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Celiac Disease: Disease Models in Understanding Pathogenesis and Search for Therapy

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Abstract

Celiac disease is a complex polygenic systemic disorder caused by dietary gluten exposure that selectively occurs in genetically susceptible people. The potential celiac disease is defined by the presence of celiac disease-specific antibodies and compatible human leukocyte antigen but without histological abnormalities in duodenal biopsies. At present, the only treatment is lifelong adherence to a gluten-free diet. Despite its effectiveness, the diet is difficult to maintain due to its cost, availability of gluten-free foods, and hidden gluten. The need to develop non-dietary treatment methods is widely recognized, but this is prevented by the absence of a pathophysiologically relevant preclinical model. Nonetheless, *in vitro* and *in vivo* models have made it possible to investigate the mechanisms of the disease and develop new treatment approaches: The use of foods with neutralized gluten, microbiota correction, cocktails of specific endoproteinase, polymer gluten binders, specific inhibitors of transglutaminases and inflammatory cytokines, and a vaccine based on allergen-specific therapy.

Introduction

Celiac disease (gluten enteropathy) is a chronic autoimmune disease caused by permanent intolerance to the gluten in genetically predisposed individuals. The mucosal changes in celiac disease (CD) may vary in severity from increased number of intraepithelial lymphocytes (IELs) (Marsh Type 1) to Type 4 or hypoplastic lesion (total villous atrophy with crypt hypoplasia) [1], [2].

Celiac disease is a disease with well-studied pathogenesis, thanks to active research work. The cause of celiac disease was determined in the middle of the 20th century in the works of Dicke who revealed a decrease in the symptoms of celiac disease in children amid starvation during the Second World War and the recurrence of the disease in them in the post-war period with the return to the traditional diet and consumption of flour-based products from cereal plants (wheat, rye), as well as Ch. Anderson who showed the link between gluten/gliadin and celiac disease. In 1952, McIver and French was the first to successfully use a gluten-free diet to treat gluten-dependent enteropathy. In 1954, the first endoscopic biopsy of the small intestine was performed (Paulley *et al*.), and in 1956, the histological picture of celiac disease was determined (Shiner). In 1989 (Sollid *et al*.), an association of celiac disease with antigens of the human leukocyte antigen (HLA) system was discovered, and in 1997 (Schuppan *et al*.), the main antigen of the disease – tissue transglutaminase – was identified.

When celiac disease is diagnosed, a strict gluten-free diet is prescribed. Even short-term consumption of food rich in gliadin gives rapid immune response and leads to a serious exacerbation of the disease. The main principles of a gluten-free diet: Exclusion of foods containing gluten as well as foods that enhance fermentative and putrefactive processes; a full-value diet with a high content of protein, calcium salts, and Vitamin D; limitation of mechanical and chemical irritants of the intestinal mucosa; correction of other metabolic disorders associated with malabsorption (anemia, impaired water, and electrolyte balance, polyhypovitaminosis); correction of dysbiosis; prescription of cytoprotectors; and absorbents.

The celiac disease leads to a wide range of gastrointestinal and extraintestinal manifestations. Classic gastrointestinal symptoms include diarrhea, abdominal pain, and malabsorption. The pathophysiology includes abnormal liver enzyme levels, arthritis, dermatitis herpetiformis, headache, anemia, muscle pain, depression, rash, short stature, delayed puberty, psychiatric disorders, osteoporosis, and infertility [3], [4], [5].

The article describes the developed models of celiac disease as well as the proposed treatment strategies.

Genetic Predisposition, Role of Gluten

HLA is the main genetic determinant of the disease. HLA alleles are located in the MHC II on the short arm of chromosome 6 (6p) [6]. HLA DQ encodes α- and β-chains that form the DQ αβ-heterodimeric protein, which is located on the surface of specialized antigen-presenting cells and contributes to the interaction of gluten peptides with T cells [5], [7].

An association has been established between the level of wheat consumption and the frequency of HLA-DQ2/-DQ8 alleles worldwide [8], [9], [8]. High positive correlation (r ¼ 0.88, $p < 0.05$) between wheat consumption and the prevalence of CD (biopsy proved) was found [10].

Among the molecules that determine the predisposition to this disease, in the European population, 95% have the DQ2 heterodimer and 5% have DQ8. However, only 3% of carriers of these alleles suffer from celiac disease. The presence of DQ2/DQ8 molecules is an essential but not sufficient condition to develop the disease. On average, 96.2% of patients are carriers of HLA-DQ2/DQ8 isoforms, but the likelihood of disease in people negative for both of these alleles is extremely low [11].

Normally, gluten is transported in the lamina propria in the presence of regulatory Foxp3+ T cells. Moreover, IELs express inhibitory natural killer CD94/NKG2A receptors that prevent uncontrolled T-cell activation [12]. In celiac disease, HLA-DQ2 or -DQ8 proteins bind gluten on APC, which leads to a T-cell response and release of pro-inflammatory cytokines (IFN-γ, TNF-α, IL18, and IL21), and that lead to the start of B-cell response with the formation of anti-gluten antibodies. Immunological markers of celiac disease are autoantibodies to the gliadin, to the deaminated forms of gliadin peptides, reticulin, endomysial, to tissue transglutaminase [13]. It has been shown on cell cultures of human intestinal explants that IFN-γ activates macrophages that secrete TNF-α and matrix metalloproteinases (MMPs) that damage enterocytes and tight junctions. In intestinal myofibroblasts, TNF-α and IFN-γ stimulate the expression of proteolytic MMP-1 and MMP-3. This release and activation of MMP induce extracellular matrix proteolysis, which leads to the change in intestine structure seen in celiac disease. MMP-3 plays a dominant role in tissue remodeling, as it is involved in the destruction of various non-collagen matrix components and activates MMP-1, the main protease that destroys collagen fibrils. Increased expression of interleukin-15 (IL-15) and IFN-α in the lamina propria causes dendritic cells to acquire a pro-inflammatory phenotype [12], [14], [15].

Gluten, Gliadin, and Its Peptides

Gluten is a storage protein in the seeds of cereal plants, consisting of prolamins and glutelin subcomponents. About 90% of the protein contained in wheat flour is gluten, which includes gliadins, a heterogeneous mixture of proteins consisting of four main groups: α-, β-, γ-, and ω- [16]. The heterogeneity of the wheat gluten proteins is high, so no surprise that many distinct gliadin and glutenin-derived T-cell epitopes exist [17].

Most gliadin peptides are digested by gastrointestinal proteases, but two remain non-cleaved - 33-mer: LQLQPFPQPQLPYPQPQLPYPQPQLPYPQPQPF (p57–89) and 25-mer: LGQQQPFPPQQPY (p31–43) [1]. The peptides are resistant to proteolytic digestion due to their high content of proline [18], and the presence of the amino acid glutamine makes the peptides excellent substrates for tissue transglutaminase-2 (TG-2). TG-2 catalyzes the deamidation of amino acids which lead to the replacement of glutamine by negatively charged glutamic acid in protein molecules [19], [20]. The transformation of gliadin peptides by tissue transglutaminase leads to the intense affinity of gliadin peptides for the binding sites of DQ2 and DQ8 molecules [21].

The p57–89 peptide, deamidated by tissue transglutaminase, binds to HLA DQ2/DQ8 and induces an adaptive Th1 pro-inflammatory response. The p31–43 peptide is not recognized by T cells in the abdomen and can damage the intestinal mucosa *in vitro* and *in vivo*. The p31–43 peptide is also capable of initiating an innate immune response with IL-15 as the main mediator [1].

An important phase in the pathogenesis of the celiac disease is the penetration of gliadin peptides into the lamina propria, where gluten reactive T cells are located. There are two ways: Transcellular and paracellular. In a transcellular way, intestinal lumen sIgA binds to gliadin, receptor CD71, and the enterocyte. Then, it is transcytosis, delivering peptides to the lamina propria [22]. The second way involves the destruction of tight junctions of epithelial cells, the main protein of which is zonulin. Zonulin reversibly regulates intestinal permeability to gliadin peptides. Bacteria and gliadin are considered to be the main triggers of zonulin release [23]. Toxic gliadin peptides, by damaging zonulin-dependent structures, reducing F-actin content in enterocytes, inhibiting epithelial cell growth, and stimulating apoptosis of enterocytes, disrupt tight intercellular junctions, disruption, and

reorganizing the enterocyte cytoskeleton, thereby increasing the intercellular permeability of the small intestine mucosa [22], [23].

Gliadin binds to the CXCR3 chemokine receptor on epithelial cells. This interaction recruits an adapter protein, myeloid differentiation factor 88. Subsequently, the activated myeloid differentiation factor 88 induces zonulin release into the lumen. The released zonulin binds to the epidermal growth factor receptor and protease-activated receptor 2 in the intestinal epithelium. This complex initiates a signaling pathway that leads to the phosphorylation of zonula occludens proteins and the destruction of the tight junction of small intestine cells [24]. In genetically susceptible individuals, gliadin can recruit and stimulate other CXCR3-expressing cells, which lead to an inflammatory reaction [24].

Sollid *et al.*, 2012, had published a nomenclature and listing of celiac disease-relevant gluten T-cell epitopes restricted by HLA-DQ molecules. The names of the epitopes contain gene loci and short terms the proteins of origin (α-gliadin, γ-gliadin, ω-gliadin, low-molecular-weight glutenin, high-molecular-weight glutenin, hordein, scaling, and avenin). According to this nomenclature, it was found 23 variants of DQ2.5 restricted epitopes; one DQ2.2 restricted epitope; four variants of DQ8 restricted epitopes, and three variants of DQ8.5 restricted epitopes [17]. In the 2020 year, the authors published an update of this article and the total number of epitopes increased on the nine variants for different genes [25].

IL-15 in Disease Development

IL-15 is a pleiotropic cytokine that can stimulate inflammation through several pathways and regulate the accumulation of IELs, disrupting T_{regs} inhibitory activity and TGF-β signaling, as well as allowing activation of disease-specific CD4+ T cells [26].

Increased production of IL-15 and apoptosis of enterocytes were registered when examining duodenal biopsy samples from patients with disease incubated with the p31–43 peptide [27]. In C57BL/6 mice, in intestinal tissues, the p31–43 peptide-induced release of zonulin and pro-inflammatory cytokines, including IL-15, under the action of gliadin-stimulated macrophages [28].

