Chapter

The Emerging Role of the Autophagy Process in Children with Celiac Disease: Current Status and Research Perspectives

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Abstract

Celiac disease (CD) affects approximately 1% of the population in Europe and North America, but the number of patients currently undiagnosed is estimated to be far higher than that of diagnosed cases owing to the presence of prevalent forms with nonspecific symptoms. The toxicity of gliadin in children with CD is not destroyed through digestion with gastropancreatic enzymes. An innate immunity to gliadin plays a key role in the development of CD. Autophagy, a physiological catabolic process, plays also a crucial role in the pathogenesis of several inflammatory diseases. Recent studies have described functional involvement of the regulation of autophagy within a pediatric CD cohort. Furthermore, the contribution of autophagy has been highlighted in the degradation and in the reduction of extracellular release of gliadin peptides, thus suggesting novel molecular targets to counteract gliadin-induced toxicity in CD.

Keywords: gluten, autophagy, celiac disease, gluten-free foods, gluten-free diet

1. State of the art

Celiac disease (CD) is an immune-mediated disorder triggered by gluten ingestion in genetically susceptible subjects. About 1% of the European and North American population are affected, but the number of CD cases currently undiagnosed is suspected to be far superior to known cases due to the prevalence of forms with nonspecific symptoms. An increasing incidence of CD has been observed in developing countries, possibly due to westernization of the local diet, changes in wheat production/preparation, and increasing simplicity of diagnostic techniques. The principal determinant of genetic susceptibility to CD is the major histocompatibility class II HLA molecules. The HLA-DQ2 haplotype is expressed in the majority of patients with CD, whereas the HLA-DQ8 haplotype is expressed only in a minority of patients. However, although the presence of the DQ2 and DQ8 haplotypes is a necessary condition, it is not sufficient for the development of CD. In point of fact, only 10% of people with a genetic predisposition goes on to develop CD.

Gluten is a protein complex rich in proline and glutamine and is found in wheat, rye, and barley. The term gluten refers to a group of prolamins of wheat (gliadin and glutenin). Other prolamins are found in rye (secalin) and barley (hordein) and are genetically similar to each other.

It is particularly interesting that maize, while containing prolamins, causes no mucosal damage in celiac patients, most likely because a different phylogenetic evolution of maize prolamins makes its consumption safe for celiac subjects and not toxic.

Gluten is poorly digested in the human intestine regardless of the presence of celiac disease. Its oligopeptides cross the intestinal mucosa and reach the submucosa where they are deamidated by transglutaminase type 2 (tTG2). Deamidation promotes high affinity binding with HLA DQ2 and DQ8 expressed on the surface of T lymphocytes. In celiac patients, this process triggers an inflammatory and immune-mediated response, typical of the disease. T lymphocytes recognize the HLA complex and release various cytokines including IL-15 and IFN-γ. These molecules induce the activation and clonal expansion of B cells which produce antibodies against gluten as well as autoantibodies against tTG2. Other cytokines stimulate fibroblasts and inflammatory cells to secrete matrix metalloproteinases with consequent tissue remodeling and further release of tTG2 in the extracellular compartment. At the same time, there is an increase in intraepithelial lymphocytes with cytolytic activity which determines epithelial damage.

The typical histological lesions of celiac disease are villous atrophy, intraepithelial lymphocytosis, and crypt hyperplasia. Villous atrophy consists of decreased villous height and alteration of normal crypt/villous ratio (3:1) until total disappearance of villi. Intraepithelial lymphocytosis is defined as a number of intraepithelial lymphocyte (IEL) greater than 30 per 100 enterocytes. Crypt hyperplasia is the extension of the regenerative epithelial crypts associated with changes in the presence of more than one mitosis per crypt.

These elementary lesions associated with celiac disease are identified through duodenal biopsies from endoscopic evaluation. Multiple biopsies of the duodenum (at least one biopsy of the bulb and four of the distal part of the duodenum) are required to make a diagnosis as elementary lesions are not exclusive and frequently may be patchy [1].

In children, adding biopsies of the bulb increases diagnostic reliability, owing to the fact that in 10% of pediatric patients, villous atrophy is exclusively located in the duodenal bulb [2].

Histological changes can be classified according to the Marsh classification which identifies three entities: (1) type 1 or infiltrative lesions (normal villous and crypt architecture, normal villous/crypt ratio, and an increased number of intraepithelial lymphocytes); (2) type 2 or hyperplastic lesion (normal villous architecture, hyperplasia of the glandular element with an increased number of mitoses, and increased intraepithelial lymphocytes); and (3) type 3 or destructive lesion (varying degrees of villous atrophy associated with hyperplasia of the glandular crypt and increased intraepithelial lymphocytes). Oberhuber et al. [3] proposed a different classification dividing the Marsh type 3 lesion into three subgroups according to the severity of villous atrophy: (3a) mild villous atrophy and pathological increase of IELs; (3b) moderate villous atrophy and pathological increase of IELs; and (3c) total villous atrophy and pathological increase of IELs.

