP-GHAN) presented the latest edition of the Celiac Guidelines in a North American Pediatric Population with the modified criteria for diagnosis of celiac disease [71].

Primary immunologic screening for WA are skin prick tests and in vitro wheat-specific IgE assays. However, the accuracy of these tests is insufficient because frequently due to the result interpretation for the most part ambiguous owing to the cross-reactivity with grass pollens (mainly in adults), low sensitivity of commercial tests, which do not contain insoluble gliadin fraction allergens of wheat [72]. These limitations can be partially overcome using in vivo diagnosis: prick-by-prick testing with raw material and oral wheat-containing food challenge. Up to the present time, a significant number of wheat proteins and its components are examined as novel allergens with the aim of increasing the diagnostic accuracy of the in vitro IgE assays [29].

A diagnosis of NCGS due to the lack of specific clinical plausibility is based on exclusion criteria of CD and WA. Provided that diagnosis of CD and WA is ruled out, the condition of NCGS needs to be confirmed by a gluten provocation (gluten-containing diet.
after GFD period) via open and/or, more often blind tests, depending on objective (e.g. vomiting, diarrhea) and subjective (e.g. abdominal pain, nausea, headache, tiredness) symptoms [10]. The double-blind placebo-controlled food challenge has confirmed the occurrence of gluten sensitivity in only a small proportion of patients. In addition, up to half of NCGS patients produce anti-gliadin antibodies, which can be identified. However, these antibodies can be detected in various disorders as well as in healthy persons, and therefore these antibodies cannot be considered as a specific biomarker of NCGS [73]. Thus, more thorough investigation of NCGS pathogenesis is required for the detection of its specific biomarkers to set the diagnostic tools.

**GLuten INtolerance THERAPEUTIC STRATEGIES**

The number of people diagnosed with gluten intolerance tends to increase. Consequently, there is a need for appropriate therapy. Available treatment strategies are based on pathogenesis and symptomatology, the degree of functional impairment and the presence of other coexisting diseases. It is well established that if CD is left untreated, it is associated with risk of severe complications such as anemia, infertility, and intestinal lymphoma. It is likely that untreated NCGS may lead to similar consequences.

Undoubted conditions of current and developed therapy strategies should be safety, effectiveness, cost and accessibility of outcomes. Alternatives to the GFD therapies are under progress and mainly represent immunomodulatory approaches, enzymatic preparations for stimulation of gastrointestinal degradation of gluten peptides, agents for TG2 inhibition, or enhancing the tightness of the intestinal barrier to prevent the entry of gluten peptides, and development of vaccines aimed to restore gluten tolerance at alias (Tables 2 and 3). Some of candidate preparations described below are passing clinical trials and showing promising results. In order to improve the health and well-being of individuals with gluten intolerance the most appropriate in each specific case therapeutic strategy should be selected.

**MEDICAL NUTRITIONAL THERAPY**

**Gluten-Free Diet**

Lifelong GFD is the only strategy currently available for CD treatment and in several cases for NCGS treatment. Individuals with celiac disease are more susceptible to pancreatic insufficiency, dysbacteriosis, as well as accelerated bone loss due to an increase in inflammatory signaling molecules. According to some physicians’ opinions gluten-free diets can be deficient in fiber as well as other vitamins and minerals, mainly folic acid, vitamin B12, iron and vitamin D family [92]. CD patients with adherence to a GFD consume daily a large amount of products with high content of sugar, with hydrogenated fats, low fibers and protein content. Such gluten-free foods have a high glycemic load and can contribute to the occurrence of hyperinsulinemia and insulin resistance resulting in an increased risk of metabolic syndrome [93]. Therefore, to correct dietary treatment of patients with gluten intolerance there is a need for the nutrient supplementation. In addition to the above, it is necessary to mention that some CD patients treated with GFD continue to have histological abnormalities and a small number of patients develop refractory celiac disease [94]. Thus, to improve the treatment of these patients original therapeutic dietary supplements and potential alternatives to GFD are needed. At the same time, GFD is commercially inaccessible for the most needy and strict adherence to the diet is complicated by cross-contamination and/or the presence of small amounts of the gluten

Table 2. Developing enzymes for gluten intolerance medicinal treatment.