In healthy individuals, normal CD8$\alpha\beta$+TCR$\alpha\beta$+ epithelial lymphocytes express inhibitory NK cell receptors, including CD94/NKG2A [29], whereas in patients with celiac disease elevated levels of IL-15 enhance the cytolytic activity of IELs. They are NKG2D and CD94/NKG2C molecules on the surface of lymphocytes, which bind to MHC I and are a target for natural killer NK receptors. Finally, IL-15 activation results in innate cytotoxic destruction of small intestinal epithelial cells, which enhance intestinal permeability to various lumen macromolecules, including immunogenic gluten peptides [30].

Microbiota

It is now recognized that the intestinal microbiota has a profound impact on human health participating in the regulation of metabolic functions and immune homeostasis. This balanced micro-ecosystem provides the host with a natural defense against the invasion of potential pathogens. Environmental factors can disrupt intestinal homeostasis which can lead to pro-inflammatory responses to harmless food antigens and the development of chronic inflammation [31], [32]. A lot of studies associate problems with intestinal microbiota with impaired immune homeostasis and the risk of developing immune-mediated diseases [31], [32]. For example, the prevalence of IgA deficiency in patients with CD is 10–15 times higher than that in the general population [33].

The microbiota can influence the pathogenesis of disease in different ways: By modulating the digestion of gluten peptides, generating both toxic and/or tolerogenic peptides, which can influence the acquisition of dietary tolerance to the antigen; affecting intestinal permeability through the release of zonulin and the production of tight junction proteins; promoting the maturation of the mucosa; and regulating the activity of the immune system through the expression of cytokines and pro-inflammatory or anti-inflammatory peptides [34].

Short-chain fatty acids produced by the microbiota contribute to epithelial cell proliferation through the activation of G-linked protein receptors and can influence the immune system of the intestine through their histone deacetylase inhibiting properties [34].

In a study by Rossi *et al*. showed that enzymatic transamidation of gliadins by microbial transglutaminase specifically reduces the Th1 immune response in the small intestine of sensitized HLA-DQ8 transgenic mice. Analysis of the interaction of peptides with HLA-DQ8 showed that peptides p13 and p23 in the form of glutamate can interact through salt bridges with specific amino acids of the HLA-DQ8 alpha chain, suppressing a specific immune response [35].

A lot of studies have shown that patients with celiac disease have a decrease in the number of *Lactobacillus* and *Bifidobacterium* and an increasing number of *Bacteroides, Escherichia coli, Proteobacteria,* and *Staphylococcus* (potentially pathogenic bacteria) compared to healthy subjects [36], [37], [38]. In the same time in recent systematic review, fecal and

duodenal microbiota in pediatric CD patients was investigated. No clear celiac signature was identified in this review [39].

In the last few years, the salivary microbiome has started to attract the interest due to its potential role in the disease pathogenesis. Salivary microbiome could correlate better with the duodenal bacterial environment compared to fecal microbiota [40].

Caminero *et al*. have shown that opportunistic pathogens, such as *Pseudomonas aeruginosa,* produce proteases that can degrade undigested gluten to the peptides that permeate better through the barrier and stimulate human gluten-specific T cells [41]. In the last article, authors show that small intestinal microbiota influences CD pathogenesis [42], [43].

In animal models, one can radically change the intestinal microbiota by introducing probiotics, pathogens, and genetically modified microorganisms, as well as by keeping animals in gnotobiotic conditions that will destroy the entire microbiota [44]. All these approaches have been used to study the influence of intestinal microbiota on the development of gluten sensitivity. Switching humanized gnotobiotic mice from a low-fat diet rich in plant polysaccharides to a Western-type (high in fat/sugars) diet significantly altered the composition of the transplanted human microbiome, with notable increases in the *Erysipelotrichia* and *Bacilli* classes [44].

Another study looked at changes in commensal microbiota composition in DQ8 transgenic mice after the administration of dietary gluten with or without indomethacin. They found that *E. coli* and *E. rectal-Clostridium* groups were reduced in gluten-sensitized mice and that all bacterial groups assessed (*Prevotella, E. coli, Clostridium leptum, E. rectal,* and *Clostridium histolyticum*) were significantly reduced with the administration of both indomethacin and gliadin to DQ8 transgenic mice. Experiments using non-obese DQ8 diabetic transgenic mice are proof of the concept that the microbiota generates gluten-associated immune-mediated mucosal damage. Under microbial-free conditions, mice develop more aggressive gluten-induced pathology compared to mice colonized with altered Schedler's flora (benign microbiota) devoid of opportunistic pathogens. However, in the presence of microbiota with opportunistic pathogens or in the event of disorders secondary to the use of antibiotics, mice develop severe pathology caused by gluten. These findings reinforce the role of the intestinal microbiota in the inflammatory response associated with gluten consumption [45].

Papista *et al*. showed in the gluten-sensitive mouse model that *Saccharomyces boulardii* KK1 strain hydrolyzed toxic 28 kDa α-gliadin peptides and its consumption was accompanied by an improvement in enteropathy and a decrease in histological damage and production of pro-inflammatory cytokines [46].

Laparra *et al*. studied the effect of *B. longum* CECT 7347 in neonatal rats treated with gliadin alone or treated with gliadin and sensitized with IFN-γ [47]. Results indicate that administration of *B. longum* CECT 7347 leads to a reduction of CD4+ and CD4+/Foxp3+ cell populations and an increasing number of CD8+ T-cell populations, an increasing number of NFκB and IL-10 expression but the reduction of TNF-α production.

Disease Models

The goal of research for any disease is to find an effective treatment method [48]. There is an active search for therapeutic agents that can help to escape from or supplement a gluten-free diet to reduce the side effects associated with random exposure to gluten. The need to develop non-dietary treatment methods is now widely recognized, but this is prevented by the absence of a pathophysiologically relevant preclinical model dependent on gluten and HLA. To understand the complex pathophysiology, *in vitro* and *in vivo* models of the disease have been developed, which are of prime importance for testing new treatment methods. The main elements of celiac disease pathogenesis are DQ2 or DQ8 variants of the HLA-DQ gene, small intestinal villous atrophy, high levels of IL-15 expression, crypt atrophy, and infiltration of T cells and macrophages. The perfect model should reflect all the main elements of pathogenesis.

In vitro Systems of Celiac Disease

One of the *in vitro* models of celiac disease is the epithelial layer, which includes the Caco-2 and IEC-6 epithelial cell lines. The effect of gluten on these cells leads to rearrangement of the cytoskeleton, destruction of the integrity of tight junctions, and increased expression of inflammatory cytokines. It was shown that a peptide p31–p43 induces IL-15 expression by Caco-2 cell surface cells and thereby stimulates T-cell proliferation [49]. In contrast, the p57–68 peptide does not induce cell surface expression of IL15 by Caco-2 cells but activates T cells when presented in the context of DQ2. This difference is associated with the property of p31–43 to disrupt the delivery of endocytic vesicles to epithelial cells. Only p31–43 peptide (but not p57–68) induces expression of TG2 by Caco-2 cells [49].

Lindfors *et al*. showed that *Bifidobacterium lactis* reduces the toxicity of gluten peptides on Caco-2 [50]. They found that *B. lactis* exerted a protective effect on epithelial cells against cellular damage induced by gliadin incubation. Furthermore, it was observed that the addition of 106 and 107 CFU/ml, but not 105 CFU/ml, of *B. lactis,* was able to

preserve tight junctions in comparison to epithelial cells maintained in the presence of gliadin alone.

De Palma *et al*. studied the role of enterobacteria (*E. coli* CBL2 and *Shigella* CBD8), bifidobacteria (*Bifidobacterium longum* CECT 7347 and *Bifidobacterium bifidum* CECT 7365), and gliadins in the phenotypic and functional features of MDDC (monocyte-derived dendritic cells) cell culture and coculture with Caco-2 cells [51]. Results show that microbiota may influence MDDC: Induction of production of inflammatory cytokines and morphological changes.

Mixtures of antigen-presenting cells with T cells of intestinal origin from patients with celiac disease have been widely used to model the presentation of gluten epitopes. There have been identified specific alleles of DQ2 and DQ8 that are capable of presenting gliadin derivatives, as well as specific gliadin derivatives that are presented by these alleles [52], [53]. The antigen-presenting cell/T-cell model provides important data on adaptive immune reactions to gluten, which can later be used to select personalized therapy.

In vitro intestine-on-chip systems of celiac disease can now be developed. Human-induced pluripotent stem cells are the basis of these systems and can be generated from both diseased and healthy individuals, which means that they can be stratified based on their load of genetic risk factors. The first human intestinal organoids were grown from intestinal crypts derived from human biopsy material [54]. When cultured in an extracellular matrix gel in the presence of specific growth factors, it is possible to maintain the stem cell niche and the proliferative and differentiation capacity of crypt cells *in vitro*, allowing them to grow out into complex three-dimensional "budding" structures. The closed configuration of human intestinal organoids renders them less ideal for studying transport over the intestinal barrier or interactions with commensal microbes or pathogens. Organ-on-chip systems are microfluidic devices in which cells are cultured in continuously perfused microchannels engineered to mimic the physical microenvironment of tissues and organs [55]. Mechanical forces induce epithelial cells to spontaneously form polarized 3D villus-like structures that contain cells expressing markers characteristic of differentiated IECs.

Combining human-induced pluripotent stem cells and human intestinal organoid technology, *in vitro* models of the intestine can be created from cells that contain the spectrum of celiac disease-associated genetic risk factors. A celiac disease-on-chip model system has great potential to improve our understanding of disease etiology and accelerate the development of novel treatments and preventive therapies for celiac disease and other complex diseases [56].

At the moment, cell culture after intestinal biopsy is the best *in vitro* model because of the presence of different cell types and intercellular interactions. The disadvantage of this model is the absence of hormonal and neurological signals delivered by other organs or cellular systems. To ensure such signals are not overlooked, animal models of celiac disease have been developed. They in particular play an important role in understanding complex immunological reactions and pathophysiological mechanisms.

Information about *in vitro* models is summarized in Table 1.

In vivo models of celiac disease

Animal models with spontaneously occurring disease

Spontaneous animal models are models that do not require sensibilization to develop a disease. The majority of studies on spontaneous animal models have been done on dog and monkey models.

The first animal model for celiac disease was the Irish Setter, as studies conducted in the 1980s showed that when it was switched to a diet containing wheat at an early age, it developed partial villous atrophy and IELs infiltration [57], [58]. Nevertheless, this gluten-dependent enteropathy was identified in later studies as independent of canine MHC II and, therefore, was not a CD4+ T-cell-mediated disease [59].

