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Immunopathogenesis and Therapeutic Approaches in Pediatric Celiac Disease

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Summary

Celiac Disease is an autoimmune enteropathy with increasing incidence worldwide in both adults and children. It occurs as an inflammatory condition with destruction of the normal architecture of villi on consumption of gluten and related protein products found in wheat, barley and rye. However, the exact pathogenesis is not yet fully understood. A gluten-free diet remains the main modality of therapy to date. While some patients continue to have symptoms even on a gluten-free diet, adherence to this diet is also difficult, especially for the children. Hence, there is continued interest in novel methods of therapy and the current research focus is on the promising novel non-dietary modalities of treatment. Here, we critically reviewed the existing literature regarding the pathogenesis of celiac disease in children including the role of *in-utero* exposure leading to neonatal and infant sensitization and its application for the development of new therapeutic approaches for these patients.

Keywords

Celiac disease; IL-15; gluten sensitivity; autoimmunity; villous atrophy

INTRODUCTION

Celiac disease (CD) is a chronic inflammatory disorder of the small bowel seen in genetically predisposed individuals [1]. European Society for Pediatric Gastroenterology and Nutrition (ESPGHAN) defines it as an immune-mediated condition seen after ingestion of

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Declaration of Interests

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gluten containing diet in genetically predisposed individuals with variable combination of clinical symptoms, CD specific antibodies and HLA DQ2 or DQ8 positivity [2].

The first description of celiac disease dates back to 250 A.D. by a Greek physician named, Aretaeus of Cappadocia [3]. Dutch physician, William Dicke was the first one to identify the role of wheat in triggering celiac disease [4], while the histological features were initially described by John W. Paulley [5]. The revolutionary discovery of CD specific antibodies was made in early 1990s which then provided a basis of serological diagnosis of CD [6].

It is a T cell-mediated disorder which gets activated on infiltration of gliadin into the lamina propria [7]. This triggers the release of pro-inflammatory cytokines, including IFN- γ and IL-15. This further activates cytotoxic T cells that cause the characteristic villous atrophy in CD patients [8]. Although there is strong predisposition in children with high levels of HLA DQ2 and DQ8, it does not explain the entire risk of acquiring celiac disease. Therefore, it is considered due to interplay of many factors, including environmental and epigenetics. Gluten free diet remains the cornerstone for treatment. But adhering to this diet can be difficult, especially for the children and hence, there is a need to develop novel non-dietary therapeutic approaches. Though many clinical trials have been ongoing in adults with CD, they are yet to be conducted in pediatric population.

EPIDEMIOLOGY

Once considered a disease of European origin, it is now believed to be widely distributed with increasing prevalence throughout the world [9]. Recent studies show that prevalence in USA is 0.71% (1 in 141) [10], which is comparable to the incidence in European countries, including Finland 1.01% (1 in 99) [11], Sweden 0.53% (1 in 188) [12] among others. CD is prevalent even in African (0.5% in Egypt and 0.8% in Libya) and Middle East countries (0.8% in Iran and 0.6% in Turkey), but the reporting has been very low, possibly due to the limited availability of resources [8,13]. Although limited data is available from Asia [14], several studies have been conducted in India and prevalence was found to be high, especially in the northern part which is best described as the 'celiac belt' of India, and this can be attributed to an extent to their staple diet of wheat [15]. The worldwide distribution of CD is not surprising given the globalization of food market [8]. CD epidemiology has been typically described as an iceberg phenomenon, where the size of the iceberg is represented by the total number of cases, diagnosed or undiagnosed, while the tip of the iceberg indicates the already diagnosed cases usually presenting with classical symptoms [13]. The submerged part of the iceberg comprises of the cases that went undiagnosed owing to minimal, absent or non-classical symptoms [13]. Celiac disease is also commonly associated with other autoimmune disorders, including type 1 DM [16], autoimmune thyroid disorders [17] and rheumatoid arthritis [18].

CLINICAL FEATURES

Traditionally considered a primary gastrointestinal disease, it is now well established that celiac disease is a multiorgan disorder. The clinical manifestations may vary depending on the age of presentation, duration of disease, amount of gluten in diet, presence of other auto

immune diseases [19]. Children less than 2 years present primarily with symptoms like diarrhea, vomiting, malnutrition, abdominal bloating and anemia, whereas older children present with loose stools, growth delay, pubertal delay and so on [20]. Usually when some autoimmune disease is associated with celiac disease, like diabetes mellitus, CD is often silent and is recognized only after serological screening for CD in these patients after the autoimmune disease has been recognized [21]. This underlines the importance of screening for CD antibodies in such patients.

According to the Oslo definition [22], the term classical CD has been used to describe patients with signs and symptoms of malabsorption like diarrhea, steatorrhea, weight loss. Pediatric equivalent of classical CD refers to children presenting with symptoms, including failure to thrive, muscle wasting, diarrhea, abdominal distension, loss of appetite. Non-classical CD patients do not have signs and symptoms of malabsorption. Potential CD term is preferred over latent CD and is used to describe people with normal small intestinal mucosa with increased risk of CD as identified by positive serology in first degree relatives. Refractory CD (RCD) is used to describe persistent or recurrent signs and symptoms of malabsorption without villous atrophy in spite of a gluten free diet for more than a year.