Diagnosis of CD is based not only on histology but also on the presence of specific serological markers which should be performed in patients on a gluten-containing diet.

Anti-tTG2 is circulating, gluten-dependent, autoantibodies that target transglutaminase 2, the principal self-antigen involved in pathogenesis of CD. IgA anti-tTG2 has high sensitivity (97%) and specificity (91%) and is deemed the single most

reliable test for detection of CD in primary care in cases of clinical suspicion or as a screening test in relatives of celiac patients or in people with an increased risk of developing CD. In comparison with the anti-endomysial antibody, the anti-tTG2 IgA assay has greater sensitivity and reproducibility. It is important to remember that IgA deficiency is more common among celiac patients than in the general population; therefore, in cases of low serum IgA levels, IgG class anti-tTG2 should be evaluated.

Anti-endomysial antibodies (EMA) are directed against the intermyofibril substance of smooth muscle, which may correspond to either a reticulin-like structure or a surface component of smooth muscle fibrils. These are detected by indirect immunofluorescence on monkey esophagus cells and on human umbilical cord cells as a substrate. The EMA assay specificity is high (100%), but it is also IgA-based and the EMA IgG assay is not widely available. Anti-endomysial antibodies are considered a confirmatory assay and should be used only in cases of borderline positive or possibly false positive results for anti-tTG2.

Testing for antibodies directed against native gliadin is no longer recommended. However, antibodies against synthetic deamidated IgG class gliadin peptides (anti-DPG) have a diagnostic role in cases of IgA deficiency.

Histological evaluation and serological markers are important for diagnosis but also for testing efficacy of alternative therapies for celiac disease, as mentioned below.

Currently, the only effective treatment for celiac disease is a strict gluten-free diet (GFD). The aim of dietary regimen is recovery of intestinal damage (usually within 24 months), disappearance of serological markers, and resolution of symptoms, when present. Moreover, a GFD improves nutritional parameters including body mass index and bone mineral density.

Celiac patients should avoid all products containing wheat, barley, and rye for life. Complete elimination of gluten is very difficult, especially due to contamination of other foods with traces of gluten.

Strict avoidance of gluten is demanding especially in Mediterranean countries where gluten ingestion in normal diet is high as well as in adolescence and in asymptomatic children diagnosed by screening. Therefore, CD subjects should be monitored annually for adherence to the GFD. Verification of the disappearance of the specific antibodies is important during follow-up.

The definition "gluten-free" is reserved for foods having less than 20 parts per million of gluten. The lowest quantity of gluten known to be responsible for mucosal damage ranges from 10 to 50 mg per day. A gluten intake of less than 10 mg per day is unlikely to cause mucosal damage.

Nevertheless, some patients may be more sensitive than others are to gluten exposure. Therefore, compliance to GFD should be strict and lifelong.

The gluten-free diet has numerous difficulties; a significant lifestyle change is required by patients, and it may be challenging especially in Western countries where gluten is contained in a lot of foods.

Furthermore, gluten-free products are more expensive [4] and are known to have poor palatability and high fat content.

Hypothetical gluten exposure in restaurants may also be a source of anxiety for celiac patients [5].

It is extremely important that patients know potential hidden sources of gluten and obtain precise information about gluten-free substitute and their fiber and nutrient content. Celiac patients should have high-fiber diets and frequently need supplementation of iron, folic acid, vitamin B12, and vitamin D. It may be very useful to refer patients to a dietitian at the time of diagnosis.

Serological markers are used to assess adhesion to gluten-free diet and its efficacy. First of all, in a patient with persistent symptoms, despite a gluten-free diet, it is mandatory to verify strictly compliance to GFD and in particular to investigate

the possibility of inadvertent gluten exposure. Second, it is important to exclude other causes of persistent symptoms. It is necessary to underline the hypothetic presence of alternative diagnosis (i.e., lactose or fructose intolerance, irritable bowel syndrome, microscopic colitis, pancreatic insufficiency, and small intestinal bacterial overgrowth). Another reason for the recurrence of clinical manifestations is refractory celiac disease, whereby CD patients present symptoms of malabsorption and villous atrophy despite a GFD for more than 12 months. In the primary form, there is no initial response to a diet, while in the secondary one, a relapse occurs after an initial response to a GFD. Patients with refractory CD are at risk of developing enteropathy-associated T cell lymphomas.