In a study by Bethune *et al*., rhesus monkeys developed pathology in response to gluten, but, there is no association of the disease with MHC II [60]. Interestingly, one of the rhesus monkeys developed dermatitis, similar to the skin manifestation of celiac

Table 1: *In vitro* models of celiac disease

No.	Name of model	Description of model	Results	References
1	Epithelial layer	Caco-2 + IEC-6 epithelial cell lines	Gluten on these cells leads to rearrangement of the cytoskeleton, destruction of the integrity of tight junctions, and increased expression of inflammatory cytokines	[49]
2	Cell culture	Caco-2 cells	*Bifidobacterium lactis* protects tight junctions	[50]
3	Cell co-cultures	MDDC + Caco-2 cells	*Escherichia coli CBL2, Shigella CBD8* induced pathogenic effects: morphological changes, increased attachment/spreading ability, induction production of IFN-γ *B. longum* CECT 7347 reduced IFN- γ production; reversed the gliadin-reduced zonula occludens protein 1 expression in Caco-2 cells	[51]
4	Antigen-presenting cell/T cell model	CD4 + T cells from small intestinal biopsies of CD patients	Gliadin was presented by HLA-DQ2 receptors. Gliadin reactive T cells were found in the intestinal mucosa of CD patients but not in biopsies of control patients.	[51]
5	Organoid culture	Long-term culture of the human small intestine and colon tissues.	Developed protocols allow a robust and long-term culture of primary human epithelial cells isolated from the small intestine, colon, adenoma (carcino) mas, and Barrett's esophagus.	[53]
6	Organoid-on-chip system	Microfluidic devices where cells are cultured in continuously perfused microchannels engineered to mimic the physical microenvironment of tissues and organs.	Mechanical forces induce epithelial cells to spontaneously form polarized 3D villus-like structures that contain cells expressing markers characteristic of differentiated IECs.	[55]

CD: Celiac disease, INF: Interferon.

disease. This monkey spontaneously developed antibodies specific for epidermal transglutaminase and TG, and dermatitis resolved after the introduction of a gluten-free diet [61].

Transgenic models

Transgenic and knockout mice were created to investigate the pathways of celiac disease. Mice that express the HLA-DQ2/-DQ8 genes are most commonly used. These mice have demonstrated that HLA DQ2/DQ8 molecules can promote a powerful inflammatory T-cell response to gliadin, but this fact alone is not sufficient for gluten enteropathy development characterized by villus atrophy.

Induced disease in transgenic mouse models can be used to better understand the environmental factors that break tolerance. The mechanisms by which bacteria influence the pathogenesis of celiac disease can be studied by manipulating the composition of the microbiota or exposing transgenic mouse models to certain bacteria. For example, in mice expressing human HLA-DQ8, the composition of microbiota influences the degree of gluten-induced immunopathology. Protection against gluten-induced immunopathology in pure SPF mice was lost after the addition of a center adhesive strain of *E. coli* isolated from a patient with the disease. Similarly, antibiotic treatment leading to the growth of *Proteobacteria* resulted in more severe gluten-induced pathology in normal SPF mice [62].

Among transgenic models, mice with increased expression of human IL-15 under the control of an enterocyte-specific promoter are of the greatest interest. Such mice have an increased number of CD8+ cells in the small intestine and develop antibodies to TG2 (IgA) [63], [64]. In another study, mice were crossbred with DQ8 transgenic mice. As a result, IL-15 DQ8 transgenic mice developed an increased number of CD3 and IELs in response to gliadin feeding [65].

A recent study by Abadie *et al*. describes the DQ8-Dd-villin-IL-15 transgenic mouse model of CD [66]. This model represents the first animal model that develops villous atrophy in an HLA- and gluten-dependent manner without administration of any adjuvant. When creating this model, we used the assumption that tissue destruction in CD requires not only an antigen-specific CD4 T-cell response but also the expression of stress signals by the target tissue, which are necessary for the start of tissue destruction by cytotoxic T cells.

Induced models

A study in rats found that coadministration of a *Shigella* or *E. coli* inoculum with gliadin into intestinal loops led to increased tight junction disruption and transport of gliadin peptides into the lamina propria. These results confirm the theory that the intestinal microbiome plays an important role in the development of celiac disease [67]. In a neonatal rat model sensitized with IFN-γ and orally administered gliadin, *B. Longum* CECT 7347 reduced TNF-α and increased IL-10 in intestinal tissue samples [47].

There are also mouse models where enteropathy is induced. In one mouse model by Freitag *et al*. [68], induction was achieved by transferring CD4+CD25-CD45R Blow cells from gliadin-sensitized mice to recipients deficient in T and B cells (Rag 1-/-) [68]. Recipient mice gained less weight and experienced severe duodenitis after gluten feeding compared to recipients on a gluten-free diet or compared to control mice. During the process of feeding with gliadin, the histological features of the mucous membrane deteriorated, which is characteristic of celiac disease.

Another model included Balb/c mice that were raised on a gluten-free diet for three generations. The resulting offspring from 10 weeks of age were fed gluten-containing food for 30 days. In these mice, the number of infiltrating CD3 + IELs increased, and they developed villous atrophy and intestinal crypt hyperplasia [47].

Araya *et al*. in his induced model used 8-week-old male C57BL/6 mice, which were injected with 25-mer (p31–43) gliadin peptide into the intestinal lumen to avoid digestion by pancreatic enzymes and slaughtered 72 h/l. The administration of the peptide led to pathological changes: There were observed stable edema and cell infiltration in the lamina propria, an increased number of IELs, and a higher histological index compared to control mice [69].

A recent study by Vijaykrishnaraj *et al*. speaks of the preferred use of BALB/c mouse models over transgenic mice for this kind of research to better understand the connection between innate and humoral immune responses [70]. To address this issue, this study was designed to develop gluten sensitivity in BALB/c mice through the intraperitoneal and intragastric routes of administration over an extended period to imitate gluten-associated inflammatory diseases.

To study the effects of gliadin administration, germ-free Wistar rats were used immediately after birth and up to 63 days of age [71]. This resulted in villus shortening, crypt hyperplasia, and an increased number of intestinal lymphocytes. Further supporting the achievement of gluten sensitivity, transplantation of IELs from gliadin-treated rats into intestinal loops caused pathology in naive recipients and showed that transplantation of IELs contributed to the development of enteropathy. Continuing this work, the same group determined that commensal bacteria affect the ability of gliadin to induce intestinal permeability [67]. In particular, they discovered that administration of gliadin and IFN-γ with *B. bifidum* into intestinal loops resulted in only a small concentration of gliadin in the lamina propria

(translocation), but when *Shigella* was administered with gliadin and IFN-γ, they found a large amount of translocated gliadin. This study demonstrated that the composition of commensal bacteria influences the degree of gliadin-induced intestinal permeabilization in an MHC II-independent manner.

Even though there is no ideal model for celiac disease, the use of *in vitro* and *in vivo* model systems makes it possible to explore new therapies for the disease in the context of individual manifestations of the disease. This approach is useful for screening new enteropathy treatment strategies but does not give a 100% result when transferring research results to humans. Information about *in vivo* models is summarized in Table 2.

Approaches to Celiac Disease Therapy

The main source of immunogenic gluten is wheat. For a gluten-free diet, there have been seeking various alternatives to wheat and ways to modify wheat making it less toxic for patients with celiac disease.

In animal models and cell cultures, the demonstrations of celiac disease were studied in the presentation of cereals alternative to wheat: Sorghum and quinoa. *In vitro* studies of sorghum did not show an inflammatory reaction. A 5-day test in two patients with celiac disease did not reveal gastrointestinal symptoms or serological changes [47]. Incubation with cell culture after an intestinal biopsy from patients with celiac disease on a gluten-free diet with prolamins from quinoa cultivars showed that four out of 15 cultivars had gluten-toxic epitopes with values within the range considered acceptable for gluten-free labeling. However, two cultivars had epitopes capable of stimulating adaptive and innate immune responses [72].

Creation of gluten-free wheat

The development of gluten-free wheat might seem to be a good therapeutic path; however, it is a challenging task as gluten plays a key role in determining the structure of bread. Wheat species and varieties differ significantly in levels of T-cell-stimulating gluten epitopes [73].

C173 is an experimental line of wheat with spontaneous deletions of several gliadins and glutenin. Incubation mucosal biopsy samples from patients on the gluten-free diet and untreated patients with C173 did not reduce the villus-to-crypt ratio but were associated with increased levels of IFN-gamma, IL-2, IL-10, and anti-tTg antibodies [74]. The absence of histological changes suggests that this wheat is less toxic; however, the production of inflammatory cytokines sparks great concern. After long-lasting exposure to C173, histological changes may develop.

Another mechanism of wheat toxicity reduction is fermentation using combinations of fungal and lactobacilli proteases for the complete hydrolysis of

Table 2: *In vivo* models of celiac disease

No.	Animal	Symptoms	Comments	References
Animal models with Spontaneously occurring disease				
1	Irish Setter	partial villous atrophy and intraepithelial lymphocyte infiltration after diet with wheat	Enteropathy was independent of canine MHC II and therefore was not a CD4+ T-cell-mediated disease	[57], [58]
2	Rhesus monkeys	Pathology in response to gluten dermatitis, spontaneously developed antibodies specific for epidermal transglutaminase and TG2	There is no association of the disease with MHC II dermatitis resolved after the introduction of a gluten-free diet	[60], [61]
Transgenic models				
3	Knockout mice with HLA-DQ2/-DQ8 genes expression	Inflammatory T-cell response to gliadin	On such mice were shown the influence of microbiota on the severity of CD	[62]
4	Transgenic mice IL-15	increased expression of human IL-15, CD8+ in the small intestine, develop antibodies to TG2 (IgA)		[63], [64]
5	IL-15 DQ8 transgenic mice (first generation of crossbreed IL-15 and DQ8 mice)	developed an increased number of CD3 and IELs in response to gliadin feeding		[65]
6	DQ8-Dd-villin-IL-15 transgenic mouse	Develops villous atrophy in an HLA- and gluten-dependent manner without administration of any adjuvant		[66]
Induced models				
6	Rats + coadministration of a *Shigella/E. coli* + gliadin	TJ destruction, transport of gliadin peptides into the lamina propria		[67]
7	Neonatal rat + IFN-γ + gliadin		*B. longum* CECT 7347 reduced TNF-α and increased IL-10 in intestinal tissue samples	[47]
8	Mice (C57BL/6) + CD4+ CD25-CD45R BlowCD25 cells from gliadin-sensitized mice	Gliadin-primed CD4+ CD45RBlowCD252 T cells drive gluten-dependent small intestinal damage after adoptive transfer into lymphopenic mice	CD4+ T-cell immunity to gluten leads to a breach of oral gluten tolerance and small intestinal pathology in lymphopenic mice, similar to human coeliac disease.	[68]
9	C57BL/6 mice + p31-43 peptide	Increased cell death, expression of inflammatory mediators, and effects of p31–43 were dependent on MyD88 and type I IFNs and e enhanced by coadministration of the TLR3 agonist polyinosinic: polycytidylic acid.	Gliadin peptide p31–43 activates the innate immune pathways *in vivo*, such as IFN-dependent inflammation, relevant to CD.	[69]
10	BALB/c mice + gliadin (intraperitoneal and oral)	Gluten-mediated inflammation, IL-15 immune response		[70]
10	Wistar rats + gliadin	Villus shortening, crypt hyperplasia, and an increased number of intestinal lymphocytes	Transplantation of IELs contributed to the development of enteropathy.	[71]
11	Wistar rats + gliadin + IFN-γ + *Bifidobacterium bifidum/Shigella*	Same as in [62]. + *Bifidobacterium bifidum*: low concentration of gliadin in lamina propria + *Shigella*: High concentration of gliadin in lamina propria	Microbiota influences the degree of gliadin-induced intestinal permeabilization.	[67]

TJ: Tight junction, CD: Celiac disease, INF: Interferon, IL; Interleukin, IEL: Intraepithelial lymphocytes.

gluten [75]. Incubation of the duodenal mucosa of patients on a gluten diet and healthy individuals with this fermented wheat flour did not increase IFN-γ mRNA levels [76]. This fermented bread does not alter intestinal permeability in patients [75]. Clinical studies give positive results [75], [76].