DIAGNOSTIC TESTS

Diagnosis of CD has undergone a lot of modification in past few years. Traditionally, intestinal biopsies are considered gold standard for confirming the diagnosis of celiac disease [2]. But, according to the current ESPGHAN recommendations, in symptomatic children, if there is positive anti-endomysium antibodies (anti-EMA) and anti-tissue transglutaminase (anti-tTG) IgA antibody titers more than 10 times the normal with positive HLA DQ2/8, then intestinal biopsy can be avoided [2]. The anti-tTG IgA antibodies titer and histological lesions have an inverse correlation with the age [23]. Thus, as the age of presentation increases, the antibody titers decrease and the histological lesions become less evident [22]. Interestingly, Makovicky et al. [24] highlighted the importance of performing biopsies for the accurate diagnosis of CD in pediatric population. These investigators reported that certain pediatric patients had active CD even when seronegative. The lack of association between autoantibodies and disease activity justifies the use of biopsy for diagnosis and need for revision of current diagnostic criteria.

In case of an IgA-deficient person, anti-deamidated gliadin peptides (anti-DGP) antibodies come into play and serve as the marker antibodies. Also, it has been suggested by Amarri and colleagues [25] that anti-DGP antibodies have a superior diagnostic accuracy compared to anti-EMA and anti-tTG antibodies in children below 2 years of age. IgG anti DGP antibodies are much more sensitive and specific compared to IgA, making them the investigation of choice [24, 26].

HLA DQ typing has a high negative predictive value and hence, is recommended by ESPGHAN as a test for screening in asymptomatic but at risk children [2]. An algorithm for diagnosis of CD as suggested by ESPGHAN is summarized in Figure 1.

PATHOGENESIS

Gluten is a protein present in wheat, rye and barley. The complex interaction of this protein with genetic, environmental and immunologic factors forms the basis for the pathogenesis of CD.

1. GENETIC PREDISPOSITION

HLA is the single most important genetic risk determinant for CD, with HLA class II molecules accounting for 35% of the genetic risk [27]. Celiac disease patients mostly express HLA DQ2 and DQ8 positivity, with stronger association with HLA DQ2, especially HLA DQ 2.5. Disease susceptibility depends on the dosage effects of HLA DQ2.5 heterodimer [28]. Individuals with homozygous DR3-DQ2 haplotype or heterozygous DR3-DQ2/DR7-DQ2 have the highest levels of 2.5 heterodimers, and thus, even the highest risk of CD. Refractory CD patients have higher levels of homozygosity for HLA DR3-DQ2 haplotypes compared to other CD patients [29]. The gene dosage effect was proven in an Italian study conducted in twins, where heritability estimates in monozygotic and dizygotic twins were found to be more than 80% and 50%, respectively [30].

1.1 ROLE OF HLA—The molecular basis for the linking of CD with HLA DQ is thought to be related to the physiochemical properties of MHC molecules [28]. They have positively charged pockets that can bind strongly to the negatively charged molecules. Lysine at β 71 position of HLA DQ2, renders it the property to bind to negatively charged peptides at positions P4, 6 and 7 [31]. Additionally, both HLA DQ2 and 8 lack aspartic acid at position β 57, which renders them basic at position P9 and this site can also strongly bind to negatively charged gluten peptides [32]. HLA DQ8 polymorphism at β 57 position enables it to switch from an interaction with negatively charged TCR residue to an interaction with negatively charged gluten peptide residue [28].

The immunological basis of HLA gene dosage effect is that the expression of HLA DQ and the number of available T cell stimulatory gluten peptides are critical limiting factors to reach the threshold for disease development. Homozygous individuals express more HLA DQ2.5 and HLA DQ8 on the surface of antigen presenting cells (APCs) and can therefore, induce a larger magnitude of T cell response. But, some variants of HLA DQ2, such as HLA DQ2.2, are not associated with CD. This can be explained in the way that DQ2.2 carries a phenylalanine at position 22 of α -chain, whereas DQ2.5 carries a tyrosine at the same position. This tyrosine is able to form stable hydrogen bonds with the main peptide chain, and can therefore, form more stable complexes with gluten peptides which results in sustained presentation to T cells, thereby increasing the possibility of reaching a pathogenic threshold for T cell response [33].

Thus, it is possible that the T cell response must reach a threshold to be pathogenic to cause CD. This can be attained by selecting HLA molecules that form more stable complexes with gluten peptides (DQ2.5 vs DQ2.2), or by increasing the number of HLA molecules (dosage effect).

1.2 ROLE OF NON-HLA LOCI IN CD PATHOGENESIS—Although HLA is the most important genetic determinant of CD, recently many non-HLA genomic regions have also been found to be associated with CD. Genetic wide association studies (GWAS) have identified more than 58 non-HLA risk variants within 40 different loci [34]. These non-HLA loci have a very small impact on the predisposition to CD and together they account for only about 5% of the genetic heritability [35]. This indicates that still 50% of the genetic heritability factors are yet to be discovered. These non-HLA loci have also been found to be rich in immune genes, especially for those encoding for chemokines, cytokines, lymphocyte differentiation which all may mediate T cell immune response in CD. NK cell and IFN- γ pathways that are selectively enhanced for CD are also encoded. Some of these are even involved in T cell selection in thymus, like ETS1 (E26 Transcription Specific 1), Thymocyte expressed molecule in selection (THEMIS) and few of them codify cytokines like Interleukin 2 (IL-2) and IL-21 [7].