2. The autophagy process

Eukaryotic cells digest their cytoplasmic content through different processes that come under the general term autophagy (from the Greek words *auto* meaning "self" and *paghein* meaning "to eat"). Autophagy includes different forms of digestive pathways such as macroautophagy, microautophagy, chaperone-mediated autophagy, and noncanonical autophagy. Generally, the term autophagy refers to macroautophagy, and this process depends on specialized autophagy-related proteins (ATGs) to digest different targets, such as organelles, large aggregates of proteins, and microorganisms. Autophagy also plays a key role in direct microorganisms and virus clearance, in the control of inflammation through the inhibition of inflammasome, in antigen presentation, in regulating T cell homeostasis, and the secretion of immune mediators [6]. It is worth nothing that autophagy impairment plays a crucial role in several diseases, in particular proteopathies, such as Parkinson's [7] and Huntington's disease [8].

As analytically described by Codogno et al. [9], there are four types of autophagy:

- Macroautophagy: organelles or other cargos (proteins, lipids, or nucleic acids) are sequestered in the autophagosome, a double-membrane vesicle, and delivered to the lysosome for degradation.

- Microautophagy: small cytosolic materials are degraded after their engulfment in lysosomes through membrane invagination processes.

- Chaperone-mediated autophagy: proteins with the specific KFERQ target sequence are recognized by chaperone Hsc70 protein and then degraded by lysosomes action.

- Noncanonical autophagy: under specific circumstances autophagosome formation in macroautophagy can bypass the canonical steps. To date two noncanonical pathways have been described: Beclin-1-independent autophagy and a pathway that bypasses the action of specific autophagy-related proteins such as ATG5, ATG7, and LC3.

To date, several autophagy-related genes (*ATG*s) have been described and exert a finely coordinated function at different stages of the pathway. It is widely accepted that autophagy consists of six sequential steps: (1) initiation; (2) nucleation or phagophore formation; (3) ATG5-ATG12 conjugation, interaction with ATG16L, and multimerization at the phagophore; (4) LC3 processing and insertion into the nascent autophagosome; (5) capture of random/selective targets for degradation; and (6) fusion of the autophagosome with the lysosome.

3. Autophagy and autoimmune diseases

Autophagy plays a crucial role in the pathogenesis of several autoimmune diseases. In particular, in Crohn's disease, an inflammatory bowel disease is caused by a combination of environmental, immune, and bacterial factors in genetically susceptible individuals. It has been demonstrated that genetic polymorphisms in the *ATG16L* and *IRGM* autophagy-related genes lead to a strong predisposition for the development of Crohn's disease [10]. Despite this significant association, the role played by the ATG16L protein in the disease pathogenesis is still under debate. In particular, the ATG16L protein is known to suppress the inflammatory process, as demonstrated in Atg16L-deficient mice, which were find to be highly susceptible to colitis induced by dextran sulfate sodium. Subsequently the symptoms developed by these mice were effectively treated with injections of anti-IL-1β and IL-18 antibodies [11]. Recently, nucleotide polymorphisms within the key regulatory autophagy gene *ULK1* have been shown to increase susceptibility to Crohn's disease, thus demonstrating that autophagy might contribute to the pathogenesis of this disease [12]. Similarly, the ATG5 protein, another essential component of the autophagic machinery, is implicated in the development of systemic lupus erythematosus (SLE), multiple sclerosis (MS), and rheumatoid arthritis (RA). SLE is an autoimmune disease in which the patient's immune system attacks healthy tissues. Different single nucleotide polymorphisms (SNPs), identified near and within the *ATG5* locus, are associated with SLE initiation and/or development, although the pathogenetic mechanisms involved are still unknown. In another study, it was demonstrated that T cells in SLE patients are autoreactive and autophagy promotes their survival and contributes to their persistence in autoimmune conditions [13]. MS is a demyelinating disease that affects the brain and the spinal cord. Changes in the expression of the ATG5 protein correlates also with immune-mediated myelin injury in MS-derived mice and in affected patients. Specifically, ATG5 is overexpressed in circulating T cells of relapsing–remitting MS patients compared with healthy controls. ATG5 altered expression seems to extend T cell survival and proliferation during active disease; moreover, ATG5 expression profiles correlate with the severity of the disease in mice models [14]. In RA, anti-citrullinated protein antibodies are the most powerful biomarkers in the diagnosis of this disease. During inflammation, the arginine residues of self-proteins are converted to citrulline (a nonessential alpha amino acid) by the peptidylarginine deiminase enzyme, in a process known as citrullination, thus leading to an altered immune response. Presentation of these peptides is blocked after treatment with 3-methyladenine, an autophagy modulator drug, or by reducing ATG5 protein expression, confirming a key role of autophagy in RA pathogenesis [15].

The clinical spectrum of celiac disease is broad, and often it may be not so easy to discern between poor compliance, difficult acceptance of therapy, or presence of disease complications.