Another method of creating less toxic wheat involves transamidation of α-gliadin-derived peptides by incubating commercial wheat flour with microbial transglutaminase and lysine methyl ether. T cells obtained from 12 patients with celiac disease (treated and untreated) were incubated with modified wheat for 48 h. As a result, the binding of toxic peptides to DQ2 decreased and the expression of IFN-γ was decreased [77].

The other functional food is wheat flour enriched in L-theanine, developed by Ribeiro *et al*. [78]. They use enzymatic transamidation of wheat flour by ethylamine as an amine nucleophile. Potentially, this transformed wheat could be used as food for celiac patients on a gluten-free diet [78].

Sánchez-León *et al*. showed that CRISPR/Cas9 technology can be used to precisely and efficiently reduce the number of α-gliadins in the seed kernel, providing bread and durum wheat lines with reduced immunoreactivity for gluten-intolerant consumers. They precisely modify the immunoreactive α-gliadin genes by two sgRNAs. CRISPR/Cas9 efficiently and precisely targeted conserved regions of the α-gliadin genes in both bread and durum wheat, leading to high-frequency mutagenesis in most gene copies. Immunoreactivity of the CRISPR-edited wheat lines was reduced [79].

The same group of authors previously reported the downregulation of gliadin genes by RNAi [80], [81]. The effectiveness of seven plasmid combinations, encompassing RNAi fragments from a-, c-, x-gliadins, and LMW glutenin subunits, for silencing the expression of different prolamin fractions was studied. Six plasmid combinations provided a strong reduction in the gluten content as measured by moAb and for two combinations, this reduction was higher than 90% in comparison with the wild type. Celiac disease epitope analysis in peptides identified in LC-MS/MS showed that lines from three plasmid combinations were devoid of celiac disease epitopes from the highly immunogenic a- and x-gliadins. Both CRIPSR/Cas9 and RNAi are highly effective for obtaining wheat lines lacking celiac disease epitopes [80], [81].

Changing the gluten profile: Glutenases and gluten-binding molecules

Gluten triggers an inflammatory cascade in the mucous lining of the small intestine. One of the therapeutic approaches is to either modify gluten so that it is no longer immunogenic or alter the body's ability to "see" gluten and react to it.

Glutenases are enzymes that cleave off glutamine and proline residues from gluten, reducing the immunogenicity of gluten. They are supposed as supplements to a gluten-free diet to reduce the consequences of hidden gluten consumption.

ALV003 supplementary contains two glutenases, a glutamine-specific cysteine endoprotease and a proline-specific prolyl endoprotease from *Sphingomonas*. Studies in rats show that ALV003 saves enzymatic activity in the stomach and the duodenum [82]. A Phase 2A clinical study revealed that patients with gluten enteropathy who consumed 2 g of gluten every day with ALV003 for 6 weeks had no serological changes and had fewer symptoms and morphological changes compared to patients with celiac disease who received a placebo [83].

Endopeptidase derived from *Aspergillus Niger* is used in AN-PEP. *In vitro* studies have shown that it saves its activity at pH levels comparable to those in the lumen and is resistant to pepsin [84]. The use of an *in vitro* multi-chamber system imitating the gastrointestinal tract has shown that "swallowing" bread with AN-PEP leads to the efficient breakdown of gluten within the physiological time. *In vitro* studies have also shown that AN-PEP eliminates the property of gluten to stimulate T cells [85].

Engineered gliadin peptidase Kuma010 specifically degrades peptides after the PQ dipeptide motif, which is found throughout the immunogenic fraction of gliadin [86]. In the last work, the authors redesign the active site of the Kuma010. The resulting protease, Kuma030, specifically recognizes tripeptide sequences that are found throughout the immunogenic regions of gliadin, as well as in homologous proteins in barley and rye. Gliadin treated with Kuma030 loses its ability to stimulate a T-cell response [87].

Another cocktail consists of aspergillopepsin from *A. niger* and dipeptidyl peptidase IV (DPPIV) exopeptidase. Aspergillopepsin is not specific for gluten immunotoxic epitopes but can help break down big proteins into peptides, so exposing target residues more quickly to specific enzymes. DPPIV enhances the ability of aspergillopepsin to break down gluten; unfortunately, it is inactive at pH < 4 and, therefore, requires concomitant use of antacids [88]. Aspergillopepsin can be used together with ALV003.

Another enzyme cocktail usually used in dietary supplements is STAN 1. A clinical study showed that consumption of STAN-1 and 1 g of gluten daily for 12 weeks did not alter serology in patients with gluten enteropathy who previously had positive serological reactions despite a gluten-free diet [89].

Glutenases physically alter gluten which is why it loses its toxicity. Another way of immune response prevention is to use a polymer to bind gluten. The gluten-binding process prevents degradation and absorption, allowing the gluten to go through the organism unnoticed.

Poly(hydroxyethyl methacrylate-co-styrene sulfonate sodium) is a polymer that has a high affinity for α-gliadin peptides in an acidic environment. Testing of this binder in HLA-DQ8 mice revealed selectivity in the sequestration of gliadin and hordein as compared to other nutrients, as well as a reduction in the villous damage caused by the gluten [90], [91]. A pre-clinical study in the NOD-DQ8 mouse model showed that BL-7010 was effective in preventing gluten-induced villus-to-crypt ratio reduction, intraepithelial lymphocytosis, and changes in paracellular permeability [92]. The study also demonstrated that BL-7010 interacts with high affinity with gliadin and does not interact with tested vitamins and digestive enzymes. A clinical study has been conducted to check the safety and systemic effects of a single ascending administration and repeated administration of the polymer (NCT01990885).

A novel research avenue in the quest for an alternative to the gluten-exclusion diet is the development of complex three-dimensional chemical systems with specific biofunctionality. Extensive molecular self-association between the chitosan and gluten proteins in an aqueous environment after the reduction of the disulfide bonds was studied. The strategy of the supramolecular assembly was successful in decreasing gluten digestibility and its capacity to stimulate a T-cell-mediated immune response. This type of tunable approach is a promising nanotechnology tool to change biomaterials features as their immunogenicity [93].

Microbiome modification: Probiotics and helminths

Microbiota and some parasites can have immunomodulatory effects. It is interesting to find organisms that can influence celiac disease pathogenesis. At present, the use of probiotics and helminths is under study.

The amount of *B. longum* in the microbiome of patients is often reduced compared to healthy individuals. The addition of *B. longum* to culture of Caco-2 cells sensitized by gliadin results in a decrease in TNF-α production and consequently may be associated with inhibition of inflammation [94].

Probiotics have been studied in HLA transgenic mice. Studies have shown that probiotic therapy can reduce gluten-induced inflammation and could be used as supporting therapy on a gluten-free diet. The effects of different *Lactobacilli* sp. on DQ8 transgenic mice showed modulation of innate and adaptive immune response [95], [96]. They found that effects were strain specific. *B. longum* NCC2705 strain produced a serine protease inhibitor (serpin) with immune modulating properties and prevented gliadin-induced inflammation in genetically susceptible mouse model of CeD [95].

Based on *in vitro* and *in vivo* models, a clinical trial was conducted on the use of probiotics. There are a few studies on the effect of probiotics in patients with CeD with cohorts ranging from 22 to 109 patients [97], [98], [99], [100], [101], [102], [103], [104], [105], [106]. Most of these studies used *Bifidobacterium* strains, while a few studies used mixture of *Lactobacilli* spp. and *Bifidobacterium* spp. Most of these studies demonstrated modulation of gut microbiota, decreased inflammatory cytokines causing reduction in CeD symptoms.

Helminths have been considered as potential intestinal immunomodulators [107]. Studies of peanut allergy in mouse models have shown that helminths are safe and able to suppress the production of peanut-specific IgE [108].

Extending this potential therapy to people with celiac disease, a Phase 1a/2b study examined human hookworm (*Necator Americanus*) to suppress the immune response of patients. Patients received the helminth and 20 weeks later they underwent a 5-day gluten challenge. The intervention did not prevent histological or systemic inflammatory responses compared to controls, but there was a tendency toward reduced histological lesions and inflammatory processes in response [109].

Stop the inflammatory cascade

Another celiac disease treatment method is to target key molecules in the inflammatory cascade. The addition of Vitamin C *in vitro* to gliadin-exposed duodenal biopsy samples from treated patients leads to reduction of production of IFN-γ, TNF-α, IL-6, IFN-α, IL-15, and IL-17 [110]. Antibodies to IL-15 and its receptor successfully inhibited histological changes of villous in one of the mouse strains with IL-15 overexpression [111], [112].

Protection of tight junctions and prevention of changes in intestinal permeability

Gluten induces changes in proteins of tight junction and modulates intestinal permeability, and this plays a key role in the pathogenesis of celiac disease. Larazotide and TG2 inhibitors are therapeutic agents aimed at tight junction integrity maintenance as a way to prevent the activation of inflammatory cascades [113], [114]. TG2 inhibitors influence on the changes in tight junction permeability in culture of Caco-2 cells and inhibit anti-TG2 antibody production and associated crypt cell proliferation in duodenal biopsy sample cultures from untreated patients with celiac disease. In HLA-DQ8, transgenic mice injected with larazotide acetate were found inhibition of gliadin-induced inflammatory cell infiltration and save tight junction integrity.