Some of the genes have been identified by GWAS studies that could potentially be related to CD pathogenesis and hence, their blockade could serve as a potential treatment option. These genes are discussed below.

1.2.1 THEMIS: Thymocyte expressed molecule in selection (THEMIS) was identified on the peak of 6q22.33 in the celiac disease-specific GWAS [36]. THEMIS is basically involved in T cell maturation with mainly positive selection of thymocytes towards maturation [37]. Studies in knockout mice have also suggested its role in suppressive action of CD4⁺ T cells, thereby implicating that the defect might lead to inflammation [38]. Recent study conducted in Europe to evaluate its expression in active and treated CD patients compared to controls, found higher expression of THEMIS in duodenal mucosa of active CD patients compared to treated CD and controls [39]. This might potentially be related to the dysregulation of T cell function, thereby promoting the occurrence of CD.

1.2.2 PTPRK: Protein tyrosine phosphatase, receptor kappa (PTPRK) was also found to be expressed on 6q22.33 in celiac GWAS along with THEMIS [36]. Many studies have suggested the role of PTPRK in maintaining intestinal barrier through cell-cell adhesion promoted by homophilic binding [40]. Thus, PTPRK gene might be involved in CD pathogenesis by causing dysregulation of intestinal barrier function. Recently, lower expression of PTPRK in active disease patients compared to treated CD has been found [39]. Further studies are required to evaluate its exact role in CD pathogenesis and if this could serve as a potential treatment option.

1.2.3 FUT2: This gene expressed on 19q13.33, encodes for an enzyme, α -1, 2-fucosyltransferase (FUT), that influences the expression of blood group antigens (A, B, H) in mucus and other body secretions [41]. These antigens are also expressed in gastrointestinal mucosa where they control the microbial flora. Therefore, it has been suggested that they play a role in protection from pathogenic microbes and influence the development of mucosal immunity [36]. Studies have shown FUT2 gene secretor status is associated with diverse intestinal microbiota which in turn, increases the secretion of inflammatory cytokines, including IFN- γ , IL-12 and others [42]. Thus, by influencing the intestinal microbe composition, FUT2 gene may be involved in the pathogenesis of CD.

2. GLUTEN

Gluten is a protein present in wheat, rye and barley. The alcohol soluble component of gluten is gliadin, which is considered to be the toxic component and is responsible for the pathogenesis of CD [4, 43].

Most of the gliadin peptides are digested by pancreatic, gastric and intestinal enzymes except the peptides 33mer (P55-87) and 25mer (P31-55) and hence, these two active peptides are responsible for the activation of immune response in celiac intestine [43, 44]. The 33mer is deamidated by TG2 enzyme, then binds to HLA DQ2/8 and initiates Th1 cell response, whereas the P31-43 contained in the 25mer is not recognized by the T cells, but, it induces an innate immune response with production of IL-15 as the primary component [45]. Also, the 25mer can induce proliferation of enterocytes via epidermal growth factor (EGF) and interleukin 15 (IL-15), resulting in crypt hyperplasia, one of the characteristic findings in celiac mucosa remodeling in CD.

2.1 Mechanism of inducing proliferation of IL-15 and EGF by P31-43 gliadin peptides—Gliadin peptides P31-43 mimic the role of EGF in regulating cell proliferation and rearrangement of actin in multiple cell lines in CD intestine [46]. They enhance the EGF-mediated pathway by increased phosphorylation of EGF receptors (EGFR) [44]. This occurs as a result of delayed endocytosis. It has now been shown that P31-43 can influence the endocytic pathways because it bears similarity to a region of hepatocyte growth factor-regulated substrate [HRS] kinase in a small region of proline/glutamine rich domain [47]. HRS is located on the membrane of early endocytic vesicles and is involved in endocytic maturation. P31-43, because of its homology and location, interferes with the correct localization at the level of early endosomes, thereby delaying their maturation into late endosomes. As a result, there is decreased degradation of EGFR, which is endocytosed in these vesicles, hence, prolonged activation of EGFR and IL-15. Consequently, at the molecular level there is increased proliferation of enterocytes, alteration in permeability, and increased innate immune response [44].

3. ENVIRONMENT

While there may be many carriers of HLA DQ2 and DQ8, not all of them develop CD. This indicates the significance of interaction with environmental factors.

3.1 Gut microflora and CD—Gut microflora is a collection of various different microorganisms that exist in the gastrointestinal tract. These organisms comprise the gut microbiota that performs many functions in the bowel including aiding in digestion and absorption of food, tolerance to non-self-antigens in food and resistance to pathogens [48].

During the first few years of life, the microbial population is not stabilized, and during this period, microorganisms gradually inhabit the intestine and exert their effect on development of the immune system. During early postnatal period, intestinal microbiota stimulates the development of both local and systemic immunity, and later they induce inhibitory mechanisms to keep both mucosal and systemic immunity regulated [49]. Additionally, this microflora induces a transient physiological inflammation in the gut with expansion of

mucosal-associated lymphoid tissue [50]. It has been postulated that microbial colonization of animals in germ free conditions results in an increase in immunoglobulin levels, specific antibodies and changes in mucosa-associated lymphoid tissue [51]. Also, this could alter the synthesis of sugar chains in membrane-associated glycoproteins, which could modify the intestinal barrier permeability [52]. This impaired functioning of the intestinal barrier could lead to an increase in the antibodies against antigens on intestinal lumen, and they might have a predictive role in the development of inflammatory and autoimmune diseases, including CD, especially in patients with positive antibodies with no clinical symptoms [53]. Though the role of intestinal microflora is to protect the body against harmful pathogens, in certain conditions, some bacterial species, especially the ones found in low abundance are capable of causing disease by producing inflammation, infections or even increased risk of cancer. Therefore, infections are considered one of the triggers for autoimmune diseases, such as CD.