It is important to emphasize experimental therapies, in terms of alternative treatment (versus gluten-free diet) or GFD adjuvant.

Recent advances in the "non-dietary" treatment of CD include engineering gluten-free grains, degrading immunodominant gliadin peptides, decreasing intestinal permeability, and inducing oral tolerance to gluten with a therapeutic vaccine.

4. Non-dietary therapies

As outlined above, there are many reasons behind the need to identify new therapeutic options for celiac disease, especially non-dietary therapies. The purpose is to offer a better quality of life to celiac patients.

Over the years several studies regarding alternative therapies have been conducted.

The aim of experimental research is to find a drug that reduces bowel inflammation despite gluten exposure. Evaluation of mucosal damage is the best way to verify the efficacy of alternative drugs, but it is an invasive procedure and especially distressing for children. Noninvasive markers of efficacy could be serological normalization (using tTG-IgA title) or the improvement of clinical symptoms.

In celiac patients exposed to gluten, increased intestinal permeability has been observed and is due to defects in tight junctions, which are structures involved in regulating the passage through the paracellular space. Increased permeability determines the passage of gluten peptides, which reach lamina propria and stimulate inflammatory response [16].

Larazotide acetate is an oral peptide derived from the zonula occludens toxin secreted by *Vibrio cholerae*. It is believed to be involved in the modulation of tight junctions, consequently preventing gliadin passage through the epithelial barrier. Numerous trials have been conducted to verify the efficacy of larazotide. It has been shown to reduce symptoms in patients on a gluten-free diet [17].

Significant effects of larazotide on serological markers have not been demonstrated, and mucosal damage healing does not appear to be the focus of the evaluation of this drug's efficacy.

Another target of alternative drug is the degradation of toxic gluten peptides making them non-immunogenic. Several *endopeptidases* have been studied for this purpose.

AN-PEP (*Aspergillus niger* prolyl endoprotease) is an *Aspergillus niger*-derived endopeptidase which was studied in a randomized trial where celiac patients were divided into two groups: one of which received AN-PEP and the other a placebo. All patients followed a diet with gluten exposure (7 g of gluten daily) for 2 weeks. AN-PEP appeared to be well-tolerated, but no difference as regards mucosal damage was observed between two groups [18].

ALV003 is a combination of two types of glutamine-specific endoprotease, EP-B2 from barley and a prolyl endopeptidase (SC PEP from *Sphingomonas capsulata*); it is active at gastric pH and able to detoxify gluten [19].

It has been observed that ALV003 can prevent mucosal damage, secondary to gluten exposure, in celiac patients with moderate gluten consumption [20].

Over the years, the emerging role of gut *microbiota* in different diseases has been noted. Variations in the composition of microbiota could play a causative role in the pathogenesis of inflammatory and autoimmune diseases.

The microbial community is composed of more than 1000 species of microbes which exert various functions on the immune system, including protecting the body against pathogens, harvesting nutrients and energy from diet, and fermenting nondigestible carbohydrates.

A specific role of intestinal microbiota in the development of celiac disease has been suggested.

Frequent infectious diseases and consequent antibiotic treatments (with secondary effects on the intestinal microbiota) have also been associated with the onset of celiac disease in genetically susceptible infants [21].

In celiac patients, a prevalence of *Bacteroides* spp. has been found [22] along with a reduction in numbers of *Bifidobacterium* species [23].

Furthermore, regardless of GFD, celiac patients presented less variability of *Bacteroides* species in biopsy samples of the duodenal microbiota in comparison with controls [24].

Several studies regarding the association of *Bifidobacterium* and celiac disease have been conducted. *Bifidobacterium infantis* and *Bifidobacterium lactis* were found to contrast the increase of permeability, secondary to gluten ingestion, in the celiac bowel. They are also thought to play a role as downregulators of immune response in celiac patients [25–27].

Therefore, a therapeutic function of *Bifidobacteria* may be considered and it could be investigated through further studies.

BL-7010 is an orally available polymer with a high affinity for gliadins. Polymeric binders are used to reduce the intestinal absorption of endogenous or exogenous molecules.

BL-7010 sequesters gliadin and masks it from enzymatic degradation. Through this mechanism, it is thought to prevent the formation of immunogenic peptides that trigger the immune system.

It is chemically stable at a wide pH range, is non-biodegradable, and is water-soluble. It exerts its function locally in the gastrointestinal tract and is not absorbed systemically; therefore, it has a very good safety profile as was demonstrated in preclinical studies. In vitro and in mouse models have shown that BL-7010 is able to bind gliadin and to prevent barrier dysfunction, changes in IELs, and villous/crypt ratios induced by gliadin [28].