Preliminary placebo-controlled clinical trial of selective oral transglutaminase 2 inhibitor was made. Three groups of adults with well-controlled celiac disease assigned 10 mg, 50 mg, and 100 mg of

selective oral transglutaminase 2 inhibitor daily. In the end point, duodenal-biopsy samples were taken and IEL density was measured. Treatment by TG-2 inhibitor attenuated gluten-induced duodenal mucosal damage in patients with celiac disease [115].

Larazotide acetate (also known as AT1001) has been evaluated in several clinical trials. A 12 mg of AT1001 were safe and not immune in 21 patients adhering to a gluten-containing diet. The preliminary results of this study showed that AT1001 maintains intestinal permeability on the same level and reduces interferon gamma stimulation and gastrointestinal symptoms [116]. Following this study, a placebo-controlled dose-ranging study was conducted at 10 clinical centers in which AT1001 was studied in 86 patients. The patients were randomized and receive AT1001 in different concentration or placebo 3 times per day. Intestinal permeability varied greatly and no difference was found between patients on a gluten-free diet and patients on a gluten-containing diet. AT1001 was well tolerated and turned out effective in reducing gastrointestinal symptoms [117].

Vaccines: Nexvax2®

The identification of immunogenic gluten peptides is crucial for developing immunological treatment methods differ from to the gluten-free diet. It has been shown that several epitopes stimulate T-cell activity due to the high cross-reactivity of the 33-mer peptide. Nexvax2 is an epitope-specific vaccine for the treatment of celiac disease that supposedly prevents clinical and histological relapse after gluten consumption in patients with the HLA-DQ2.5 genotype. Composition consists of three synthetic peptides of 15/16 amino acids corresponding to the immunogenic determinants of gluten [118].

Two Phase 1b clinical trials were done. Unfortunately, results are not appropriate. Systemic administration of Nexvax2® causes transient activation of T cells and engages non-T-cell populations within 2–4 h, and recapitulates gastrointestinal symptoms associated with ingestion of gluten in celiac disease. These findings are consistent with gluten-specific memory CD4+ T cells being activated by epitopes in Nexvax2®, and being responsible for clinical features observed following gluten ingestion in CeD [119].

Conclusion

During the past 20 years studies in cell cultures, biopsies and model animals help to find out the mechanisms of celiac disease and suggest new therapies. Today, the mechanisms of the development of celiac disease have been studied in detail. Therapies being developed and tested are based on a deep understanding of intermolecular processes. Researchers are trying to target each stage of the disease.

Celiac disease is a multifactorial, widespread disease. So far, there are no proven alternatives to a gluten-free diet. Moreover, strict adherence to the diet is not always possible due to hidden gluten in food. Thus, the search for alternative treatment options is an urgent task. Today, many alternative therapies are being tested: Creation of non-toxic wheat by genome correction by CRISP/Cas9 and i-RNA technologies, enzymatic digestion of gluten, application of polymer gluten binders; probiotic therapy; and immunomodulatory therapy: Use of antibodies to IL-15, selective inhibition of TG-2; and use a epitope-specific vaccine.

In vitro and *in vivo* models of celiac disease help to advance in testing new strategies of treatment and start clinical trials. Clinical trials are currently in progress, but only few have reached later clinical trial phases: Larazotide acetate and gluten-specific proteases from a bacterial mix (ALV003), IL-15 monoclonal antibodies (AMG 714), and vaccination (Nexvax2). However, a recent Phase 2b study showed that ALV003 did not improve histologic and symptoms scores in patients with moderate-to-severe symptoms versus placebo [120]. IL-15 monoclonal antibodies (AMG 714) had investigated in Phase 2a study. The study failed to meet its primary outcome. However, patients in the higher schedule AMG 714 had better outcomes on IEL density. In addition, patient reported that both symptom score and fecal consistency were decreased suggesting global clinical improvement [121]. Finally, vaccination (Nexvax2) is another possible therapeutic strategy aimed at desensitizing patients with CD to gliadin peptides. Although abdominal pain and vomiting were major side effects, the trial passed Phase 1 [122].

Pharmaceuticals to target various steps in the coeliac disease are under development. Not all of them could be used as independent drugs but integrated approach to treatment celiac disease will allow to improve patients quality of life and reverse to health.

References

1. Barone MV, Troncone R, Auricchio S. Gliadin peptides as triggers of the proliferative and stress/innate immune response of the celiac small intestinal mucosa. Int J Mol Sci. 2014;15(11):20518-37. https://doi.org/10.3390/ijms151120518
   PMid:25387079
2. Re VD, Magris R, Cannizzaro R. New insights into the pathogenesis of celiac disease. Front Med (Lasusanne). 2017;4:137. https://doi.org/10.3389/fmed.2017.00137

PMid:28913337

3. Jericho H, Guandalini S. Extra-intestinal manifestation of celiac disease in children. Nutrients. 2018;10(6):755. https://doi.org/10.3390/nu10060755
PMid:29895731
4. Guandalini S, Assiri A. Celiac disease: A review. JAMA Pediatr. 2014;168(3):272-8. https://doi.org/10.1001/jamapediatrics.2013.3858
PMid:24395055
5. Alessio F, Carlo C. Celiac disease. N Engl J Med. 2012:367(25):2419-26. https://doi.org/10.1056/nejmcp1113994
PMid:23252527
6. Kårhus LL, Thuesen BH, Skaaby T, Rumessen JJ, Linneberg A. The distribution of HLA DQ2 and DQ8 haplotypes and their association with health indicators in a general Danish population. United European Gastroenterol J. 2018;6(6):866-78. https://doi.org/10.1177/2050640618765506
PMid:30023064
7. Tye-Din JA, Cameron DJ, Daveson AJ, Day AS, Dellsperger P, Hogan C, *et al*. Appropriate clinical use of human leukocyte antigen typing for coeliac disease: An Australasian perspective. Intern Med J. 2015;45(4):441-50. https://doi.org/10.1111/imj.12716
PMid:25827511
8. Lionetti E, Gatti S, Pulvirenti A, Catassi C. Celiac disease from a global perspective. Best Pract Res Clin Gastroenterol. 2015;29(3):365-79. https://doi.org/10.1016/j.bpg.2015.05.004
PMid:26060103
9. Murad H, Jazairi B, Khansaa I, Olabi D, Khouri L. HLA-DQ2 and-DQ8 genotype frequency in Syrian celiac disease children: HLA-DQ relative risks evaluation. BMC Gastroenterol. 2018;18:70. https://doi.org/10.1186/s12876-018-0802-2
10. Bradauskiene V, Vaiciulyte-Funk L, Martinaitiene D, Andruskiene J, Verma AK, Lima JP, *et al*. Wheat consumption and prevalence of celiac disease: Correlation from a multilevel analysis. Crit Rev Food Sci Nutr. 2023;63(1):18-32. https://doi.org/10.1080/10408398.2021.1939650
PMid:34184959
11. Poddighe D, Rebuffi C, De Silvestri A, Capittini C. Carrier frequency of HLA-DQB1*02 allele in patients affected with celiac disease: A systematic review assessing the potential rationale of a targeted allelic genotyping as a first-line screening. World J Gastroenterol. 2020;26(12):1365-81. https://doi.org/10.3748/wjg.v26.i12.1365
PMid:32256023
12. Kupfer SS, Jabri B. Celiac disease pathophysiology. Gastrointest Endosc Clin N Am. 2012;22(4):639-60. https://doi.org/10.1016/j.giec.2012.07.003
PMid:23083984
13. Yagur VE, Kapralov NV, Dostanko NY, Polyanskaya AV. Celiac disease. Med J. 2016;3:48-56.
14. Shumilov PV, Yu GM, Netrebenko OK, et al. Modern ideas about the pathogenetic mechanisms of celiac disease: A decisive role in the clinical course. Pediatrics. 2016;95(6):110-9.
15. Schuppan D, Junker Y, Barisani D. Celiac disease: From pathogenesis to novel therapies. Gastroenterology. 2009;137(6):1912-33. https://doi.org/10.1053/j.gastro.2009.09.008
PMid:19766641
16. Paolella G, Lepretti M, Martucciello S, Nanayakkara M, Auricchio S, Esposito C, *et al*. The toxic alpha-gliadin peptide 31-43 enters cells without a surface membrane receptor. Cell Biol Int. 2018;42(1):112-20. https://doi.org/10.1002/cbin.10874
PMid:28914468
17. Sollid LM, Qiao SW, Anderson RP, Gianfrani C, Koning F. Nomenclature and listing of celiac disease relevant gluten T-cell epitopes restricted by HLA-DQ molecules. Immunogenetics. 2012;64(6):455-60. https://doi.org/10.1007/s00251-012-0599-z
PMid:22322673
18. Sollid LM, Jabri B. Triggers and drivers of autoimmunity: Lessons from coeliac disease. Nat Rev Immunol. 2013;13(4):294-302. https://doi.org/10.1002/cbin.10874
PMid:23493116
19. Galipeau HJ, Verdu EF. Gut microbes and adverse food reactions: Focus on gluten related disorders. Gut Microbes. 2015;5(5):594-605. https://doi.org/10.4161/19490976.2014.969635
PMid:25483329
20. Lebwohl B, Sanders DS, Green PH. Coeliac disease. Lancet. 2018;391(10115):70-81. https://doi.org/10.1016/S0140-6736(17)31796-8
PMid:28760445
21. Zakharova IN, Dmitrieva YA, Dzebisova FS. Celiac disease and associated diseases. Russian Bull Perinatol Pediatr. 2014;3:44-9.
22. Khaleghi S, Ju JM, Lamba A, Murray JA. The potential utility of tight junction regulation in celiac disease: Focus on larazotide acetate. Ther Adv Gastroenterol. 2016;9(1):37-49. https://doi.org/10.1177/1756283X15616576
PMid:26770266
23. Sturgeon C, Fasano A. Zonulin, a regulator of epithelial and endothelial barrier functions, and its involvement in chronic inflammatory diseases. Tissue Barriers. 2016;4(4):e1251384. https://doi.org/10.1080/21688370.2016.1251384
PMid:28123927
24. Lammers KM, Lu R, Brownley J, Lu B, Gerard C, Thomas K, *et al*. Gliadin induces an increase in intestinal permeability and zonulin release by binding to the chemokine receptor CXCR3. Gastroenterology. 2008;135(4):194-204. https://doi.org/10.1053/j.gastro.2008.03.023
PMid:18485912
25. Sollid LM, Tye-Din JA, Qiao SW, Anderson RP, Gianfrani C, Koning F. Update 2020: Nomenclature and listing of celiac disease-relevant gluten epitopes recognized by CD4+T cells. Immunogenetics. 2019;72(1-2):85-8. https://doi.org/10.1007/s00251-019-01141-w
PMid:31735991
26. Tye-Din JA, Galipeau HJ, Agardh D. Celiac disease: A review of current concepts in pathogenesis, prevention, and novel therapies. Front Pediatr. 2018;6:350. https://doi.org/10.3389/fped.2018.00350
PMid:30519552
27. Maiuri L, Ciacci C, Ricciardelli I, Vacca L, Raia V, Auricchio S, *et al*. Association between innate response to gliadin and activation of pathogenic T-cells in celiac disease. Lancet. 2003;362(9377):30-7. https://doi.org/10.1016/S0140-6736(03)13803-2
PMid:12853196
28. Thomas KE, Sapone A, Fasano A, Vogel SN. Gliadin stimulation of murine macrophage inflammatory gene expression and intestinal permeability are MyD88-dependent: Role of the innate immune response in Celiac disease. J Immunol. 2006;176(4):2512-21. https://doi.org/10.4049/jimmunol.176.4.2512
PMid:16456012
29. McDonald BD, Jabri B, Bendelac A. Diverse developmental pathways of intestinal intraepithelial lymphocytes. Nat Rev Immunol. 2018;18(8):514-25. https://doi.org/10.1038/s41577-018-0013-7
PMid:29717233