3.2 Infections—Another major environmental factor having an influence on the risk of developing CD is early infection. It has been postulated that infections at an early age might cause CD due to increased permeability among epithelial cells allowing entry of proteins, such as gliadin across the lumen to reach the lamina propria and induce the immune response [54, 55]. There is disruption of the normal balanced state of gut microbiota caused by infections termed as ‘dysbiosis’ and these microbes are considered ‘pathobionts’.

In Spain, a study was conducted to investigate dysbiosis in patients with CD, both before and after treatment with Gluten Free diet (GFD) [56]. They found that *Bacteroides* and *Clostridium* were more abundant in biopsies of CD patients, regardless of the treatment, while *E. coli* and *Staphylococcus* were higher in untreated CD patients and normal on treatment with GFD. *Bifidobacterium* levels were lower in CD patients compared to controls. Similar findings were also reported by Di Cagno et al [57] and Collodo et al [58].

Furthermore, infections cause chronic inflammation and tissue damage owing to disruption of intestinal barrier, which can reduce the gluten tolerance. They might even initiate the process of CD in genetically predisposed individuals. One of the proposed mechanisms is termed as ‘antigen mimicry’, that is, similarity between epitopes of autoantigens and epitopes of harmless environmental antigens. So, the pathogenic microbes might activate the APCs and cause abnormal presentation and processing of self antigens, thereby initiating autoimmunity and chronic intestinal inflammation [59].

3.3 Perinatal exposure—Recent studies have described a link between CD in susceptible individuals and infectious agents, which may occur even during perinatal period. Prenatal treatment with antibiotics for the pregnant mother toward term pregnancy can disrupt the diverse balanced microbiota necessary for the development of an appropriate immune response in the offspring. Infants born to mothers treated with antibiotics in the peripartum period were reported to have an increased incidence of asthma and CD in a study [60].

Non-digestible oligosaccharides in breast milk promote function as prebiotics causing proliferation of lactobacilli and bifidobacteria, causing fermentation of oligosaccharides into short chain fatty acids emphasizing the importance of breastfeeding. Infants who are not

breastfed have theoretically increased risk for developing CD due to inadequate colonization and failure of development of a normal mucosal immune system [58].

Increased risk for developing CD has also been reported in children born via scheduled caesarean section compared to those who had emergency caesarean section where the infants had exposure to the maternal vaginal flora during the initial phase of early labor, again emphasizing the importance of normal flora [61].

A prospective Norwegian mother and child cohort study reported an increased Odds Ratio (1.48) for developing CD in the infants of mothers who were treated with iron supplementation during pregnancy [62]. The increased risk persisted even after adjusting for maternal CD, age, sex of the child, and the time of gluten introduction. Even though the exact mechanism is unknown, it is postulated that proteins related to iron metabolism—hepcidin and lactoferrin influence the innate immunity [63]. Maternal and fetal iron status influence gut microbiomes as depicted by a 2-fold increased incidence of CD in patients with hereditary hemochromatosis [64]. A clear mechanism for the role of iron in the pathogenesis is yet to be established and thus it is premature to argue against maternal iron therapy when clinically indicated.

Role of maternal gluten consumption during late gestation and the risk of CD in the offspring was evaluated by the TEDDY study group (environmental determinants of diabetes in young) but no significant correlation was found [65].

In a study conducted at Sweden, the investigators compared the infants who developed CD to healthy infants born in the years 1987–1997. After adjusting the maternal variables, exposure to neonatal infections was found to be one of the major factors predisposing infants to develop CD [66]. Plot et al [67] suggested that some infections like cytomegalovirus (CMV), Epstein Barr virus (EBV), Rubella might be protective against development of CD by establishing an immunological background that does not favor the progression of CD while several other infections, like rotavirus, HCV, adenovirus, may intensify the gut response to gluten and further predispose to CD [67].

3.3 Diet—Dietary factors are especially important in the pathogenesis of CD in neonates where the gut is immature and immunological system is still developing and acquiring tolerance to self-antigens. It was found that in high-risk infants, there were five-fold more chances of developing CD autoimmunity if gluten was introduced within the first three months of life. Again, the risk was also higher if gluten was introduced for the first time after 7 completed months of age. One of the explanations for this phenomenon has been given by Szajewska et al. (PREVENTCD group) [68] according to which early introduction (<3 months) of gluten containing food might lead to intolerance because the immune system is not fully developed, whereas late introduction (after 7 months) of gluten might cause intolerance due to exposure to higher amounts of gluten at first. Only one study has described the amounts of gluten intake and risk of CD. This was undertaken by Ivarsson et al [69] in Sweden, where they defined cut-off for gluten containing flour at 58 g/day and if consumption falls in upper one third of this cut-off, it was considered as large amount. The definition of large amounts is, therefore, arbitrary and requires further exploration. In the

United States, a prospective observational study conducted by Norris et al [70] on children over a period of ten years (1994–2004) also concluded that CD risk was five-times higher in children with exposure to gluten before three months of age or after seven months. Therefore, according to current ESPGHAN guidelines [2], gluten containing foods should be introduced around 4–6 months of age while the child is still breastfeeding and the quantity should be increased gradually.

