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Immunogenetic Pathogenesis of Celiac Disease and Non-celiac Gluten Sensitivity

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Abstract Celiac disease is the most common oral intolerance in Western countries. It results from an immune response towards gluten proteins from certain cereals in genetically predisposed individuals (HLA-DQ2 and/or HLA-DQ8). Its pathogenesis involves the adaptive (HLA molecules, transglutaminase 2, dendritic cells, and $CD4^+$ T-cells) and the innate immunity with an IL-15-mediated response elicited in the intraepithelial compartment. At present, the only treatment is a permanent strict gluten-free diet (GFD). Multidisciplinary studies have provided a deeper insight of the genetic and immunological factors and their interaction with the microbiota in the pathogenesis of the disease. Similarly, a better understanding of the composition of the toxic gluten peptides has improved the ways to detect them in food and drinks and how to monitor GFD compliance via non-invasive approaches. This review, therefore, addresses the major findings obtained in the last few years including the re-discovery of non-celiac gluten sensitivity.

Topical Collection on *Small Intestine*

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Introduction

Celiac disease (CD) is an autoimmune disorder with a systemic and chronic inflammatory immune response against gluten and gluten-related prolamins from wheat (gliadin), barley (hordeins), rye (secalins), and certain oat varieties (avenins) in genetically predisposed individuals [1–4]. CD represents the most common food intolerance in western society, with an estimated prevalence of around 1 % of the population [5].

Gluten consumption by CD patients triggers a clinical symptomatology which typically appears in the form of diarrhea, abdominal distension, and vomits ultimately leading to nutrient malabsorption, fatigue, and malnutrition [1]. This classical form of presentation is common in children, whereas the differential diagnose in adults is more complex due to the appearance of moderate intestinal symptoms but also extra-intestinal manifestations such as osteoporosis, dermatitis herpetiformis, gluten-ataxia, and other neuropathological syndromes [6]. The only treatment for CD is a permanent and strict gluten-free diet (GFD), although its compliance is difficult to maintain due to gluten's common presence as a food additive [7] and even in non-dietary sources such as the plastics used in orthodontics [8]. Therefore, dietary transgressions (either accidental or deliberate) are common representing up to 50 % of the CD patients where it prevents mucosal healing and therefore maintains mucosal atrophy [9, 10]. Recurrent dietary transgressions and prolonged gluten consumption (e.g., in non-diagnosed individuals) contribute to the refractory forms of CD (RCD) where the patients are unable to respond to the GFD and maintain an inflammation. There are two forms, a type I that responds to immunomodulators and a

type II that is associated with an increased incidence of associated T-cell lymphoma [11].

In recent years, the diagnosis of CD and its complications has substantially improved with the analysis of biopsy specimens through the advances made in flow cytometry techniques which objectively show an increase of both total ($CD3^+$) and gamma-delta ($CD3^+$ $TCR\gamma\delta^+$) intraepithelial lymphocytes (IELs) in the CD mucosa [12]. Moreover, given that the latter remain increased even on those patients following a GFD, as opposed to the total IEL numbers which come back to normal in such group of patients, the profiling of IEL is a tool not only for CD diagnosis but also for monitoring GFD compliance and to help in the differential diagnosis of type I and type II RCD [12]. Still, the common diagnostic tools for CD in many hospitals are the genetic markers (HLA-DQ2/DQ8), the specific serological antibodies (anti-transglutaminase antibodies), and the histopathological analyses of the duodenal specimens [6]. The latter is usually based on the Marsh-Oberhuber histological classification which ranges from a normal mucosa (marsh 0) to the appearance of lymphocytic infiltration (marsh 1), crypt hyperplasia (marsh 2), and different levels of villous atrophy (marsh 3a–c), although this classification is still subjective. More objective and practical classifications have been proposed in the last years such as the one by Corazza and Villanacci or that by Ensari (Fig. 1) [13••].

Pathogenesis of Celiac Disease

Immune response against gluten in CD patients is a consequence of both innate and adaptive abnormal immune responses which together promote a pro-inflammatory environment, a massive intraepithelial infiltration, and the appearance of its characteristic villous atrophy and crypt hyperplasia (Fig. 1) [13••, 14] which ultimately leads to the clinical manifestations of the disease. The adaptive immune response mainly takes place in the *lamina propria* of the intestinal mucosa while the innate immune response preferentially involves the epithelial layer.