30. Cukrowska B, Sowińska A, Bierła JB, Czarnowska E, Rybak A, Grzybowska-Chlebowczyk U. Intestinal epithelium, intraepithelial lymphocytes and the gut microbiota-Key players in the pathogenesis of celiac disease. World J Gastroenterol. 2017;23(42):7505-18. https://doi.org/10.3748/wjg.v23.i42.7505
PMid:29204051

31. Verdu EF, Galipeau HJ, Jabri B. Novel players in coeliac disease pathogenesis: Role of the gut microbiota. Nat Rev Gastroenterol Hepatol. 2015;12(9):497-506. https://doi.org/10.1038/nrgastro.2015.90
PMid:26055247

32. Cenit MC, Codoñer-Franch P, Sanz Y. Gut microbiota and risk of developing celiac disease. J Clin Gastroenterol. 2016;50:148-52. https://doi.org/10.1097/MCG.0000000000000688
PMid:27741161

33. Kumar V, Jarzabek-Chorzelska M, Sulej J, Karnewska K, Farrell T, Jablonska S. Celiac disease and immunoglobulin a deficiency: How effective are the serological methods of diagnosis? Clin Diagn Lab Immunol. 2002;9(6):1295-300. https://doi.org/10.1128/cdli.9.6.1295-1300.2002
PMid:12414763

34. Cénit MC, Olivares M, Codoner-Franch P, Sanz Y. Intestinal microbiota and celiac disease: Cause, consequence or coevolution? Nutrients. 2015;7(8):6900-23. https://doi.org/10.3390/nu7085314
PMid:26287240

35. Rossi S, Giordano D, Mazzeo MF, Maurano F, Luongo D, Facchiano A, *et al*. Transamidation down-regulates intestinal immunity of recombinant α-gliadin in HLA-DQ8 transgenic mice. Int J Mol Sci. 2021;22(13):7019. https://doi.org/10.3390/ijms22137019
PMid:34209932

36. D'Argenio V, Casaburi G, Precone V, Pagliuca C, Colicchio R, Sarnataro D, *et al*. Metagenomics reveals dysbiosis and a potentially pathogenic, *N. Flavescens* strain in duodenum of adult celiac patients. Am J Gastroenterol. 2016;111(6):879-90. https://doi.org/10.1038/ajg.2016.95

37. Quagliariello A, Aloisio I, Cionci NB, Luiselli D, D'Auria G, Martinez-Priego L, *et al*. Effect of *Bifidobacterium* breve on the intestinal microbiota of coeliac children on a gluten free diet: A pilot study. Nutrients. 2016;8(10):660. https://doi.org/10.3390/nu8100660
PMid:27782071

38. Tian N, Faller L, Leffler DA, Kelly CP, Hansen J, Bosch JA, *et al*. Salivary Gluten degradation and oral microbial profiles in healthy individuals and celiac disease patients. Appl Environ Microbiol. 2017;83(6):e03330-16. https://doi.org/10.1128/AEM.03330-16
PMid:28087531

39. Abdukhakimova D, Dossybayeva K, Poddighe D. Fecal and duodenal microbiota in pediatric celiac disease. Front Pediatr. 2021;9:652208. https://doi.org/10.3389/fped.2021.652208
PMid:33968854

40. Poddighe D, Kushugulova A. Salivary microbiome in pediatric and adult celiac disease. Front Cell Infect Microbiol. 2021;11:625162. https://doi.org/10.3389/fcimb.2021.625162
PMid:33680992

41. Caminero A, Galipeau HJ, McCarville JL, Johnston CW, Bernier SP, Russell AK, *et al*. Duodenal bacteria from patients with celiac disease and healthy subjects distinctly affect gluten breakdown and immunogenicity. Gastroenterology. 2016;151(4):670-83. https://doi.org/10.1053/j.gastro.2016.06.041
PMid:27373514

42. Caminero A, McCarville JL, Galipeau H, Deraison C, Bernier SP, Constante M, *et al*. Duodenal bacterial proteolytic activity determines sensitivity to dietary antigen through protease-activated receptor-2. Nat Commun. 2019;10(1):1198. https://doi.org/10.1038/s41467-019-09037-9

43. Caminero A, Meisel M, Jabri B, Verdu EF. Mechanisms by which gut microorganisms influence food sensitivities. Nat Rev Gastroenterol Hepatol. 2018;16:7-18. https://doi.org/10.1038/s41575-018-0064-z

44. Turnbaugh PJ, Ridaura VK, Faith JJ, Rey FE, Knight R, Gordon JI. The effect of diet on the human gut microbiome: A metagenomic analysis in humanized gnotobiotic mice. Sci Transl Med. 2009;1(6):6ra14. https://doi.org/10.1126/scitranslmed.3000322
PMid:20368178

45. Galipeau H, McCarville JL, Moeller S, Murray JA, Alaedini A, Jabri B, *et al*. Gluten-induced responses in NOD/DQ8 mice are influenced by bacterial colonization. Gastroenterology. 2014;146(5):S-833. https://doi.org/10.1016/S0016-5085(14)63025-0

46. Papista C, Gerakopoulos V, Kourelis A, Sounidaki M, Kontana A, Berthelot L, *et al*. Gluten induces coeliac-like disease in sensitised mice involving IgA, CD71 and transglutaminase 2 interactions that are prevented by probiotics. Lab Invest. 2012;92(4):625-35. https://doi.org/10.1038/labinvest.2012.13
PMid:22330344

47. Laparra JM, Olivares M, Gallina O, Sanz Y. *Bifidobacterium* longum CECT 7347 modulates immune responses in a gliadin-induced enteropathy animal model. PLoS One. 2012;7(2):e30744. https://doi.org/10.1371/journal.pone.0030744
PMid:22348021

48. Hall N, Rubin G, Charnock A. Intentional and inadvertent non-adherence in adult coeliac disease. A cross-sectional survey. Appetite. 2013;68:56-62. https://doi.org/10.1016/j.appet.2013.04.016
PMid:23623778

49. Caputo I, Secondo A, Lepretti M, Paolella G, Auricchio S, Barone MV, *et al*. Gliadin peptides induce tissue transglutaminase activation and ER-stress through Ca2+ mobilization in caco-2 cells. PLoS One. 2012;7(9):e45209. https://doi.org/10.1371/journal.pone.0045209
PMid:23049776

50. Lindfors K, Blomqvist T, Juuti-Uusitalo K, Stenman S, Venäläinen J, Mäki M, *et al*. Live probiotic *Bifidobacterium* lactis bacteria inhibit the toxic effects induced by wheat gliadin in epithelial cell culture. Clin Exp Immunol. 2008;152(3):552-8. https://doi.org/10.1111/j.1365-2249.2008.03635.x
PMid:18422736

51. De Palma G, Kamanova J, Cinova J, Olivares M, Drasarova H, Tuckova L, *et al*. Modulation of phenotypic and functional maturation of dendritic cells by intestinal bacteria and gliadin: Relevance for celiac disease. J Leukoc Biol. 2012;92(5):1043-54. https://doi.org/10.1189/jlb.1111581
PMid:22891290

52. Molberg O, Kett K, Scott H, Thorsby E, Sollid LM, Lundin KE. Gliadin specific, HLA DQ2-restricted T cells are commonly found in small intestinal biopsies from coeliac disease patients, but not from controls. Scand J Immunol. 1997;46(3):103-9. https://doi.org/10.1046/j.1365-3083.1997.d01-93.x
PMid:9315123

53. Lundin KE, Scott H, Hansen T, Paulsen G, Halstensen TS, Fausa O, *et al*. Gliadin-specific, HLA-DQ(alpha 1*0501,beta 1*0201) restricted T cells isolated from the small intestinal mucosa of celiac disease patients. J Exp Med. 1993;178(1):187-96. https://doi.org/10.1084/jem.178.1.187
PMid:8315377

54. Sato T, Stange DE, Ferrante M, Vries RG, Van Es JH,

Brink SV, *et al*. Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium. Gastroenterology. 2011;141(5):1762-72. https://doi.org/10.1053/j.gastro.2011.07.050

PMid:21889923

55. Bhatia SN, Ingber DE. Microfluidic organs-on-chips. Nat Biotechnol. 2014;32(8):760-72. https://doi.org/10.1038/nbt.2989

PMid:25093883

56. Moerkens R, Mooiweer J, Withoff S, Wijmenga C. Celiac disease-on-chip: Modeling a multifactorial disease *in vitro*. United European Gastroenterol J. 2019;7(4):467-76. https://doi.org/10.1177/2050640619836057

PMid:31065364

57. Batt RM, McLean L, Carter MW. Sequential morphologic and biochemical studies of naturally occurring wheat-sensitive enteropathy in Irish setter dogs. Dig Dis Sci. 1987;32(2):184-94. https://doi.org/10.1007/BF01297107

PMid:3026759

58. Hall EJ, Batt RM. Abnormal permeability precedes the development of a gluten sensitive enteropathy in Irish setter dogs. Gut. 1991;32(7):749-53. https://doi.org/10.1136/gut.32.7.749

PMid:1906829

59. Polvi A, Garden OA, Houlston RS, Maki M, Batt RM, Partanen J. Genetic susceptibility to gluten sensitive enteropathy in Irish setter dogs is not linked to the major histocompatibility complex. Tissue Antigens. 1998;52(6):543-9. https://doi.org/10.1111/j.1399-0039.1998.tb03085.x