<table>
<thead>
<tr>
<th>Therapeutic agent (glutenase)</th>
<th>Predominant cleavage sites of highly immunotoxic antigen - 33-mer peptide from α2-gliadin (residues 57 to 89)</th>
<th>Refs.</th>
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<tr>
<td>EP-B2 (barley endoprotease B, isoform 2 from <em>Hordeum vulgare</em> )</td>
<td>LQ ↓ LQFPQPQ ↓ LLYPQPQ ↓ LLYPQPQ ↓ LLYPQPQPF*</td>
<td>[74]</td>
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<tr>
<td>ASP (Aspergillopepsin from <em>Aspergillus niger</em>)</td>
<td>have not been yet identified</td>
<td>[75]</td>
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<tr>
<td>Ttc (wheat protease Triticain-alpha-GM from <em>Triticum aestivum</em>)</td>
<td>LQL ↓ Q ↓ LPQPOQ ↓ LLYPQPQ ↓ LYPQPQ ↓ LLYPQPQ ↓ QP ↓ QPF</td>
<td>[76]</td>
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<tr>
<td>AN-PEP (prolyl endoprotease from <em>Aspergillus niger</em>)</td>
<td>QLQP ↓ FP ↓ QPQLP ↓ Y** QLQP ↓ FP ↓ QPQLP ↓ YQPF</td>
<td>[77, 78]</td>
</tr>
<tr>
<td>Pep-N/Pep-O/PEP (general aminopeptidase type N/endopeptidase/prolyl endopeptidyl peptidease from pooled cytoplasmic extracts of <em>L.sanfranciscensis, L.alimentarius, L.brevis, L.hilgardii, Aspergillus oryzae and Aspergillus niger</em>)</td>
<td>L ↓ Q ↓ LQ ↓ FPQPOQ ↓ LPYQPQPOQ ↓ LPYQPQPOQ ↓ LPYQPQPOQPF**</td>
<td>[79]</td>
</tr>
<tr>
<td>Enzymes from probiotic VSL#3 (mixture of lactic acid and bifidobacteria)</td>
<td>have not been yet identified***</td>
<td>[80]</td>
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</table>

* - arrows indicate predominant cleavage sites with the enzymes in mimic stomach conditions
** - selected 33-mer α2-gliadin peptides (residues 58 to 69 and 63 to 76)
*** - gluten detoxification was shown for food manufacturing procedures (sourdough fermentation)
Table 3. Developing non-enzymatic agents for gluten intolerance medicinal treatment.

<table>
<thead>
<tr>
<th>Therapeutic agent</th>
<th>Proposed Mechanism of Action</th>
<th>Refs.</th>
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<tr>
<td>Inhibitors of the zonulin (e.g., Larazotide acetate)</td>
<td>Inhibition of Zonulin (modulator of intestinal tight junctions)</td>
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<tr>
<td>Inhibitors of tissue transglutaminases (e.g., dihydroisoxazoles)</td>
<td>Inhibition of Transglutaminase 2</td>
<td>[82, 83]</td>
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<tr>
<td>Peptides that block the binding of immunogenic gliadin fragments to HLA-DQ2</td>
<td>Blocking of HLA-DQ2-mediated antigen presentation</td>
<td>[84]</td>
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<tr>
<td>Recombinant IL-10</td>
<td>Suppression of gliadin-dependent T-cell activation</td>
<td>[85]</td>
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<tr>
<td>Antibodies neutralizing IL-15 or IFN-γ</td>
<td>Neutralization of cytokine production</td>
<td>[86, 87]</td>
</tr>
<tr>
<td>Peptide vaccination (NexVax®)</td>
<td>Immune tolerance induction</td>
<td>[88, 89]</td>
</tr>
<tr>
<td>Polymeric binders (e.g., P(HEMA-co-SS))</td>
<td>Reduction of gluten exposure</td>
<td>[90, 91]</td>
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components in some foods and even medicines. Concerning the latter, specialized databases were developed that categorize the gluten status of medications, allowing clinicians to identify drugs that are safe for patients with celiac disease (for instance, the drug information service (DIS) at Robert Wood Johnson University Hospital (RWJUH) [19].