There have been some studies that show protective role of breast-feeding in delaying CD. One of the studies, conducted by Ivarson et al [69] which is among the largest case control studies till date, showed that in children <2 years of age, risk of CD was reduced if gluten was introduced while the child is still breastfeeding and the risk was increased if large quantity of gluten was introduced in diet. The main reasons of protective effects of BF could be that human milk contains anti-inflammatory and anti-microbial agents, enzymes, hormones, and other substances that are involved in the maturation of gut and immune system and also in the protection from infections [71]. It might also help in the induction of tolerance to gluten because of possible transfer of small amounts of gluten and gluten-specific IgA antibodies through breast milk.

IMMUNOPATHOGENESIS

Though the exact mechanism underlying the pathogenesis of celiac disease still remains unknown, the pathognomonic feature is immune-mediated enteropathy on consumption of gluten containing diet.

The **adaptive immune response** to CD is mediated by T helper cells. Gluten gains access to peyer's patches physiologically through M cells and additionally during periods of increased intestinal permeability as in infections. They are then processed by dendritic cells and presented to CD4 T helper cells. Normally, gluten peptides cause a Th2 immune response whereas in CD patients, gluten peptides induce a Th1 cell response in the presence of deamidated gluten peptides in APCs. The deamidation of gluten peptides is carried out by tTG enzyme [72]. This enzyme is normally present in an inactive state intracellularly and released upon tissue damage. Normally, gliadin peptides carry only a few negative charge residues, but in active CD, tTG levels are highly increased and therefore, deamidation produces a larger negative charge on these peptides, and they can bind with higher affinity to HLA DQ2 and DQ8 [4]. These HLA molecules then present the gluten peptides to CD4+ T cells and there is release of pro-inflammatory cytokines such as TNF- α , IL-1 β , IL-15, IL-21, IL-22 and others. These cytokines then activate cytotoxic CD8⁺ T cells which in turn promote tissue remodeling and damage [73]. There is also increased production of IFN- γ , which further elaborates the expression of HLA DQ and thus, increased presentation of gliadin. So, this functions as a self amplifying loop in the presence of gliadin and causes ongoing intestinal damage and inflammation [74]. The damage to intestinal mucosa, in turn, activates more of tissue transglutaminase that will alter more of gliadin peptides into negatively charged particles, promoting interaction with HLA DQ and hence, the commencement of second subset of self amplifying loop [74]. There is also stimulation of the B cells, which then secrete anti-gluten and anti-tTG antibodies. Although the exact role of these autoantibodies in the pathogenesis of CD is not known, it has been considered that

anti tTG IgA antibodies can inhibit blood vessel proliferation and increase their permeability to large molecules and lymphocytes, which could potentially expand the inflammatory response in small intestine [72]. Lebreton et al [75] studied the effect of anti-gliadin IgA antibodies in celiac pathogenesis and their interaction with CD71. They concluded that anti-gliadin IgA antibodies bind to CD71 on the apical membrane of enterocytes, bypasses lysosomal degradation and this allows the transcytosis of the bound gliadin peptides. Recently, a new subset of T cells has been found to be involved in CD pathogenesis, Th17 cells, especially via the release of one of the isotypes of IL-17 cytokine, IL 17A [76]. Ivan Monteleone et al [77] used real-time PCR and ELISA techniques to show that the levels of IL-17A were increased in biopsy specimens of patients with active CD as compared to inactive CD indicating they might be involved in villous atrophy. They are usually co-expressed with IFN- γ . Also, they are found to promote the secretion of granulocyte-colony stimulating factor by epithelial cells, thereby increasing neutrophils production and promoting inflammation [78].

Role of Intraepithelial Lymphocytes (IELs)

Increased population of IELs is one of the characteristic findings in CD but their role in the pathogenesis has not been fully identified. Few recent studies showed that IELs might be related to the development of refractory CD (RCD) and enteropathy-associated T cell lymphoma [79, 80]. IELs may be involved in causing epithelial cell damage by conversion from antigen specific T cells to NK like cells that cause stress-induced damage to epithelial cells and this in turn, may be mediated by IL-15 [80]. In active CD, IELs have increased expression of NKG2D and NKG2C receptors, which interact with their ligands MICA and HLA-E on the intestinal epithelial cells. This interaction leads to death of intestinal epithelial cells and release of enzymes like perforins and granzymes, resulting in tissue damage and villous atrophy. They may undergo T cell monoclonal rearrangement, which can lead to the development of RCD. They also initiate the anti-apoptotic pathway by activation of JAK (Janus kinase), STAT5 (Signal Transducer and Activator of Transcription) and anti-apoptotic factor Bcl-xL, which again promotes the development of RCD [81].

The **innate immune response** occurs in the epithelial cells of the intestinal mucosa. It is mediated by increased production of cytokines, predominantly IL-15, on exposure to gliadin peptides (P31-43) by the macrophages, dendritic cells and enterocytes. This subsequently causes differentiation of intraepithelial lymphocytes into cytotoxic CD8⁺ T cells that express the NK cells marker NKG2D. IL-15 specifically upregulates the expression of NKG2D and licenses these cells to kill IEC expressing the stress-induced ligand MICA [82,83]. Apart from this, it lowers the threshold of TCR activation, seen *in vitro*, which can thereby help in CD8⁺ T cell-mediated destruction of cells not expressing the cognate antigen, and also increased ability for recognition of self antigens [84,85], again resulting in villous atrophy and crypt hyperplasia. In the following section, role of IL-15 in CD is critically discussed.