PMid:9894853

60. Bethune MT, Borda JT, Ribka E, Liu MX, Phillippi-Falkenstein K, Jandacek RJ, *et al*. A non-human primate model for gluten sensitivity. PLoS One 2008;3(2):e1614. https://doi.org/10.1371/journal.pone.0001614

PMid:18286171

61. Sardy M, Karpati S, Merkl B, Paulsson M, Smyth N. Epidermal transglutaminase (TGase 3) is the autoantigen of dermatitis herpetiformis. J Exp Med. 2002;195(6):747-57. https://doi.org/10.1084/jem.20011299

PMid:11901200

62. Galipeau HJ, McCarville JL, Huebener S, LitwinO, Meisel M, Jabri B, *et al*. Intestinal microbiota modulates gluten-induced immunopathology in humanized mice. Am J Pathol. 2015;185(11):2969-82. https://doi.org/10.1016/j.ajpath.2015.07.018

PMid:26456581

63. Yokoyama S, Takada K, Hirasawa M, Perera LP, Hiroi T. Transgenic mice that overexpress human IL-15 in enterocytes recapitulate both B and T cell-mediated pathologic manifestations of celiac disease. J Clin Immunol. 2011;31(6):1038-44. https://doi.org/10.1007/s10875-011-9586-7

PMid:21938511

64. Korneychuk N, Ramiro-Puig E, Ettersperger J, Schulthess J, Montcuquet N, Kiyono H, *et al*. Interleukin 15 and CD4+ T cells cooperate to promote small intestinal enteropathy in response to dietary antigen. Gastroenterology. 2014;146(4):1017-27. https://doi.org/10.1053/j.gastro.2013.12.023

PMid:24361466

65. DePaolo RW, Abadie V, Tang F, Hall JA, Wang W, Jabri B, *et al*. Co-adjuvant effects of retinoic acid and IL-15 induce inflammatory immunity to dietary antigens. Nature. 2011;471(7337):220-4. https://doi.org/10.1038/nature09849

PMid:21307853

66. Abadie V, Khosla C, Jabri B. A mouse model of celiac disease. Curr Protoc. 2022;2(8):e515. https://doi.org/10.1002/cpz1.515

67. Cinova J, De Palma G, Stepankova R, Kofronova O, Kverka M, Sanz Y, *et al*. Role of intestinal bacteria in gliadin-induced changes in intestinal mucosa: Study in germ-free rats. PLoS One. 2011;6(1):e16169. https://doi.org/10.1371/journal.pone.0016169

PMid:21249146

68. Freitag TL, Rietdijk S, Junker Y, Popov Y, Bhan AK, Kelly CP, *et al*. Gliadin-primed CD4+CD45RBlowCD25-T-cells drive gluten-dependent small intestinal damage after adoptive transfer into lymphopenic mice. Gut. 2009;58(12):1597-605. https://doi.org/10.1136/gut.2009.186361

PMid:19671544

69. Araya RE, Castro MF, Carasi P, McCarville JL, Jury J, Chirdo FG, *et al*. Mechanisms of innate immune activation by gluten peptide p31-43 in mice. Am J Physiol Gastrointest Liver Physiol. 2016;311(1):G40-9. https://doi.org/10.1152/ajpgi.00435.2015

PMid:27151946

70. Vijaykrishnaraj M, Kumar BV, Muthukumar SP, Kurrey NK, Prabhasankar P. Antigen-specific gut inflammation and systemic immune responses induced by pro-longing wheat gluten sensitization in BALB/c murine model. J Proteome Res. 2017;16(10):3514-28. https://doi.org/10.1021/acs.jproteome.7b00199

PMid:28809572

71. Stepankova R, Tlaskalova-Hogenova H, Sinkora J, Jodl J, Fric P. Changes in jejunal mucosa after long-term feeding of germfree rats with gluten. Scand J Gastroenterol. 1996;31(6):551-7. https://doi.org/10.3109/00365529609009127

PMid:8789893

72. Ciacci C, Maiuri L, Caporaso N, Bucci C, Del Giudice L, Massardo DR, *et al*. Celiac disease: *In vitro* and *in vivo* safety and palatability of wheat-free sorghum food products. Clin Nutr. 2007;26(6):799-805. https://doi.org/10.1016/j.clnu.2007.05.006

PMid:17719701

73. Spaenij-Dekking L, Kooy-Winkelaar Y, van Veelen P, Drijfhout JW, Jonker H, van Soest L, *et al*. Natural variation in toxicity of wheat: Potential for selection of nontoxic varieties for celiac disease patients. Gastroenterology. 2005;129(3):797-806. https://doi.org/10.1053/j.gastro.2005.06.017

PMid:16143119

74. Carroccio A, Di Prima L, Noto D, Fayer F, Ambrosiano G, Villanacci V, *et al*. Searching for wheat plants with low toxicity in celiac disease: Between direct toxicity and immunologic activation. Dig Liver Dis. 2011;43(1):34-9. https://doi.org/10.1016/j.dld.2010.05.005

PMid:20554485

75. Di Cagno R, De Angelis M, Auricchio S, Greco L, Clarke C, De Vincenzi M, *et al*. Sourdough bread made from wheat and nontoxic flours and started with selected lactobacilli is tolerated in celiac sprue patients. Appl Environ Microbiol. 2004;70(2):1088-96. https://doi.org/10.1128/AEM.70.2.1088-1096.2004

PMid:14766592

76. Di Cagno R, Barbato M, Di Camillo C, Rizzello CG, De Angelis M, Giuliani G, *et al*. Gluten-free sourdough wheat baked goods appear safe for young celiac patients: A pilot study. J Pediatr Gastroenterol Nutr. 2010;51(6):777-83. https://doi.org/10.1097/MPG.0b013e3181f22ba4

PMid:20975578

77. Marino M, Casale R, Borghini R, Di Nardi S, Donato G, Angeloni A, *et al*. The effects of modified versus unmodified wheat gluten administration in patients with celiac disease. Int Immunopharmacol. 2017;47:1-8. https://doi.org/10.1016/j.intimp.2017.03.012

PMid:28343108

78. Ribeiro M, Lopes S, Picascia S, Gianfrani C, Nunes FM.

Reinventing the nutraceutical value of gluten: The case of l-theanine-gluten as a potential alternative to the gluten exclusion diet in celiac disease. Food Chem. 2020;324:126840. https://doi.org/10.1016/j.foodchem.2020.126840

PMid:32344339

79. Sánchez-León S, Gil-Humanes J, Ozuna CV, Giménez MJ, Sousa C, Voytas DF, Barro F. Low-gluten, non-transgenic wheat engineered with CRISPR/Cas9. Plant Biotechnol. J. 2018;16(4):902-10. https://doi.org/10.1111/pbi.12837

PMid:28921815

80. Gil-Humanes J, Pistón F, Tollefsen S, Sollid LM, Barro F. Effective shutdown in the expression of celiac disease-related wheat gliadin T-cell epitopes by RNA interference. Proc Natl Acad Sci USA. 2010;107(39):17023-8. https://doi.org/10.1073/pnas.1007773107

PMid:20829492

81. Barro F, Iehisa JC, Giménez MJ, García-Molina MD, Ozuna CV, Comino I, *et al*. Targeting of prolamins by RNAi in bread wheat: Effectiveness of seven silencing-fragment combinations for obtaining lines devoid of coeliac disease epitopes from highly immunogenic gliadins. Plant Biotechnol. J. 2016;14(3):986-96. https://doi.org/10.1111/pbi.12455

PMid:26300126

82. Siegel M, Garber ME, Spencer AG, Botwick W, Kumar P, Williams RN, *et al*. Safety, tolerability, and activity of ALV003: Results from two Phase 1 single, escalating-dose clinical trials. Dig Dis Sci. 2012;57(2):440-50. https://doi.org/10.1007/s10620-011-1906-5

PMid:21948339

83. Lahdeaho M, Maki M, Kaukinen K, Laurila K, Adelman D. ALV003, a novel gluteanase, attneuates gluten-induced small intestinal mucosal injury in Celiac Disease patients: A randomized phase 2A clinical trial. In: Federation UEG. Vienna: United European Gastroenterology Week; 2011. p. 57.

84. Stepniak D, Spaenij-Dekking L, Mitea C, Moester M, de Ru A, Baak-Pablo R, *et al.* Highly efficient gluten degradation with a newly identified prolyl endoprotease: Implications for celiac disease. Am J Physiol Gastrointest Liver Physiol. 2006;291(4):621-9. https://doi.org/10.1152/ajpgi.00034.2006

PMid:16690904

85. Mitea C, Havenaar R, Drijfhout JW, Edens L, Dekking L, Koning F. Efficient degradation of gluten by a prolyl endoprotease in a gastrointestinal model: Implications for coeliac disease. Gut. 2008;57(1):25-32. https://doi.org/10.1136/gut.2006.111609

PMid:17494108

86. Gordon SR, Stanley EJ, Wolf S, Toland A, Wu SJ, Hadidi D, *et al*. Computational design of an α-gliadin peptidase. J Am Chem Soc. 2012;134(50):20513-20. https://doi.org/10.1021/ja3094795

PMid:23153249

87. Wolf C, Siegel JB, Tinberg C, Camarca A, Gianfrani C, Paski S, *et al*. Engineering of Kuma030: A gliadin peptidase that rapidly degrades immunogenic gliadin peptides in gastric conditions. J Am Chem Soc. 2015;137(40):13106-13. https://doi.org/10.1021/jacs.5b08325

PMid:26374198

88. Ehren J, Moron B, Martin E, Bethune MT, Gray GM, Khosla C. A food-grade enzyme preparation with modest gluten detoxification properties. PLoS One. 2009;4(7):6313. https://doi.org/10.1371/journal.pone.0006313

PMid:19621078

89. Korponay-Szabo IR, Tumpek J, Gyimesi J, Laurila K, Papp M, Maki M, *et al*. Food-grade gluten degradation enzymes to treat dietary transgressions in coeliac adolescents. J Pediatr Gastroenterol Nutr. 2010;50:68.