Dendritic cells (DC), found at the interface of the innate and adaptive immune responses, are the most potent antigen-presenting cells as they determine the outcome (pro-inflammatory or tolerogenic) of antigen-specific immune responses. In resting conditions, DC promote the maintenance of immune tolerance towards nutrients and commensals at the time that they initiate immune responses towards invading pathogens [15]. Nevertheless, CD patients fail to recognize gluten as a dietary antigen. Following gluten peptide deamination by the enzyme tissue transglutaminase (TG2) [16••], DC can accommodate such peptides in the MHC-II molecules, including HLA-DQ2 or HLA-DQ8, performing therefore antigen presentation to $CD4^+$-naïve T-cells inducing their differentiation towards gluten-specific Th1/Th17 pro-inflammatory T-cells resulting in a disruption of the oral tolerance to gluten (Fig. 2). These T-cells produce a bulk of pro-inflammatory cytokines, including IFN-γ, TNF-α, IL-18, and IL-21 [17–19], which attract other immune cells to the intestine establishing a positive pro-inflammatory feedback which leads to tissue damage. Such pro-inflammatory profile also constitutes a stress signal to the intestinal epithelial cells (IECs), mainly mediated by IL-15, which as a consequence increase their surface MHC class I polypeptide-related sequence A, MICA (Fig. 2). Similarly, IEL acquire innate-like lymphokine-activated killer (LAK) activities by the expression of NKG2D receptors in an IL-15-dependent manner, allowing them to target $MICA^+$ cells (the IEC in this case) hence inducing their apoptosis (Fig. 2) [20, 21]. It is however, currently unknown, if the innate immune response elicited at the epithelial layer precedes or is a consequence of the *lamina propria*-adaptive response. We have previously suggested that the first trigger of the mucosal lesion is the IL-15 production by IEL following gluten exposure [22, 23] which would lead to an increased epithelial permeability indirectly weakening the tight junctions between the IEC but also directly inducing IEC apoptosis. This would favor the transport of gluten peptides to the *lamina propria* where IL-15-activated DC would recognize gluten peptides (following their deamination by the TG2 enzyme) hence initiating the secondary antigen-specific adaptive immune response responsible for the clinical

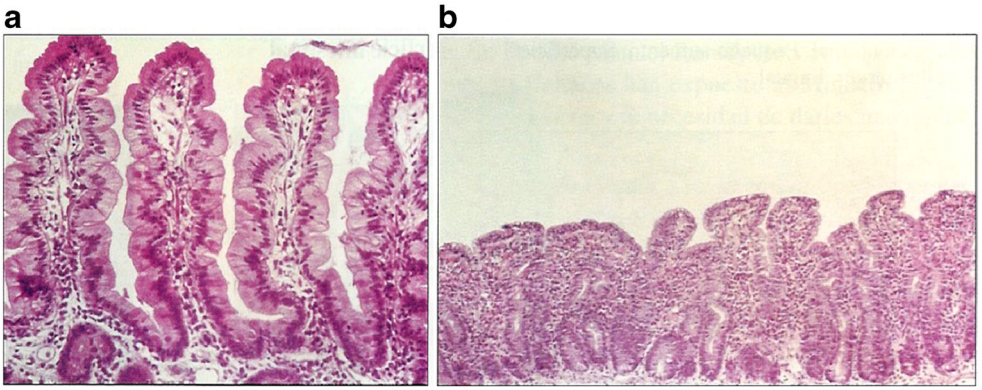

Fig. 1 Histological mucosal architecture of celiac duodenum. **a** Duodenal mucosa of a non-celiac disease patient. **b** Duodenal biopsy specimen from a celiac patient at diagnosis showing a Corazza grade B2 or Ensari type 3 of intestinal damage including lymphocyte infiltration, epithelial hyperplasia, and villous atrophy