ROLE OF IL-15

Although IL-15 is upregulated in all cases of CD, the pattern of upregulation is different in different types of CD. For instance, IL-15 in patients with active CD is increased in both intestinal epithelium and lamina propria while those with potential CD do not express

increased IL-15 in intestinal epithelial cells [86]. This indicates that there might be a role of IL-15 in different layers of the intestine depending on the subtype of CD in the patient (Figure 2).

Function in loss of oral tolerance to Gluten—IL-15 is one of the key regulators of immune response in CD patients. Since it is upregulated in both IECs and lamina propria of small intestine, it can affect various immune mechanisms, which contribute overall to CD. IL-15 is found to play a role in loss of oral tolerance to gluten in CD patients. It inhibits the regulatory T cells, and interferes with the activity of CD4+ CD25+ FoxP3+ regulatory T cells in intestinal mucosa of CD patients [8]. One study [86] used a mouse model to show that when IL-15 is increased in lamina propria, it can combine with retinoic acid to alter the tolerance of dendritic cells in intestine, thereby preventing the generation of FoxP3+ regulatory T cells to dietary gluten and promoting the development of Th1 cells-induced inflammatory response and hence, loss of tolerance to oral gluten. Also, it was found that there was increased production of anti-TG2 antibodies in mice with overexpression of IL-15 in lamina propria, without any villous atrophy. This indicates that the site of IL-15 overexpression decides the type of pathological changes. In case of increased IL-15 only in lamina propria, there is development of potential but not active CD, because of the absence of villous atrophy [87].

Role of IL-15 in enterocyte damage—Villous atrophy is mainly mediated by cytotoxic T cells in CD that identify stress signals to kill epithelial cells. In healthy persons, IELs predominantly express the inhibitory CD94/NKG2A receptors with lower levels of NKG2D receptors [87]. On the contrary, in CD patients, there is a much higher expression of NKG2D and CD94/NKG2C receptors and absence of NKG2A expression [86]. It is IL-15 that increases the expression of CD94 and NKG2D on IELs by increasing their transcription and also upregulates the expression of their ligands [MICA and HLA-E] on the enterocytes, thereby promoting apoptosis of enterocytes [87]. Moreover, IL-15 acts as a costimulatory molecule for the NKG2D cytolytic pathway and promotes the release of IFN- γ and perforin granzymes against enterocytes [88, 89]. It further lowers the threshold of IELs for activation, thereby resulting in destruction of epithelial cells in the presence of low affinity self antigens in a TCR-dependent manner [86].

Role of IL-15 in Refractory CD—In RCD, sustained high expression of IL-15 results in the expansion of CD3- cytotoxic IELs that have a highly activated NK cell like phenotype (Figure 2). In turn, this activates the anti-apoptotic pathway involving the phosphorylation of JAK3 and STAT5, thereby promoting villous atrophy. The anti-apoptotic effects of IL-15 promote the emergence of T cell clones, subsequently causing malignant complications of CD and also RCD. They are characterized by ongoing accumulation of aberrant clonal IELs, including CD8-, CD4- TCR $\alpha\beta$, etc. in the intestinal epithelium. Levels of TNF- α and IL-6, in particular, are increased in cases of RCD in contrast to uncomplicated CD patients where IL-21 and IFN- γ were increased [7]. Thus, in the future blocking IL-15 could serve as a potential form of treatment of RCD.

TRANSLATIONAL APPLICATION OF IMMUNOPATHOGENESIS: TREATMENT

Current treatment: Gluten Free Diet (GFD)

Till date, the only effective treatment found for CD is gluten-free diet [90]. Lifelong adherence to it is thought to produce remission in these patients. There is some evidence showing that GFD can even reduce the risk of malignancies in these patients [91]. But, there is still no consensus as to the amount of gluten acceptable in GFD and the daily amount of gluten that can be permitted in CD patients [92].

Though it is a safe and effective form of treatment, it can be difficult to be compliant with this diet, especially for the adolescents, teenagers and also during travel, parties and other social gatherings. Multiple studies have been performed earlier to assess the quality of life (QOL) of patients on GFD. One study done in adult CD patients showed that their QOL was reduced while on a GFD and they continued to have some GI discomfort [93, 94]. Another common complaint of patients on GFD is constipation because of the lack of fibers in their diet [95].

FUTURE TREATMENT MODALITIES

Many studies are currently under investigation to find other modalities of treatment for CD patients keeping in view the difficulty to be compliant with GFD and its effect on the QOL of patients. The immunological understanding of the pathogenesis of the disease has helped in the development of targeted approaches in the treatment strategies (Figure 3).

1. Minimizing the exposure to gluten in grains

McAllister and Kagnoff [96] suggested selective breeding and genetic manipulation of the disease-activating grains to achieve this goal. Gil-Humanes and colleagues [97] used RNA interference technology to decrease the expression of gliadins in wheat. This resulted in decreased immunogenicity of glutens and hence, decreased expression of T cell response. Another possibility could be enzymatic treatment of gluten containing grains to decrease their expression. A pilot study done by Di Cagno et al [98] showed that the use of lactobacilli and fungal proteases reduces the amount of gluten to <10 ppm during sourdough fermentation. They then enrolled CD patients in remission for the study and found that they could tolerate well but a registered well-controlled clinical trial is yet to be performed, before introducing this as a treatment modality.