Another therapeutic target identified in recent years is a vaccine called *Nexvax2*. The aim of this drug is to restore immune tolerance to gluten using peptide-based immunotherapy to induce T cells to make a regulatory response. Murine models were used for the study, and three immunogenic peptides have been identified. These peptides elicit an immune response in patients with celiac disease who carry the HLA-DQ2 immune recognition gene. Tolerance is based on reduction of CD4 T cell proliferation, decreasing IL-2 and IFN-γ levels and promoting expression of T cells with regulatory functions. It was reported that patients treated with the vaccine (especially with high doses) presented gastrointestinal side effects similar to celiac symptoms. This finding corroborates the hypothesis that the vaccine has a similar action to gluten ingestion as regards intestinal immunity [29].

Another attempt at immuno-modulation was made using *CCX282-B* an antagonist of the CCR9 chemokine receptor. Increased blood levels of T cells expressing CCR9 have been found in celiac patients. It is thought that CCX282-B may prevent T cell migration from the blood to the intestinal mucosa. Unfortunately the results of this study are not yet available.

A further consideration is the hygiene hypothesis, whereby excess hygiene is thought to trigger the inflammatory process through immune imbalance resulting in autoimmune disorders. The effect of helminth infections on immunity has also been studied, in particular their role in celiac disease and in inflammatory bowel disease.

Interestingly, it has been reported that the *Necator americanus* species of hookworm enabled individuals with celiac disease to tolerate escalating challenges with dietary gluten [30]. The exact mechanism responsible for tolerance is not known, and further studies are expected.

As previously described, reasons justifying the search for an alternative therapies for celiac disease are numerous, and several studies have been conducted to assess their efficacy. The aim is to identify a new drug and, at the same time, to define new hypothetical therapeutic targets to improve celiac patients' quality of life.

Over the years we have studied the autophagy process. Our results have highlighted the possible contribution of this process to the degradation and the reduction of extracellular release of gliadin peptides and suggest novel molecular targets to counteract gliadin-induced toxicity in CD.

5. Autophagy and celiac disease

The primary link between autophagy and celiac disease is that autophagy is conventionally described as a catabolic pathway where the cytoplasmic material sequestered by autophagosomes is degraded. Therefore, the exogenous gliadin

peptides content might be a potential target of the autophagy clearance process. The autophagy escape, on the other hand, might specifically lead to MHC antigen presentation by dendritic cells or to other unspecific exocytic/endocytic processes between different cells.

Autophagy is known to modulate two crucial aspects of the adaptive immune response involved in the pathological context of the celiac condition: it can enhance priming of CD4+ T cell responses, but at the same time, by allowing the presentation of self-peptides, it may also regulate the establishment of peripheral T cell tolerance [31]. Importantly, antigen presentation by MHC class I or II proteins is dependent on the activity of the proteasome or the endocytic/phagocytic system, respectively, and therefore associated with the functionality of the autophagy process.

To date, however, only a few scientific publications have attempted to investigate the functional role of the autophagy process in celiac disease.

Weersma and colleagues [32] first reported the absence of association of *IL23R* and *ATG16L1* mutations with celiac disease susceptibility, in contrast to that of Crohn's disease. This result was subsequently confirmed independently by genetic analysis in a different cohort of celiac disease patients [33].

More recently, Rajaguru and colleagues [34] evaluated the expression of the LC3 autophagy marker in duodenal biopsies of celiac patients at initial presentation and after 6 months of gluten-free diet, reporting a time-related reduction of LC3 expression in dendritic cells through immunohistochemical analysis. The authors concluded that the observed typical histological pathological hallmarks in duodenal biopsies were associated with a reduction in activated dendritic cells expressing autophagic proteins. This alteration within the autophagy executor organelles may well play an important role in the pathogenesis of autoimmune disorders like celiac disease.

Comincini and collaborators [35] have recently tackled a different question regarding celiac disease, i.e., the possibility to identify novel molecular markers in order to increase the sensitivity and specificity in the diagnosis of pediatric celiac disease patients. To this end, the expression levels of two key autophagy executor genes (*ATG7* and *BECN1*) and their regulatory validated miRNAs (miR-17 and miR-30a, respectively) were analyzed by relative quantitative real-time PCR on a cohort of confirmed celiac patients compared to age-related controls, analyzing peripheral blood, and corresponding duodenal specimens. Among the investigated gene/miRNA targets, statistical analysis indicated the highest significant association as that of *BECN1* expression profiles with the pathological status in the blood, while in intestinal biopsies, all the investigated sequences were positively associated with the celiac disease condition. The authors were also able to identify specific celiac/control molecular subtypes based on specific genes and miRNA expression signatures. Overall, the authors described novel molecular markers that might be useful in increasing the accuracy in CD diagnosis and in molecular-based stratification of the patients, further reinforcing the functional involvement of the regulation of the autophagy process in digestive and autoimmune-related disorders such as CD.