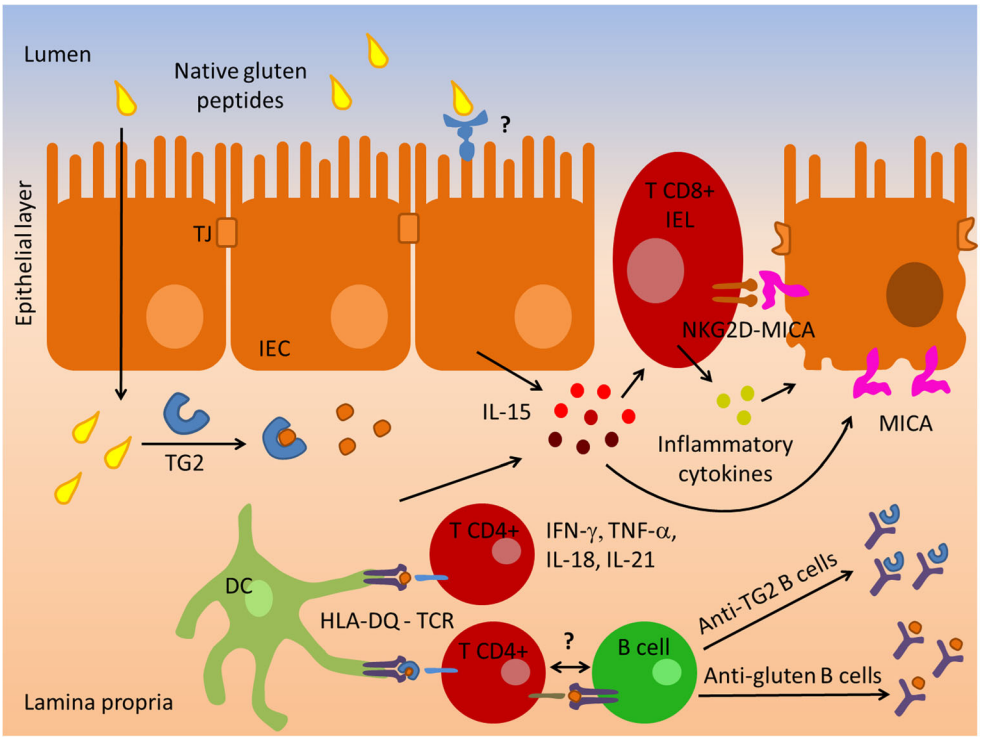

Fig. 2 Immunological response to gluten peptides. TG2 deamidates gluten peptides so HLA-DQ molecules, expressed on the surface of the dendritic cells (DC), are more likely to bind gluten peptides to present them to $CD4^+$ T-cells. These cells then become gluten-reactive and are committed to produce Th1 cytokines (IFN-γ, TNF-α, IL-18, and IL-21) and could also cooperate with B-cells on antibody synthesis at the time that they differentiate into plasmatic cells and secrete specific antibodies against TG2 or gliadin. Intestinal epithelial cells (IECs) on the other hand produce IL-15 after exposure to other gliadin peptides. Altogether, inflammatory cytokines induce IECs to express stress molecules (MICA) and their ligand (NKG2D receptor) on activated intraepithelial lymphocytes (IELs) which therefore induce IEC apoptosis increasing intestinal permeability. *IECs* intestinal epithelial cells, *TJ* tight-junctions, *TG2* tissue transglutaminase 2, *DC* dendritic cell, *IELs* intraepithelial lymphocytes, *LP* lamina propria, *TCR* T-cell receptor, *IFN-γ* interferon-γ, *TNF-α* tumor necrosis factor-α, *IL* interleukin, *MICA* MHC class I polypeptide-related sequence A, *NKG2D* natural killer cell activating factor 2D

manifestations of the disease [24]. Nevertheless, either if the innate response precedes, is a consequence, or is triggered at the same time than the adaptive immune response, there is no doubt that the HLA-DQ2 and DQ8 antigen-presenting molecules, the TG2 enzyme and the IL-15 cytokine are the central players in the pathogenesis of CD (Fig. 2).

HLA-DQ genes are of invaluable importance in the diagnosis given that virtually all CD patients carry the coding variants for DQ2 or DQ8 molecules although they are not sufficient to develop the disease [6, 25–27]. Double doses of these molecules and specific allelic combinations provide different risks to suffer from CD. The proteins encoding these molecules are expressed by antigen-presenting cells (APC) including DC. APC found at the Peyer Patches gain access to luminal antigens through M cells [28], while *lamina propria* APC can sample luminal antigens via IEC, either directly following antigen transfer at the basolateral membrane of the IEC or indirectly following phagocytosis of apoptotic IECs [28] or by direct uptake as elicited by $CX3CR1^+$ tissue-resident macrophages that are able to extend cellular projections between IECs [29, 30]. Given that under CD inflammatory conditions the epithelial integrity is compromised, APCs can also have a direct access to the luminal content [29]. Following DC acquisition of gluten peptides and their recognition as harmful antigens [15], DC carry them to the organized lymphoid tissues and mesenteric lymph nodes in a CCR7-dependent manner. Then, DC present gluten peptides to naïve T-cells [15, 31] and induce their differentiation towards pro-inflammatory T-cells [17–19] at the time that they also induce their expression of the general gut-homing α4β7 integrin and the small bowel chemokine (C–C motif) receptor 9, CCR9 [29], directing therefore T-cell migration to the duodenum [32], where they will encounter dietary gluten peptides eliciting the immune response.