One challenge with this technique is that there are many immunogenic epitopes located on different loci within the genome, so silencing all of them does not appear to be a feasible approach [99].

2. To diminish gluten absorption

Another possible way to decrease the gluten absorption is the use of high molecular weight copolymer that can combine with gliadin, prevent its absorption and hence, its interaction with T cells. This method was examined in mice with the use of polyhydroxy methylacrylate and polystyrene sulfonate copolymer to bind to gliadin [100]. This prevented its digestion to

smaller peptides and hence, no immune response occurred. But, there might be other proteins that may react with this polymer and limit its use in human [101].

3. Use of enzymes to facilitate gluten digestion (granzymes)

The toxic components of gluten, which is resistant to proteolysis by gastric, pancreatic and brush border enzymes, are proline- and glutamine-rich peptides. As a result, gluten is only partially digested by human and this partially digested form then activates immune system in susceptible individuals, causing CD [102]. Therefore, one of the approaches that could be used is the use of enzymes that can break the bond between proline and glutamine in gluten and render it non-immunogenic. Microorganisms such as *Flavobacterium meningosepticum* and *Sphingomonas capsulata* produce prolyl endopeptidases that can digest these bonds but most of them are rendered inactive in acidic pH of stomach and thus, do not even reach the intestine [98]. Gass et al. used a combination of glutamine specific endoprotease from barley (EPB2) and prolyl endopeptidase from *S. capsulata*, and found that this combination could effectively break down glutamine *in vitro* and in an *in vivo* model of rat [103]. These two enzymes could break down gluten to nontoxic levels and even remains active even in acidic pH.

Based on this principle, a drug named ALV003 is developed [104], which is an orally administered combination of two recombinant proteases-cysteine endoprotease from barley and prolyl endopeptidase from *S. capsulata*, to digest gluten to non-immunogenic fragments. This drug is under clinical trials in adults and has already successfully cleared up to phase IIA of the trial. Because of the encouraging results, it has now being planned to enter into phase III clinical trial as an adjunct to GFD [99].

4. Altering the intestinal permeability

Tight junctions between the epithelial cells in intestine regulate the movement of molecules across the intestinal barrier. The function of these tight junctions can alter the intestinal permeability.

A change in intestinal permeability is one of the postulates given in the pathogenesis of CD. Gluten can pass across the intestines through paracellular route and stimulate the immune system. Zonulins are a type of proteins in human that increase intestinal permeability, similar to *Vibrio cholera*-derived ZOT (zonula occludens toxin) and they have been found to have increased expression in patients with CD [105]. It has been suggested that gliadins interact with chemokine receptor, CXCR3, and this leads to zonulin release via MyD88-dependent pathway, which in turn increases intestinal permeability [56, 106]. Therefore, zonulin antagonism can decrease intestinal permeability.

Based on this principle, a drug named AT-1001 (larazotide acetate) is developed, which is an octapeptide derived from ZOT in *Vibrio cholera* and is found to antagonize zonulin [107]. Recently, a 12-week phase IIB clinical study was completed with 0.5mg larazotide acetate use in more than 300 CD patients showing its safety and efficacy [107].

5. Blocking of HLA-DQ

Adaptive immunity in CD is propelled by HLA-mediated activation of CD4+ T cells. HLA have epitopes that bind to deamidated gliadin peptides and form a complex that is immunogenic and hence, activates T cells. Considering this, there could be amino acid substitution within the T cells stimulatory sequences that could convert immunogenic epitope to an antagonist and block further inflammatory process [101]. But, it could be difficult to gain access to the gluten binding site on HLA, making this a challenging form of treatment.

6. Transglutaminase-2 (tTG) inhibition

Transglutaminase-2 deamidates gluten peptides, thereby creating epitopes that bind to DQ2 with increased affinity [72]. Thus, the use of tTG inhibitors could serve as a potential treatment option [108]. Many inhibitors have been developed *in vitro* [109], which includes competitive inhibitors (putrescine, cadaverine, fluorescein), reversible inhibitors [110] and irreversible inhibitors (iodoacetamide, imidazolium derivatives) [111]. But, since transglutaminase enzymes are ubiquitous in the body and are also involved in many functions to maintain homeostasis in other parts of the body, care has to be taken to develop drugs that specifically target only small intestine transglutaminase.

7. Immunomodulation

Gluten acts as an antigen in the body and therefore, introducing smaller amounts can help to develop tolerance to gluten. This is similar to desensitization techniques used for the treatment of allergies. Nex Vax2 is a vaccine being developed by a company named ImmunoSant, which is currently in phase I of the clinical trial. It uses three peptides with the aim of developing tolerance to gluten in CD patients [99].

8. Therapies targeting specific immune cells

8.1 Use of steroids—Steroids are used in the treatment of many autoimmune disorders like Crohn's disease and play the role of immunosuppression. They are especially useful in cases of refractory CD. Wall et al. showed augmented improvement with the use of prednisolone along with GFD in patients with CD [112]. But, the side effects associated with the use of steroids limit their long-term use in these patients.