In this regard, scientists attempted to develop medicinal approaches for effective gluten intolerance treatment. Nevertheless, until GFD is efficient for patients, the strict adherence to it can prevent normalization the intestinal microflora, therupon specialists recommend to supplement the diet with probiotics and prebiotics for beneficially modifying the intestinal microflora of patients with gluten intolerance and CD particularly.

**Probiotics**

Probiotics are the viable microorganisms (mainly the certain strains of lactic acid bacteria) that are believed to provide a curative effect on the well-being of the host by improving its intestinal microbial balance. The proteolytic system of lactic acid bacteria includes, in particular, an extracellular serine protease, transport systems specific for di- and oligopeptides and a variety of intracellular peptidases. Proposed model for the proteolytic pathways can be represented by the following stages: proteases have a broad specificity and are able to yield a large number of different oligopeptides (4-8 amino acid residues); oligopeptide transport is the main supplier of nitrogen into the cell, co-operation of all intracellular located peptidases is needed for complete degradation of accumulated peptides [95].

Probiotics in the light of gluten intolerance nutrition therapy can provide food (wheat) allergy attenuation and immunomodulatory activity. It is well known that the intestinal microflora is an important constituent of the gut mucosal barrier [96], at the injury of which increases environmental antigen transport. In some cases, exposure of certain antigens may lead to allergic sensitization by means of abatement of intestinal inflammation. The study by De Angelis et al. has shown the capacity of probiotic VSL#3 (containing *Streptococcus thermophilus*, *Lactobacillus plantarum*, *L. acidophilus*, *L. casei*, *L. delbrueckii*, *L. bulgaricus*, *Bifidobacterium breve*, *B. longum* and *B. infantis*) to hydrolyze wheat flour allergens at least in non-physiological conditions [97]. Earlier, it was demonstrated that the same probiotic preparation and some other lactobacillus’ are able to hydrolyze crucial for CD gliadin polypeptides during the sourdough fermentation (i.e. cereal food processing) [80, 98]. Thus, the supplement of sourdough (i.e. lactobacillus peptidases) to wheat flour potentially can serve alternative processing of the gluten proteins in gluten-free food manufacturing. Mechanisms of gluten degradation in sourdough fermentation and limitations of sourdough technology used in preparation of foods, which is tolerated by CD patients, have been described recently in detail in the review by Cabrera-Chávez and Calderón de la Barca [99]. In addition to the gluten proteins modifications via fermentation processes, a number of other trends are under development for food processing technologies aimed to produce safe foods for CD patients [99].

Certainly, worth mentioning are some bifidobacteria that are also able to cleave gliadin peptides in physiological conditions. Namely human carcinoma Caco-2 cell line was used to show the neutralization of inflammatory effects of gliadin-derived peptides in intestinal epithelial mediated by different *Bifidobacterium* strains [100]. Thus, the beneficial alterations in the gut microbiota composition by probiotics lead to improve the quality of life of patients with gluten intolerance.