HLA binding of the specific gluten peptides is essential to perform antigen presentation and explains the restriction to $HLA\text{-}DQ2^+$ or $HLA\text{-}DQ8^+$ since they are unique to accommodate the toxic gluten peptides on their DC. Moreover, the dosage effect of HLA-DQ molecules has a direct impact with risk for CD as the threshold to activate $CD4^+$ T-cell responses is higher in homozygous than in heterozygous individuals [16••]. However, for that binding to be optimal, it is crucial for the presence of negatively charged residues preferentially at positions 4 and 6 and occasionally at position 7 of the

antigen-binding groove [16••] to allow for a perfect suit into the binding pocket of HLA-DQ molecules, preferentially in HLA-DQ2.5 molecules but also, to a lesser extent, in HLA-DQ2.2 and HLA-DQ8 molecules [33••].

TG2 is a calcium-dependent enzyme that is constitutively expressed in several tissues and can be induced under injured or inflammatory conditions [34]. TG2 enzyme confers a negative charge to the gluten peptides via glutamine deamination into glutamic acid hence allowing their presentation by the APC and therefore increasing their affinity for the specific HLA-DQ molecule. Similarly, the TCR epitope repertoire on T-cells varies as they are capable of indirectly sensing the deamidation by the changes it provokes in the HLA molecule conformation [16••]. Given that the TG2 enzyme has an assorted affinity for glutamine residues on specific positions (QXP motifs), not all glutenin residues can be deaminated by this enzyme [33••]. As a consequence, many different epitopes can be presented to the T-cells conferring several levels of immunogenicity to different gluten peptides [27]. TG2 is predominantly associated to fibronectin (Fn) in the basement membrane of IECs in the healthy small bowel. However, in CD patients, TG2 is expressed in the apical surface of IECs and can also impact on the transport of gluten peptides by interacting with the transferrin receptor CD71 and the secreted form of IgA in a process known as retrotranscytosis [35]. Therefore, the role of TG2 in CD pathogenesis is not only restricted to provide an increased affinity between gluten peptides and HLA molecules as it also increases the permeability of the epithelial barrier. This may also explain the appearance of anti-TG2-specific antibodies, in addition to the expected anti-gluten ones, which have great relevance as biomarkers for the diagnosis as, indeed, when their level exceeds ten times the upper limit of normal values, they correlate with the severity of the lesion [6]. Among the different available antibodies, anti-endomysium and anti-TG2 antibodies are the recommended ones to evaluate a patient for CD [6] as both of these antibodies have a sensitivity and a specificity above 90 and 95 %, respectively, and together account for a positive predictive value for CD of 97 % [36].

Recent evidence in the literature has shown that both anti-TG2 and anti-gluten antibodies share characteristics that are unusual in the development of immunoglobulins as B-cells preferentially use *IGHV* and *IGKV* genes, with a particular dominance of *IGHV5-51* [37••]. This new insight in the biology of B-cells clarifies the specific antibody production. It is also known that few B-cells have somatic hypermutations or even contain germline-encoded sequences [38]. Thus, B-cell specificity and affinity mature during the generation of the antibodies, which conducts to hypothesize that B-cells recognizing TG2 and gluten must exist within the repertoire of naïve B-cells of CD patients [16••]. Furthermore, the epitopes recognized by anti-gluten antibodies are mostly deamidated and typically overlap to T-cell epitopes [16••, 39], suggesting that gluten-reactive T-cells cooperate in gluten-specific B-cell activation. Moreover, B-cells can also present different epitopes and thus obtain help from a variety of gluten-specific T-cells [16••] following internalization of gluten-TG2 complexes through their B-cell receptor (BCR). Gluten peptides bound to the TG2 active site or via isopeptide to the TG2 surface [40]. However, TG2 can also catalyze a cross-linkage between the BCR and the gluten peptides, as it has been demonstrated for IgD [41]. Interestingly, the epitopes of anti-TG2 antibodies match the N-terminal end of TG2 and overlap with the Fn binding site, allowing the enzyme to remain active [42••, 43••].