In the latest PubMed contribution, Manai and collaborators [36] reported that in Caco-2 cells, a widely used in vitro model for celiac disease studies, the administration of enzymatically digested gliadin (PT-gliadin) peptides significantly reduced the expression of the LC3-II autophagy-related marker. Furthermore, electron and fluorescent microscope analysis suggested a compromised function of the autophagosome apparatus. The improvement of the dysregulated autophagy process, along with a reduction of PT-gliadin toxicity, was achieved by means of a starvation induction protocol and by 3-methyladenine administration, while rapamycin, a well-known autophagy inducer, did not produce significant improvement in the clearance of extra- and intracellular fluorescent PT-gliadin amounts. Importantly, these results highlighted the possible

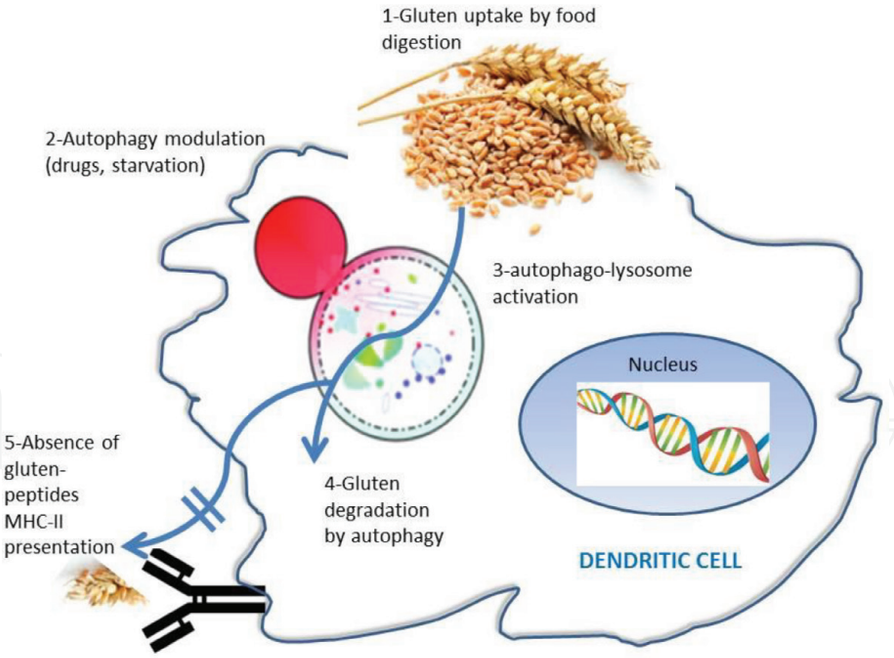

Figure 1.
Gluten degradation by autophagy process. Gluten digested peptides (1) can be internalized into different cell types, including human dendritic cells. These peptides form relatively large intracellular aggregates, partially resistant to intracellular catabolic process, and they can also be released to other surrounding cells by exocytic processes. The discussed approach, (2) based on the autophagy activation using drugs (rapamycyn, chloroquine, etc.) or by means of starvation induction, (3) leads to the fusion of autophagosomes and lysosomes to produce autophago-lysosomes, able to digest the gluten digested peptides. (4) this intracellular digestion produces a reduction of the exocytosis of the gluten peptides, and, mostly, (5) it might reduce the gluten-peptide presentation by MHC-II molecules (HLA-DQ2.5) and therefore the pathological activation of T cell in the celiac disease.

contribution of the autophagy process to the degradation and the reduction of extracellular release of gliadin peptides and suggest novel molecular targets to counteract gliadin-induced toxicity in celiac disease. A schematized summary of the autophagy-modulation strategy to counteract the gluten-derived cellular toxicity is illustrated in **Figure 1**.

6. Concluding remarks

Celiac disease is an increasingly complex disease, with a well-established genetic background but with a plethora of molecular/cellular actors involved. Despite this emerging complexity, the cellular uptake of the digested gliadin components and their ultimate fate is the key determinant for this disease. Once within a cell, gliadin peptides, as with any exogenous components, undergo different catabolic processes, including the relatively low-energy consumption processes such as exocytosis. In this scenario, autophagy protein turnover might represent a pro-survival process to counteract a surge in potentially toxic gliadin. However, for reasons still unknown, the autophagy process seems to be impaired in the celiac condition: as a result, gliadin is easily internalized in different types of cells, but no marked signs of a prominent degradation are reported. On the other hand, more and more is being learned about the process of autophagy and its molecular players, and, consequently, a relatively large number of molecular and pharmacological modulators are being put on the market and assayed in clinical trials for different pathologies. Therefore, once the alterations of the steady-state status of the autophagy process are clarified by comparing physiological to celiac pathological conditions, one could realistically hope to counteract gliadin toxicity by improving its catabolism within the cells, bearing in mind however

that the exacerbation of the fine autophagy intracellular balance might also lead to other, even more complex pathological conditions such as cancers.