8.2 CCR9 Inhibitor—CCR9 is expressed by effector and memory T cells and play a role in small intestinal homing on adhesion with chemokine CCL25 expressed on the intestinal epithelium. In a mice model, blocking of CCL25-CCR9 interaction improved the epithelial damage in ileitis indicating that this could serve as a promising therapy [113]. CCX282-B is an orally bioavailable antagonist of CCR9 which is undergoing clinical trials for use in Crohn's and CD. A phase II clinical trial for evaluation of this drug in CD patients started in 2007, has now been completed but results have not yet been released [36].

8.3 IL-15 antagonists—IL-15 is found to play a central role in the activation of cytotoxic IELs and pathogenesis of refractory celiac disease. Therefore, use of its antagonist as a treatment can have a huge impact on the improvement of patients with CD. The use of IL-15

antagonist as a treatment option has been tested in other diseases like rheumatoid arthritis but was discontinued due to limited efficacy [114], while a phase I clinical trial is currently recruiting patients to evaluate the efficacy of Mik-Beta1 monoclonal antibody to IL-15 in refractory celiac disease [36].

Expert commentary and five-year view

The past few years have expanded our knowledge of celiac disease, which is an autoimmune disease that can present at any age of life with different clinical manifestations and is found to be widespread throughout the globe. There has been advances in understanding the pathogenesis of the condition which has promoted the development of new non-dietary treatment options for these patients.

Essentially, its pathogenesis is found to be related to the interaction of three factors: gluten, genes (HLA and non-HLA) and multiple environmental factors. There is involvement of both innate and active immune system with special reference to the role of IELs in the causation of villous atrophy and enterocyte damage. In the recent past, IL-15 has emerged as a major mediator of the immune response. It upregulates the expression of NKG2C and NKG2D receptors on IELs that interact with their ligands on enterocytes and promote their apoptosis [88, 89]. They further activate the anti-apoptotic pathway by phosphorylation of JAK3-STAT5, which results in malignant clonal proliferation of enterocytes leading to the progression of refractory CD [7].

The advancement in understanding the pathogenesis has stimulated research in the field of developing treatment modalities that block one or more steps in the pathogenesis pathway. Clinical trials have been ongoing with drugs like ALV003 [104] that is based on enzymes that can digest the proline residues in gliadin peptides and another drug called larazotide acetate(AT 1001) [107] that is based on the principle of decreasing intestinal permeability to prevent the entry of immunogenic peptides in the small bowel. These studies are being conducted in adults and further investigations to establish their efficacy in children are warranted. In murine models, high molecular weight copolymers to bind with gluten and decrease its absorption have been tested [100], and this could have therapeutic implication in human which can be explored in future. Additional research needs to be conducted to investigate the role of other immunomodulators that could serve as a potential treatment for these patients in future and decrease their dependence on gluten free diet.

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Reference annotations

* Of interest

** Of considerable interest

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KEY ISSUES

- Celiac disease is a worldwide problem and the incidence is on the rise..
- Although exact pathogenesis is still unclear, it is thought to be a complex interaction of genetic, immunological and environmental factors with gluten.
- Apart from HLA DQ2/8, many non-HLA genes have also been found to be related to CD.
- Gliadin peptides (p31-43) via upregulation of EGFR promote increased proliferation of enterocytes and permeability.
- IL-15 plays a role in enterocyte damage and progression to Refractory Celiac Disease (RCD).
- Currently, gluten-free diet remains the only known mode of treatment, although clinical trials are ongoing for the development of drugs that can block one or more steps in the pathogenesis of celiac disease.
- Evidence for the role of infections, breast feeding and timing of exposure to gluten in a neonate can influence the susceptibility to CD and are being actively investigated.
- Recent data on perinatal influence including maternal exposure to medications, like antibiotics, and route of delivery render a glimpse into the complex pathophysiology and time of onset of this disease.
- GWAS studies have shown many non HLA genes associated with CD that could serve as potential therapeutic targets for treatment.

Figure 1. Diagnosis of CD

Algorithm for diagnosing CD in a suspected child based on ESPGHAN guidelines

Figure 2. Role of IL-15 in CD pathogenesis

Figure describes the various roles of IL-15 in CD immunopathogenesis. Gliadin on entering the lamina propria activates dendritic cells to secrete IL-15. (1) IL-15 acts on IELs on the epithelium to upregulate NKG2D and NKG2C receptors which interact with MICA and HLA-E ligands respectively on the epithelial cells and release perforins, granzymes, etc. that produce cell damage and villous atrophy. (2) IL-15 acts on Th1 cells to inhibit the release of regulatory T cells (Treg) and prevents development of oral tolerance to gluten. (3) IL-15 also activates the anti apoptotic pathway in IELs through JAK3 STAT5 and Bcl-x1 which initiates their aberrant differentiation and proliferation that plays a role in development of refractory CD (RCD).

Figure 3. Novel therapeutic approaches

Figure demonstrates the possible modes of intervention in CD pathogenesis that can serve as treatment options. (1) Use of genetically modified wheat varieties, gluten binders, and granzymes (ALV003) to interrupt the entry of gluten peptides into the lamina propria. (2) Use of Zonulin toxin (AT001) to decrease the intestinal permeability. (3) tTG inhibitors to prevent deamidation of gluten peptides. (4) HLA DQ binders to block their interaction with gluten. (5) Lymphocytic blockers (CCR9). (6) IL-15 inhibitors. (7) Anti-IFN- γ antibodies.