**Enzymatic Gluten Intolerance Therapeutic Strategies**

Based upon CD pathomechanism as the most studied among gluten-related diseases, detoxification of gluten components is possible if gluten is degraded into the fragments incapable to induce immune responses [101]. Some gluten Pro- and Gln-rich peptides and particularly 33-mer peptide from α2-gliadin (residues 57 to 89), are resistant to proteolysis by human gastrointestinal proteases and promote immune-mediate toxicity to the small intestine. Therefore, it has been proposed to use endopeptidases from distinct sources for effective hydrolyzation of such immunostimulatory gluten peptides. Indeed, cereal plants seem as perspective candidates for this source due to their ability to degrade gluten as one of storage proteins and due to the fact that even general functioning of plants can be supported by proteases, whose activity is distinct to human homologs [102, 103]. Thus current enzymatic strategy focuses on peptidases from germinating cereals (e.g. *Hordeum vulgare*, *Triticum aestivum* L), as well as bacteria (e.g. *Flavobacterium meningosepticum*, *Bacillus*...
Sphingomonas capsulata, Myxococcus xanthus, fungi (e.g. Aspergillus niger, Aspergillus oryzae) [74-76, 104-107], and even on post-glutamine cleaving peptidases isolated from the midgut of the stored-product pest (Tenebrio molitor) [108]. All these endoproteolytic enzymes, called glutenases, are able in varying degrees to digest and detoxify gluten peptides (Table 2).

For glutenases-based gluten intolerance treatment, it has been proposed the oral administration of an appropriately designed preparation in a therapeutic dose according to quantities of ingested gluten. To formulate the oral glutenase preparation, it is necessary to consider the factors affecting the efficiency of gluten proteolysis: activity, stability and substrate specificity of enzyme in the digestive tract. Glutenases possessing distinct pH conditions for optimal activity can be used for effective gluten peptides detoxification within different parts of the gastrointestinal tract [77, 104, 109]. Thus, for proper functioning the enzyme must be suitably protected from the unfavorable environment and activated into the intended site of action. However, bacterial prolyl endopeptidases often have limited efficiency owing to their inactivation in low pH and short-term reactivity to realize full gluten detoxification [110]. To avoid these restrictions, for instance, the prototypical enteric-coated capsule was developed containing a pharmacologically useful dose of the bacterial prolyl endopeptidases from Myxococcus xanthus, coated with the polymer, which provided sufficient resistance to gastric conditions and rapid release under duodenal conditions [109]. The commercially produced low costs fungal patented endopeptidases from Aspergillus are more effective digestive gluten than bacterial enzymes and were shown to be well-tolerated [111]. Certain advantages possess plant proteases, more precisely, enzymes from germinating cereals (wheat, barley, etc.) at the expense of their biological properties. Thus, Hartmann and co-workers demonstrated that endogenous cereal proteases expressed during the germination are capable for the extensive hydrolysis of the proline- and glutamine-rich storage proteins [112]. In particular, endoprotease EP-B2 from barley possess properties of glutenase [74]. Our recent studies demonstrated that Triticain-α, truncated cysteine protease from wheat is a promising candidate of glutenase for possible use in a novel oral therapy of gluten intolerance [76].

Due to the differences in specificity and efficiency of distinct glutenases, for better gluten digestion combined preparations consisting of several enzymes have been reported [75, 113]. This allows markedly increased hydrolytic activity against gluten. The example is the preparation of ALV003, a mix of two recombinant gluten-specific proteases with supplemental substrate specificity: modified recombinant EP-B2 from barley and prolyl endopeptidase from bacteria Sphingomonas capsulata. ALV003 was designed for an oral therapy of CD, and successfully passed phase II of clinical trials [114-116].

In addition to the possible application of glutenases in the form of medicamentous preparations, should be considered the possibility of their use as a dietary supplement. For example, already mentioned prolyl endopeptidase from Aspergillus can be successfully used as an oral dietary supplement for gluten intolerant patients to support digestion of inadvertent gluten consumption [117]. Another application of the "non-direct" oral administration of glutenase-based preparations is the treatment with glutenases of gluten-containing ingredients for cooking. De Angelis et al. demonstrated that some sourdough lactobacilli in combination with fungal proteases are capable of hydrolyzing gluten obtained from various cultivars of Triticum turgidum L. var. durum, which aimed to produce durum wheat semolina, and most likely safely can be used for diet in the cases of the gluten intolerance patients [79].

Due to the insufficient clinical data available to date on the use of glutenase preparations (such information is partially available for ALV003 only), it is too early to make the conclusions about efficacy of developed preparations in the treatment of gluten-related disorders. In particular, available biochemical data on a specific enzyme helps to select a perspective candidate for possible enzymatic preparations, but further clinical trials are only able to confirm therapeutic efficacy of the selected enzyme in the treatment of gluten intolerance.