This article focuses on the results of researches carried out by authors in the field of celiac disease.

It is of the upmost importance to investigate new therapeutic options for celiac patients, especially non-dietary therapies, in order to improve their quality of life.

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Conflict of interest statement

The authors declare no conflict of interest.

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This article is the focus of the researches carried out by authors in the field of celiac disease.

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References

[1] Rubio-Tapia A, Hill ID, Kelly CP, Calderwood AH, Murray JA. American College of Gastroenterology ACG clinical guidelines: Diagnosis and management of celiac disease. The American Journal of Gastroenterology. 2013;**108**(5):656-676

[2] Bonamico M, Thanasi E, Mariani P, Nenna R, Luparia RP, Barbera C, et al. Società Italiana di Gastroenterologica, Epatologia, e Nutrizione Pediatrica. Duodenal bulb biopsies in celiac disease: A multicenter study. Journal of Pediatric Gastroenterology and Nutrition. 2008;**47**(5):618-622

[3] Oberhuber G, Granditsch G, Vogelasng H. The histopathology of celiac disease: Time for a standardized report scheme for pathologists. European Journal of Gastroenterology & Hepatology. 1999;**11**:1185-1194

[4] Lee AR, Ng DL, Zivin J, Green PH. Economic burden of a gluten-free diet. Journal of Human Nutrition and Dietetics. 2007;**20**:423-430

[5] Barratt SM, Leeds JS, Sanders DS. Quality of life in celiac disease is determined by perceived degree of difficulty adhering to a gluten-free diet, not the level of dietary adherence ultimately achieved. Journal of Gastrointestinal and Liver Diseases. 2011;**20**:241-245

[6] Deretic V, Saitoh T, Akira S. Autophagy in infection, inflammation and immunity. Nature Reviews. Immunology. 2013;**13**(10):722-737

[7] Winslow AR, Chen CW, Corrochano S, Acevedo-Arozena A, Gordon DE, Peden AA, et al. α-Synuclein impairs macroautophagy: Implications for Parkinson's disease. The Journal of Cell Biology. 2010;**190**(6):1023-1037

[8] Martinez-Vicente M, Talloczy Z, Wong E, Tang G, Koga H, Kaushik S, et al. Cargo recognition failure is responsible for inefficient au ophagy in Huntington's disease. Nature Neuroscience. 2010;**13**(5):567-576

[9] Codogno P, Mehrpour M, Proikas-Cezanne T. Canonical and non-canonical autophagy: Variations on a common theme of self-eating? Nature Reviews. Molecular Cell Biology. 2011;**131**:7-12

[10] Naser SA, Arce M, Khaja A, Fernandez M, Naser N, Elwasila S, et al. Role of ATG16L, NOD2 and IL23R in Crohn's disease pathogenesis. World Journal of Gastroenterology. 2012;**18**(5):412-424

[11] Saitoh T, Fujita N, Jang MH, Uematsu S, Yang BG, Satoh T, et al. Loss of the autophagy protein Atg16L1 enhances endotoxin-induced IL-1beta production. Nature. 2008;**456**(7219):264-268

[12] Henckaerts L, Cleynen I, Brinar M, John JM, Van Steen K, Rutgeerts P, et al. Genetic variation in the autophagy gene ULK1 and risk of Crohn's disease. Inflammatory Bowel Diseases. 2011;**17**(6):1392-1397

[13] Gros F, Arnold J, Page N, Décossas M, Korganow AS, Martin T, et al. Macroautophagy is deregulated in murine and human lupus T lymphocytes. Autophagy. 2012;**8**(7):1113-1123

[14] Alirezaei M, Fox HS, Flynn CT, Moore CS, Hebb AL, Frausto RF, et al. Elevated ATG5 expression in autoimmune demyelination and multiple sclerosis. Autophagy. 2009;**5**(2):152-158

[15] Ireland JM, Unanue ER. Autophagy in antigen-presenting cells results in presentation of citrullinated peptides to CD4 T cells. The Journal of Experimental Medicine. 2011;**208**(13):2625-2632

[16] Mènard S, Lebreton C, Schumann M, et al. Paracellular versus trancellular intestinal permeability to gliadin peptides in active celiac disease. The American Journal of Pathology. 2012;**180**:608-615

[17] Leffler DA, Kelly CP, Green PH, et al. Larazotide acetate for persistent symptoms of celiac disease despite a gluten-free diet: A randomized controlled trial. Gastroenterology. 2015;**148**:1311-1319