90. Pinier M, Verdu EF, Nasser-Eddine M, David CS, Vezina A, Rivard N, *et al*. Polymeric binders suppress gliadin-induced toxicity in the intestinal epithelium. Gastroenterology. 2009;136(1):288-98. https://doi.org/10.1053/j.gastro.2008.09.016

91. Pinier M, Fuhrmann G, Galipeau HJ, Rivard N, Murray JA, David CS, *et al*. The copolymer P(HEMA-co-SS) binds gluten and reduces immune response in glu-ten-sensitized mice and human tissues. Gastroenterology. 2012;142(2):316-25. e1-12. https://doi.org/10.1053/j.gastro.2011.10.038

PMid:22079593

92. Mccarville JL, Nisemblat Y, Galipeau HJ, Jury J, Tabakman R, Cohen A, *et al*. BL-7010 demonstrates specific binding to gliadin and reduces gluten-associated pathology in a chronic mouse model of gliadin sensitivity. PLoS One. 2014;9(11):e0109972. https://doi.org/10.1371/journal.pone.0109972

PMid:25365555

93. Ribeiro M, Picascia S, Rhazi L, Gianfrani C, Carrillo J, Rodriguez-Quijano M, *et al*. *In situ* gluten-chitosan interlocked self-assembled supramolecular architecture reduces T-cell-mediated immune response to gluten in celiac disease. Mol Nutr Food Res. 2018;62(23):e1800646. https://doi.org/10.1002/mnfr.201800646

PMid:30289620

94. Olivares M, Laparra M, Sanz Y. Influence of *Bifidobacterium* longum CECT 7347 and gliadin peptides on intestinal epithelial cell proteome. J Agric Food Chem. 2011;59(14):7666-671. https://doi.org/10.1021/jf201212m

95. McCarville A, Dong J, Caminero A, Bermudez-Brito M, Jury J, Murray JA, *et al*. Commensal *Bifidobacterium longum* strain prevents gluten-related immunopathology in mice through expression of a serine protease inhibitor. J Appl Environ Microbiol. 2017;83(19):e01323. https://doi.org/10.1128/AEM.01323-17

PMid:28778891

96. D'Arienzo R, Maurano F, Lavermicocca P, Ricca E, Rossi M. Modulation of the immune response by probiotic strains in a mouse model of gluten sensitivity. Cytokine. 2009;48(3):254-9. https://doi.org/10.1016/j.cyto.2009.08.003

PMid:19736022

97. Smecuol E, Hwang HJ, Sugai E, Corso L, Cherñavsky AC, Bellavite FP, *et al*. Exploratory, randomized, double-blind, placebo-controlled study on the effects of *Bifidobacterium* infantis Natren life start strain super strain in active celiac disease. J Clin Gastroenterol. 2013;47(2):139-47. https://doi.org/10.1097/MCG.0b013e31827759ac

PMid:23314670

98. Olivares M, Castillejo G, Varea V, Sanz Y. Double-blind, randomized, placebo controlled intervention trial to evaluate the effects of *Biofidobacterium longm* CECT 7347 in children with newly diagnosed coeliac disease. Br J Nutr. 2014;112(1):30-40. https://doi.org/10.1017/S0007114514000609

PMid:24774670

99. Klemenak M, Dolinšek J, Langerholc T, Di Gioia D, Mičetić-Turk D. Administration of *Bifidobacterium* breve decreases the production of TNF-α in children with celiac disease. Dig Dis Sci. 2015;60(11):3386-92. https://doi.org/10.1007/s10620-015-3769-7

PMid:26134988

100. Quagliariello A, Cionci NB, Luiselli D, D'Auria G, Martinez-Priego L, Pérez-Villarroya D, *et al*. Effect of *Bifidobacterium* breve on the intestinal microbiota of coeliac children on a gluten free diet: A pilot study. Nutrients. 2016;8(10):660. https://doi.org/10.3390/nu8100660

PMid:27782071

101. Harnett J, Myers SP, Rolfe M. Probiotics and the microbiome in celiac disease: A randomised controlled trial. Evid Based Complement Altern Med. 2016;2016:9048574. https://doi.org/10.1155/2016/9048574

PMid:27525027

102. Pinto-Sánchez MI, Smecuol EC, Temprano MP, Sugai E, González A, Moreno ML, *et al*. *Bifidobacterium* infantis NLS Super strain reduces the expression of α-defensin-5, a marker of innate immunity, in the mucosa of active celiac disease patients. J Clin Gastroenterol. 2017;51(9):814-7. https://doi.org/10.1097/MCG.0000000000000687

PMid:27636409

103. Primec M, Klemenak M, Di Gioia D, Aloisio I, Cionci NB, Quagliariello A, *et al*. Clinical intervention using *Bifidobacterium* strains in celiac disease children reveals novel microbial modulators of TNF-α and short-chain fatty acids. Clin Nutr. 2019;38(3):1373-81. https://doi.org/10.1016/j.clnu.2018.06.931

PMid:29960810

104. Francavilla R, Piccola M, Francavilla A, Polimeno L, Semeraro F, Cristofori F, *et al*. Clinical and microbiological effect of a multispecies probiotic supplementation in celiac patients with persistent IBS-type symptoms: A randomized, double-blind, placebo-controlled, multicenter trial. J Clin Gastroenterol. 2019;53(3):e117-25. https://doi.org/10.1097/MCG.0000000000001023

PMid:29688915

105. Martinello F, Roman CM, de Souza PA. Effects of probiotic intake on intestinal bifidobacteria of celiac patients. Arq Gastroenterol. 2017;54:85-90. https://doi.org/10.1590/s0004-2803.201700000-07

106. Uusitalo U, Aronsson CA, Liu X, Kurppa K, Yang J, Liu E, *et al*. Early probiotic supplementation and the risk of celiac disease in children at genetic risk. Nutrients. 2019;11(8):1790. https://doi.org/10.3390/nu11081790

PMid:31382440

107. Hewitson JP, Grainger JR, Maizels RM. Helminth immunoregulation: The role of parasite secreted proteins in modulating host immunity. 2009;167(1):1-11. https://doi.org/10.1016/j.molbiopara.2009.04.008

PMid:19406170

108. Marco G, La Rotta A, Rodriguez A, Baeza M. Effectiveness of helminths in two different mice strains in a murine model of anaphylaxis. J Allergy Clin Immunol. 2022;127(2):AB246. https://doi.org/10.1016/j.jaci.2010.12.979

109. Daveson AJ, Jones DM, Gaze S, McSorley H, Clouston A, Pascoe A, *et al*. Effect of hookworm infection on wheat challenge in celiac disease--a randomised double-blinded placebo controlled trial. PLoS One. 2011;6(3):e17366. https://doi.org/10.1371/journal.pone.0017366

PMid:21408161

110. Bernardo D, Martinez-Abad B, Vallejo-Diez S, Montalvillo E, Benito V, Anta B, *et al*. Ascorbate-dependent decrease of the mucosal immune inflammatory response to gliadin in coeliac disease patients. Allergol Immunopathol (Madr). 2012;40(1):3-8. https://doi.org/10.1016/j.aller.2010.11.003

PMid:21420224

111. Yokoyama S, Watanabe N, Sato N, Perera PY, Filkoski L, Tanaka T, *et al*. Antibody-mediated blockade of IL-15 reverses the autoimmune intestinal damage in transgenic mice that overexpress IL-15 in enterocytes. Proc Natl Acad Sci USA. 2009;106(37):15849-54. https://doi.org/10.1073/pnas.0908834106

PMid:19805228

112. Malamut G, El Machhour R, Montcuquet N, Martin-Lanneree S, Dusanter-Fourt I, Verkarre V, *et al*. IL-15 triggers an antiapoptotic pathway in human intraepithelial lymphocytes that is a potential new target in celiac disease-associated inflammation and lymphomagenesis. J Clin Invest. 2010;120(6):2131-43. https://doi.org/10.1172/JCI41344

PMid:20440074

113. Rauhavirta T, Oittinen M, Kivisto R, Mannisto PT, Garcia-Horsman JA, Wang Z, *et al*. Are transglutaminase 2 inhibitors able to reduce gliadin-induced toxicity related to celiac disease? A proof-of-concept study. J Clin Immunol. 2012;33(1):134-42. https://doi.org/10.1007/s10875-012-9745-5

PMid:22878839

114. Gopalakrishnan S, Durai M, Kitchens K, Tamiz AP, Somerville R, Ginski M, *et al*. Larazotide acetate regulates epithelial tight junctions *in vitro* and *in vivo*. Peptides. 2012;35(1):86-94. https://doi.org/10.1016/j.peptides.2012.02.015

PMid:22401908

115. Schuppan D, Mäki M, Lundin KE, Isola J, Friesing-Sosnik T, Taavela J, *et al*. A randomized trial of a transglutaminase 2 inhibitor for celiac disease. N Engl J Med. 2021;385:35-45.

116. Paterson BM, Lammers KM, Arrieta MC, Fasano A, Meddings JB. The safety, tolerance, pharmacokinetic and pharmacodynamic effects of single doses of AT-1001 in coeliac disease subjects: A proof of concept study. Aliment Pharmacol Ther. 2007;26(5):757-66. https://doi.org/10.1111/j.1365-2036.2007.03413.x

PMid:17697209

117. A Double-blind Placebo-controlled Study to Evaluate Larazotide Acetate for the Treatment of Celiac Disease. Alba Therapeutics. Bethesda (MD): National Library of Medicine (US). Available from: https://www.clinicaltrials.gov/ct2/show/NCT01396213 [Last accessed on 2022 Jun 26].

118. Di Sabatino A, Lenti MV, Corazza GR, Gianfrani C. Vaccine immunotherapy for celiac disease. Front Med (Lausanne). 2018;5:187. https://doi.org/10.3389/fmed.2018.00187

PMid:29998106

119. Goel G, Mayassi T, Qiao SW, Ciszewski C, King T, Daveson AJ, *et al*. Sa1396 a single intradermal (ID) injection of nexvax2®, a peptide composition with dominant epitopes for gluten-reactive CD4+ T cells, activates T cells and triggers acute gastrointestinal symptoms in HLA-DQ2.5+people with celiac disease (CeD). Gastroenterology. 2016;150(4):S304. https://doi.org/10.1016/s0016-5085(16)31065-4

120. Murray JA, Kelly CP, Green PH, Marcantonio A, Wu TT, Mäki M, *et al*. No difference between latiglutenase and placebo in reducing villous atrophy or improving symptoms in patients with symptomatic celiac disease. Gastroenterology. 2017;152(4):787-98.e2. https://doi.org/10.1053/j.gastro.2016.11.004

PMid:27864127

121. Popa SL, Chiarioni G. Anti-IL-15 monoclonal antibody for the treatment of coeliac disease: The light at the end of the gluten free diet tunnel? Dig Med Res. 2020;3:107-110. https://doi.org/10.21037/dmr-20-28

122. Anderson RP, Jabri B. Vaccine against autoimmune disease: Antigen-specific immunotherapy. Curr Opin Immunol. 2013;25(3):410-7. https://doi.org/10.1016/j.coi.2013.02.004

PMid:23478068