NON-ENZYMATIC GLUTEN INTOLERANCE THERAPEUTIC STRATEGIES

Inhibitors of the Tissue Transglutaminases

Due to its important role in the pathogenesis of gluten intolerance, TG2 is an attractive target for the gluten-related disorders therapy. Thus unregulated deamidation activity of TG2 can be suppressed by the design of potent and selective inhibitors of TG2 followed by weaker gluten-derived peptides binding to HLA molecules.

Inhibition of TG2 by cystamine in vitro and in situ was confirmed by means of abolished reactivity of gliadin-specific T-cell clones and T-cell response [118]. Some dihydroisoxazole compounds were also tested for their ability to irreversibly inhibit human TG2 in mice and for one compound of a number of tested (KCC009) it was shown pharmacologically effective TG2 inhibition [119]. Inasmuch as TG2 is essential for the stabilization of the extracellular matrix, optimal TG2 inhibitor should have no side effects related to extra cellular matrix formation and wound healing, and also should have an activity limited to the gut mucosa. For example, TG2 active-site inhibitors L682777 and R283 ((2-oxopropyl)thio) imidazolium derivatives) inactivate the factor XIa and human erythrocyte transglutaminase [120, 121], and, therefore, are unsuitable for CD treatment. Recently, Keillor et al. reviewed the latest and most applicable inhibitors of TG2, and offered prospects for the future design of inhibitors on the basis of the conformational effects and crystallographic structures of inhibited TG2 [83].

Antibodies Neutralizing IL-15 or IFN-γ

As gluten intolerance, especially CD causes inflammation to the mucosa of the small intestine, immune-mediated disorders are characterized abnormal cytokine balance. It was suggested that gluten components can trigger IL-15 production with the subsequent cascade of reactions in some cases (CD for instance) leading to enterocytes damage. The function of cytokine IL-15 consists in stimulation of T cell proliferation, generation of cytotoxic T lymphocytes, stimulation of immunoglobulin synthesis by B cells, and generation and persistence of NK cells [122]. Since IL-15 is an inflammatory cytokine involved in immunological memory, it plays an important role in autoimmune diseases. Thus, strategies directed toward diminishing IL-15 action, including induction of proliferation intraepithelial CD8 cells could help to develop a treatment for gluten intolerance via design of IL-15 neutralizing agents. Yokoyama et al. reported recently the reasonableness of antibody-mediated blockade of IL-15 in transgenic mice [123]. One of developed agents is the monoclonal antibody Hu-Mik-β-1 that targets the cytokine receptor subunit IL-2/IL-15Rβ and blocks IL-15 transpresentation, which has also been used in clinical trials involving patients with autoimmune diseases, including CD [124]. Another agent being under the clinical trials is Tofacitinib consisting of Janus kinase inhibitor, which blocks IL-15 signaling in the same manner [125]. Therefore, in our knowledge the therapeutic strategy using cytokine therapies shows potential clinical benefits in the near future.

It has been demonstrated that gluten specific T cell clones secrete IFN-γ, often at high concentrations [126]. The IFN-γ overexpression may consequently cause increased TG2 expression in the upper small intestine of CD patients [127]. Therefore, antibodies neutralizing IFN-γ show some of the potential therapeutics benefits for gluten intolerance therapy. For the time being, such an example might become preparation Fontolizumab, as a humanized anti-IFN-
γ antibody, that most likely going to have significant effective for Crohn's disease treatment cases [128].