IL-15 is a pleiotropic cytokine which is widely expressed and tightly regulated in the organism. Its normal function includes protection against pathogens and cancerous cells by acting on innate immune cells such as NK cells and neutrophils, and it also acts on $CD8^+$ T-cells [44, 45]. Although other molecules share some functions with IL-15 and have a role in CD pathogenesis (IFN-γ and IL-21 [45]), IL-15 is overexpressed together with its receptor in the duodenal mucosa of active CD patients and is central in the CD immune response [23, 45]. As previously discussed, IL-15 induces MICA expression on IEC [46]. It also expands the survival and activation of IEL via Bcl-2 and Bcl-xL [44] and NKG2D expression [46], respectively. As a result, IEL have a decreased activation threshold, which can finally lead to self-antigen recognition [47••], hence promoting altogether IEC apoptosis and an increase of the epithelial permeability. IL-15 also induces DC maturation and activation towards a pro-inflammatory phenotype [48, 49] and blocks TGF-β signaling by regulatory T-cells (Treg) via Smad3 and phosphatidylinositol 3-kinase [50, 51] confirming their central role in CD pathogenesis.

Genetics and Epigenetics in Celiac Disease

Although most of the patients carry the HLA-DQ2.5 variant, some of them carry the HLA-DQ8, the HLA-DQ2.2 variant or other less frequent variants in particular in non-Caucasian populations such as those observed in the Amerindian autochthonous communities, and the HLA-DQ9.3 variant in the Hahn Chinese population [6, 52–54]. However, *HLA* genes account for 40 % of the CD genetic inheritability suggesting the presence of other susceptibility genes. Two genome-wide association studies (GWAS) have discovered 26 loci outside the *HLA* region [55, 56]; The Immunochip approach—which analyzes almost 200,000 single-nucleotide polymorphisms (SNPs) based on associated regions of ten autoimmune diseases—validated the results from both GWAS studies and identified 13 other regions [57]. Interestingly, and although around 95 % of the total identified SNPs are located in non-coding areas, they typically are located close to the transcription initiation sites or the 3′-untranslated region (UTR) of the genes, suggesting that

they control gene expression [58]. Gene polymorphisms associated with CD are also typically related with biological pathways common to other autoimmune diseases as well as with genes involved in the triggering of pro-inflammatory responses [57, 59]. Indeed, although the IFN-γ gene has not been related with CD pathogenesis, it has been described that 15 CD susceptibility genes, which approximately represent 30 % of the total described genes associated with CD, regulate the increased mRNA expression levels of IFN-γ found in the CD mucosa [59] while it has been also suggested that all such genetic polymorphisms would not only be related to Th pro-inflammatory responses (including the Th1, Th2, and Th17 pathways) but also with B-cell phenotype and function [59].

Having identified such novel gene variants conferring susceptibility loci to CD, the next step is to understand how they affect the expression of the genes. Expression quantitative trait loci (eQTL) analyses in CD have revealed a great proportion of DNA sequence variants that affect gene expression in cis-eQTLs [56, 60] (i.e., located at the same locus than the control as opposed to trans-eQTL which regulate gene(s) at a different locus) while several studies agree that besides being tissue-dependent, such eQTLs are also stimulus-dependent and depend on several factors including LPS, influenza virus, or IFN-β [61, 62, 63••]. Finally, the role of non-coding RNA (ncRNA), ranging from microRNAs (miRNAs, average of 22 nucleotides) to long non-coding RNAs (lncRNAs, more than 200 nucleotides), cannot be discarded. Ten percent of the SNPs associated to immune-mediated diseases overlap with lncRNAs [64], and several of them have been associated to autoimmune diseases [65] including CD where miRNAs have been found to be regulated in the mucosa by the presence of gluten peptides both in vivo and in vitro [66••, 67]. DNA methylation studies have focused on CD-related small-bowel adenocarcinoma since there is an increased risk to develop this tumor [68]. Histone acetylation/deacetylation studies have demonstrated a role in the correct functioning of the intestinal epithelium [69]. Importantly, factors such as diet and gender can all alter the intestinal epigenetic profile adding further complexity to the picture [70, 71]. Nevertheless, despite all efforts trying to unravel the genetic determinants that predispose to CD development, only 60 % of its genetic contribution can be explained so far, rendering a total of 40 % of the so-called missing heritability even when taking into account the thousands of variants with small effects in the regulation of the immune response (odds ratio (OR) <1.5) [72]. Besides, it may also be possible that although CD accumulates within families, the genetic determinants may vary between them given that what matters is the induced biological effect of the genetic polymorphism. Therefore, two different families may carry different genetic variants which however could translate into the same "susceptibility phenotype" (e.g., lower IL-15 response threshold). Therefore, it is likely that there are hundreds of different gene susceptibility variants which may be present. We hope that new tools and the systems biology approach will help to identify the different networks and the mechanistic nodes controlling CD susceptibility.