[18] Tack GJ, van de Water JM, Bruins MJ, Kooy-Winkelaar EM, van Bergen J, Bonnet P, et al. Consumption of gluten with gluten-degrading enzyme by celiac patients: A pilot-study. World Journal of Gastroenterology. 2013;**19**(35):5837-5847

[19] Gass J, Bethune MT, Siegel M, Spencer A, Khosla C. Combination enzyme therapy for gastric digestion of dietary gluten in patients with celiac sprue. Gastroenterology. 2007;**133**(2):472-480 Epub 2007 May 21

[20] Lähdeaho ML, Kaukinen K, Laurila K, Vuotikka P, Koivurova OP, Kärjä-Lahdensuu T, et al. Glutenase ALV003 attenuates gluten-induced mucosal injury in patients with celiac disease. Gastroenterology. 2014;**146**(7):1649-1658

[21] Mårild K, Kahrs CR, Tapia G, Stene LC, Stordal K. Infections and risk of celiac disease in childhood: A prospective nationwide cohort study. The American Journal of Gastroenterology. 2015;**110**(10):1475-1484

[22] Collado MC, Donat E, Ribes-Koninckx C, Calabuig M, Sanz Y. Specific duodenal and faecal bacterial groups associated with paediatric coeliac disease. Journal of Clinical Pathology. 2009;**62**(3):264-269

[23] Collado MC, Donat E, Ribes-Koninckx C, Calabuig M, Sanz Y. Imbalances in faecal and duodenal Bifidobacterium species composition in active and non-active coeliac disease. BMC Microbiology. 2008;**8**:232

[24] Sánchez E, Donat E, Ribes-Koninckx C, Fernández-Murga ML, Sanz Y. Duodenal-mucosal bacteria associated with celiac disease in children. Applied and Environmental Microbiology. 2013;**79**(18):5472-5479

[25] 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. Clinical and Experimental Immunology. 2008;**152**(3):552-558

[26] Medina M, De Palma G, Ribes-Koninckx C, Calabuig M, Sanz Y. Bifidobacterium strains suppress in vitro the pro-inflammatory milieu triggered by the large intestinal microbiota of coeliac patients. Journal of Inflammation (London, England). 2008;**5**:19

[27] De Palma G, Cinova J, Stepankova R, Tuckova L, Sanz Y. Pivotal advance: Bifidobacteria and Gram-negative bacteria differentially influence immune responses in the proinflammatory milieu of celiac disease. Journal of Leukocyte Biology. 2010;**87**(5):765-778

[28] Pinier M, Verdu EF, Nasser-Eddine M, David CS, Vézina A, Rivard N, et al. Polymeric binders suppress gliadin-induced toxicity in the intestinal epithelium. Gastroenterology. 2009;**136**:288-298

[29] Veeraraghavan G, Leffler DA, Kaswala DH, Mukherjee R. Celiac disease 2015 update: New therapies. Expert Review of Gastroenterology & Hepatology. 2015;**9**(7):913-927

[30] Croese J, Giacomin P, Navarro S, Clouston A, McCann L, Dougall A,

et al. Experimental hookworm infection and gluten microchallenge promote tolerance in celiac disease. The Journal of Allergy and Clinical Immunology. 2015;**135**(2):508-516

[31] Cuervo AM, Macian F. Autophagy, nutrition and immunology. Molecular Aspects of Medicine. 2012;**33**(1):2-13

[32] Weersma RK, Zhernakova A, Nolte IM, Lefebvre C, Rioux JD, Mulder F, et al. ATG16L1 and IL23R are associated with inflammatory bowel diseases but not with celiac disease in the Netherlands. The American Journal of Gastroenterology. 2008;**103**(3):621-627

[33] Dema B, Fernández-Arquero M, Maluenda C, Polanco I, Figueredo MA, de la Concha EG, et al. Lack of association of NKX2-3, IRGM, and ATG16L1 inflammatory bowel disease susceptibility variants with celiac disease. Human Immunology. 2009;**70**(11):946-949

[34] Rajaguru P, Vaiphei K, Saikia B, Kochhar R. Increased accumulation of dendritic cells in celiac disease associates with increased expression of autophagy protein LC3. Indian Journal of Pathology & Microbiology. 2013;**56**(4):342-348

[35] Comincini S, Manai F, Meazza C, Pagani S, Martinelli C, Pasqua N, et al. Identification of autophagy-related genes and their regulatory miRNAs associated with celiac disease in children. International Journal of Molecular Sciences. 2017 Feb;**18**(2):391

[36] Manai F, Azzalin A, Gabriele F, Martinelli C, Morandi M, Biggiogera M, et al. The in vitro effects of enzymatic digested gliadin on the functionality of the autophagy process. International Journal of Molecular Sciences. 2018;**19**(2). pii:E635