**Inhibitors of Zonulin**

The control of passing through mucosal barrier of antigenic ambient is one of the key functions of the intestine. The optimal absorption and transport of nutrients and their balance are provided by gradients created inside intestinal tight junctions (TJ). Zonulin is one of the TJ regulatory proteins, which is involved in the proper functioning of intestinal epithelial permeability at various physiological conditions, in particularly, in conditions of autoimmune diseases, including Type 1 diabetes and celiac disease [129, 130]. It was reported that gliadin affects the intestinal barrier function by releasing zonulin [131]. Lammers et al. identified the chemokines receptor CXCR3 as the target intestinal receptor for gliadin, using α-gliadin synthetic peptide library. The binding of the gliadin and/or, rather, two α-gliadin 20-mers (QVLQQSTYQLLQELCC QHLW and QQQQQQQQQQQQQILQQILQQ) to CXCR3 is crucial for the release of zonulin that subsequently increases the intestinal permeability, since CXCR3-deficient mice failed to respond to gliadin challenge in terms of zonulin release and TJ disassembly [132]. Therefore, the inhibition of zonulin overexpression can prevent trespassing gut barrier. The effective synthetic peptide inhibitor was developed and named as AT1001 or Larazotide acetate [133]. Indeed, a number of randomized, placebo-controlled studies confirmed that Larazotide acetate improved of symptoms in CD patients [134-136]. These observations enable to consider Larazotide acetate as a novel therapeutic agent targeting TJ regulation in patients with CD.

**Peptide Vaccination**

Immunological hypersensitivity appeared to be a hallmark of the allergic syndromes and autoimmune diseases. Current treatment strategies are in most cases aimed to suppress symptoms or called the allergic syndromes and autoimmune diseases. Current treatment of immunological hypersensitivity appeared to be a hallmark of Peptide Vaccination patients with CD. Currently Nexvax2® is passing clinical trials for the release of zonulin [131]. Lammers et al. reported that gliadin affects the intestinal barrier function by releasing zonulin [131]. Lammers et al. identified the chemokines receptor CXCR3 as the target intestinal receptor for gliadin, using α-gliadin synthetic peptide library. The binding of the gliadin and/or, rather, two α-gliadin 20-mers (QVLQQSTYQLLQELCC QHLW and QQQQQQQQQQQQQILQQILQQ) to CXCR3 is crucial for the release of zonulin that subsequently increases the intestinal permeability, since CXCR3-deficient mice failed to respond to gliadin challenge in terms of zonulin release and TJ disassembly [132]. Therefore, the inhibition of zonulin overexpression can prevent trespassing gut barrier. The effective synthetic peptide inhibitor was developed and named as AT1001 or Larazotide acetate [133]. Indeed, a number of randomized, placebo-controlled studies confirmed that Larazotide acetate improved of symptoms in CD patients [134-136]. These observations enable to consider Larazotide acetate as a novel therapeutic agent targeting TJ regulation in patients with CD.

**CONCLUSION**

The number of people diagnosed with gluten intolerance tends to increase. However, nowadays physicians are unable to suggest any significant treatment options regarding gluten-related disorders. On the other hand, recent numerous studies on the pathogenic mechanisms of diverse types of gluten intolerance resulted in the discovery of the developmental pathways of these disorders, which can be blocked. Thus a number of research groups are focusing on the extensive development of alternatives to GDF medicinal ap-proaches, which can be effective in gluten intolerance treatment. We suppose that physicians will get a serious arsenal of new medicaments for the treatment of gluten-related disorders in the near future possessing distinct mechanisms of action making these drugs able to block different stages of the gluten-related disorders via the distinct pathogenic mechanisms. Therefore, we are confident that this new spectrum of the medications will be a perfect tool for the development of personalized strategies in the treatment of patients with diverse types of gluten intolerance.

**ABBREVIATIONS**

- CD = celiac disease
- ESPGHAN = European Society of Pediatric Gastroenterology, Hepatology and Nutrition
- FODMAP = fermentable, oligo-, di-, monosaccharides, and polyols
- GFD = gluten-free diet
- GS = gluten sensitivity
- HLA = human leukocyte antigen
- IEL = intraepithelial lymphocytes
- IFN-γ = interferon-gamma
- IL = interleukin
- NCGS = non-celiac gluten sensitivity
- TG2 = transglutaminase 2 also known as tissue transglutaminase, tTG
- TJ = tight junctions
- WA = wheat allergy

**CONFLICT OF INTEREST**

The authors confirm that this article content has no conflict of interest.

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