The Intestinal Microbiome in Celiac Disease

Bacterial communities of the intestinal tract collaborate to maintain a tolerogenic environment and contribute to the metabolization of nutrients [73]. As for the gluten digestion, some bacteria like *Bacteroides fragilis* are overrepresented in the CD mucosa and can hydrolyze gliadin to generate toxic peptides [74] while *Bifidobacterium longum* are decreased. They can hydrolyze gliadin to generate non-toxic peptides [75]. Other bacteria that are able to digest gluten are *Lactobacillus*, *Streptococcus*, *Staphylococcus*, *Clostridium*, *Bacillus*, and *Enterococcus*, all of them from the phylum *Firmicutes* [76]. The commensal microbiota is altered in CD patients as compared with healthy controls. *Staphylococcus, Bacteroides*, and *Clostridium*, together with *Escherichia*, are increased in CD patients [77–79]. However, the microbiota composition is also influenced by the GFD, not only in CD patients, as such patients have a lower diversity of *Lactobacillus* and *Bifidobacterium* [80], but also in healthy adult volunteers following the GFD which induces an expansion of *Escherichia* and *Enterobacteriaceae* [81]. Moreover, CD patients with gastrointestinal symptoms also have a different microbiota composition compared with CD patients with extra-intestinal manifestations [82••]. The intestinal microbiota also plays an active role in the pathogenesis by shaping immune responses as it helps to maintain constitutive levels of type-1 IFN hence conferring protection towards intestinal viral infections [83, 84]. Intestinal DC produce IFN-β and IL-15 following microbial product recognition via activation of TLR3 and TLR4 receptors, respectively [85••, 86]. The microbiota can also indirectly modulate intestinal immune responses since, following the processing of dietary fiber, releases immunomodulatory short-chain fatty acids (SCFA) which signal through G-protein-coupled receptors helping to promote oral tolerance although they are decreased in the coeliac mucosa [87]. At present, it is unknown however if the changes in the CD microbiota and their metabolism precedes the disease (hence providing protection or susceptibility to CD) or on the contrary are secondary to the activation and progression of the disease.

Non-celiac Gluten Sensitivity

In addition to CD and wheat allergy, there is a third gluten-related syndrome where the immunological mechanisms are not related to the presence of IgE, like in wheat allergy, or an adaptive immune response characterized by the presence of gluten-reactive T-cells and antibodies directed against TG2 or

deaminated gluten peptides like in CD. This syndrome is thought to arise from an innate immune response to dietary gluten not coupled to a secondary adaptive immune response and is named as non-celiac gluten sensitivity (NCGS), although this term includes patients with different pathogenesis [88]. As a consequence, the diagnosis of NCGS is based on the clinical response to GFD and the exclusion of other syndromes as there is no NCGS-specific biomarker yet identified like in wheat's allergy (presence of IgE) or CD (presence of TG2 antibodies) [89, 90]. Ideally, a double-blinded placebo-controlled challenge would be an effective way to diagnose these patients and discard improvement after GFD due to the placebo effect, although it is difficult to perform in clinical practice [88]. Nevertheless, the relation of NCGS to gluten intake has not been clarified and is a matter of intense debate [91–93, 94••]. Moreover, an important fraction of the patients enrolled in those studies were HLA-DQ2 or DQ8 positive and had IgG antibodies against gluten so they could be patients with a non-severe form of CD [94••]. Besides, it has also been suggested that other components rather than gluten could cause NCGS symptoms. Such culprits could be the Fermentable Oligo-Di-Monosaccharides and Polyols (FODMAPs) [92, 94••] and the amylase-trypsin inhibitors (ATIs) [95]. Hence, ATIs from gluten-containing cereals may signal through TLR4 in monocytes, macrophages and DC from the intestinal mucosa and act as immune adjuvants for gluten in CD biopsy cultures [96]. As a GFD is an ATI-free diet, it has been suggested that patients with ATI sensitivity could follow a more liberal GFD than CD patients [95]. Finally, the GFD is a healthy diet as it avoids the consumption of processed and manufactured foods hence favoring the intake of fresh fruit and vegetables and sauce-free grilled products. Therefore, the acquisition of a healthy lifestyle and diet could also be behind, at least to some extent, of the clinical improvement and increased well-being that several individuals have reported following the introduction of the GFD.

The Maintenance of a Gluten-Free Diet

A permanent strict GFD is the only treatment for CD patients after which villous atrophy slowly returns to normality and the humoral response disappears within months [33••]. As a consequence, the patients experience a clinical and quality of life improvement which ultimately should lead to the healing of the mucosa. Nevertheless, and although the GFD also promotes a healthy lifestyle, following a strict GFD is expensive and difficult to follow for CD patients, which is why so many CD patients perform dietary transgressions [9, 10] either deliberately or involuntarily. As a consequence, there is an increasing need to be able to monitor GFD compliance since, as previously discussed, the lack of complete adherence may be a predisposing factor for development of RCD. Currently, the most common tools to address this issue are diet surveillance through questionnaires and the presence of symptoms and antibody reappearance which ultimately would lead to performance of a duodenal biopsy when required. Nevertheless, and although the presence of circulating antibodies directed towards TG2 or gluten-deamidated peptides are useful on CD diagnosis [97], they do not correlate with histological damage on early stages [98] and hence are not good markers to monitor GFD compliance. Gluten immunogenic peptides (GIP) which are resistant to gastrointestinal digestions and can be found in the fecal content after 3 to 6 days of gluten consumption [99] can be useful biomarkers to assess GFD compliance. GIP can also be detected in urine samples 16 to 34 h after gluten intake, remaining detectable until 3 to 9 h [100••]. Moreover, GIP are detected in urine samples after intake of 25 mg of gluten, which is below the minimum amount of gluten consumption known to cause histological abnormalities in CD patients (50 mg gluten/day) [100••] proving therefore their utility as non-invasive biomarkers (as opposed to a blood test) to monitor compliance to the GFD. GIP detection in urine samples has revealed that almost half of CD patients do not completely adhere to a strict GFD (48 % of adults and 45 % of children CD patients [100••]) while their levels correlate with mucosal damage as opposed to anti-TG2 and anti-gliadin serological tests, which cannot determine compliance to the GFD and/or mucosal healing in CD patients in such a precise manner as GIP detection [100••].

Future Prospects and Alternatives to the GFD

As we have discussed, a better characterization of the factors related to CD and non-CD pathogenesis may help in the development of new treatment strategies alternative to the GFD. Such approaches include the specific targeting of several key players on CD and non-CD pathogenesis including HLA molecules, TG2, IL-15, gluten-reactive T- and B-cells, and even transcellular gliadin transport [101••]. In the last years, antibodies specially blocking the IL-15 pathway have been developed to specifically treat RCD patients, such as AMG714 and Hu-Mik-β-1 [101••], although their use in non-RCD or "classical" CD patients would be problematic given the central role that IL-15 has on the innate immune system. Alternatively, food supplementation with oral proteases may enhance gluten proteolysis in the gastrointestinal tract hence degrading peptides with immunogenic properties as for instance the enzyme cocktail ALV003 (a mixture of Gln-specific cysteine endoprotease B, isoform 2 (EP-B2), from germinating barley seeds and prolyl endopeptidases (PEPs) from *Sphingomonas capsulate*) which attenuates gluten effects in CD patients on a GFD (Fig. 3) [102]. Similarly, epitope blockage with egg yolk antibodies against gliadin [103] or non-absorbable gliadin sequestering polymer BL-7010 [104] could be used to prevent gliadin effects on the

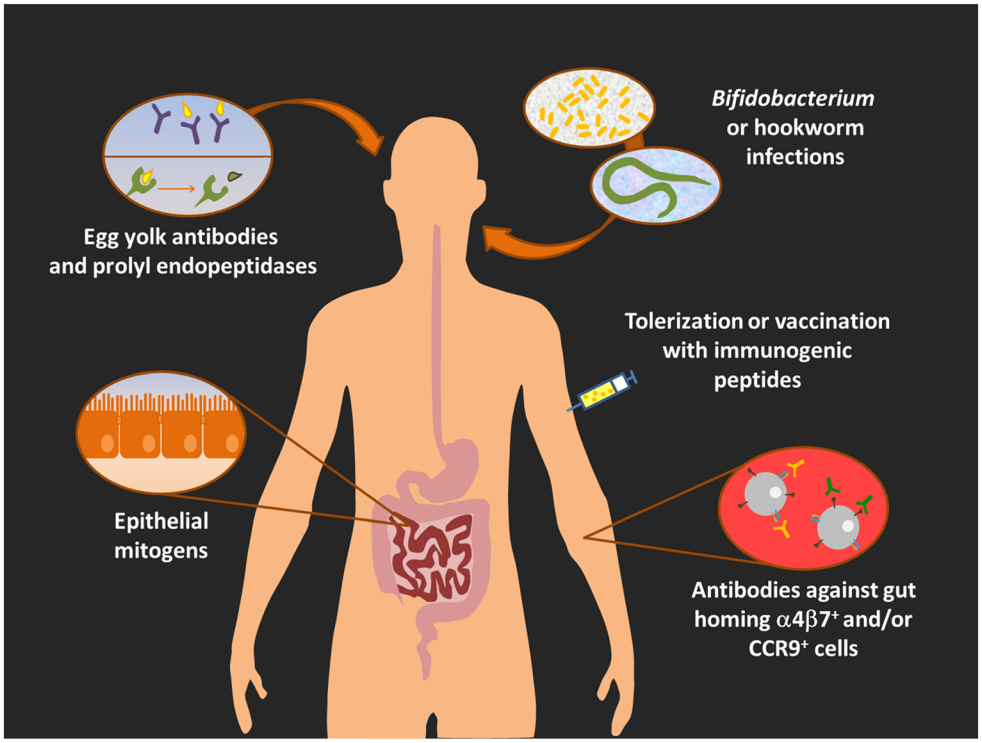

Fig. 3 Alternative therapies for CD patients. Besides targeting the key players of CD pathogenesis, other approaches could be used such as *Bifidobacterium* or hookworm infection; oral administration of egg yolk antibodies, sequestering polymers, and prolyl endopeptidases before gluten intake; treatment with epithelial mitogens and inhibitors of intestinal permeability; administration of antibodies against gut homing markers or tolerization; and vaccination with immunogenic peptides. *CD* celiac disease, *CCR9* chemokine (C–C motif) receptor 9

intestine for a short period during gluten ingestion. Another adjuvants to GFD focused on alleviation of the symptoms or on tolerance of trace amounts of gluten could be the treatment with the probiotic *Bifidobacterium infantis natren* [105], the infection with the human hookworm *Necator americanus* [106], the treatment with antibodies blocking leukocyte migration to the gastrointestine (including vedolizumab, which blocks the $\alpha 4\beta 7$ integrin, or antibodies directed towards the small-bowel chemokine receptor CCR9) [107], or even the treatment with epithelial mitogens that stimulate the growth of the epithelial layer [108] and inhibitors of intestinal permeability (i.e., larazotide acetate) [109] (Fig. 3). On the other hand, it would be desirable to permanently avoid the effects of gluten without damaging the intestine. In this regard, gluten tolerization [110] or vaccination (i.e., Nexvax2) [111] with gluten peptides could achieve this goal (Fig. 3); however, the complex composition of gluten, including multiple and different peptides and epitopes which are recognized by different HLA-DQ molecules, makes it difficult to develop a universal treatment for all the CD patients [27]. Nevertheless, we cannot forget that although expensive and difficult to follow, there is already a treatment for CD called GFD so all these new therapies should be at least as safe and affordable as the GFD which certainly would be cheaper than a drug-based therapy. Nevertheless, it is also true that such new therapies would certainly provide some relaxation on the diet allowing the patients to maintain sporadic dietary transgressions under control hence having a positive impact on their social life and well-being.

Conclusions

CD is the most important food intolerance in western countries being its only treatment at present a permanent strict GFD. As reviewed here, recent evidence has provided a deeper insight into the different factors contributing to its pathogenesis at the time that we are also getting a better understanding of the factors related to the development of another non-CD gluten intolerance syndrome like NCGS and developing new tools for a better monitoring of compliance to the GFD in a less subjective and non-invasive manner. New advances in the elaboration of gluten-free products are on the horizon, and we hope they will not only improve the palatability and the nutrition qualities but will also reduce the price of the products.

In conclusion, a better understanding of the mechanisms governing the development of gluten-related disorders together with the development of GFD compliance tests and novel adjuvant treatments is starting to have an impact on the management of patients with CD and gluten-related disorders. We hope that major complications such as osteoporosis, RCD, and malignancy will be prevented with the application of these advances to clinical practice.

Compliance with Ethical Standards

Conflicts of Interest CEH, ASP, and DB declare that they have no conflicts of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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