Physiopathology and Management of Gluten-Induced Celiac Disease

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Abstract: Proline- and glutamine-rich gluten proteins are one of the major constituents of cereal dietary proteins, which are largely resistant to complete cleavage by the human gastrointestinal (GI) digestive enzymes. Partial digestion of gluten generates approximately 35 amino acids (aa) immunomodulatory peptides which activate T-cell–mediated immune system, followed by immunological inflammation of mucosa leading to the onset of celiac disease (CD). CD is an autoimmune disease associated with HLA–DQ2/DQ8 polymorphism and dysbiosis of gut microbiota. CD is either diagnosed using duodenal mucosal biopsis or serological testing for transglutaminase 2 (TG2) specific antibodies (IgA and IgG). Current therapy for CD management is gluten-free diet, while other therapies like glutenase, probiotics, immunomodulation, jamming of HLA-DQ2, inhibition of TG2, and gluten tolerance aided by gluten tolerizing vaccines are being developed.

Keywords: celiac disease, gluten protein, probiotics

Introduction

Over the past few years, numerous studies have enriched our understanding toward digestion and absorption of food components. However, the functional role of the host digestive system for digestion of cereal dietary proteins awaits more understanding (Helmerhorst and others 2010). Gluten is one of the major constituents of the dietary proteins present in cereals inclusive of wheat, barley, oat, sorghum, and maize. It comprises of water insoluble prolamine and water soluble gliadin, which together comprise nearly 80% of the protein content within cereals (Wiser 2007). Majority of the population (>70%) in countries like India, consume a gluten-rich diet as a daily meal. As other food constituents, dietary proteins are also digested by host proteolytic enzymes into oligopeptides and subsequently into single amino acids. These digested oligopeptides and amino acids come into enterocyte via selective transporters (Helmerhorst and others 2010). However, due to the presence of high percentage of proline and glutamine in gluten, its digestion by human gastrointestinal (GI) digestive enzymes is challenging (Wieser 2007). Pepsin or trypsin are inadequate to cleave the peptide bonds C-terminal into glutamine and proline residues. Such partially digested gluten generate multiple oligopeptides, including 33-mer peptide from α 2-gliadin and 26-mer peptide from γ-gliadin (Helmerhorst and others 2010). These peptides are capable of T-cell stimulation once they reach the duodenum. There they are catalysed by the transglutaminase enzyme present in duodenal mucosa which imparts a negative charge to enhance class II major histocompatibility complex (MHC) binding with antigen presenting cells (APCs), followed by T-cell activation. This leads to inflammatory immune responses in the proximal intestine of individuals susceptible to onset of celiac disease (CD; Koning and others 2005; Jabri and Sollid 2006). Surprisingly, CD develops only in a small proportion of the population (approximately 1% to 5%). As host proteolytic enzymes cannot catabolise gluten completely, CD should have occurred in every individual consuming gluten containing diet. As this is not the case, possible protective role of other biological processes vis-à-vis gluten cleavage can be anticipated. Population-based genetic studies have highlighted strong association between onset of CD and polymorphisms within HLA-DQ2/DQ8 locus (Tjon and others 2010). In parallel with understanding the disease mechanism, there has been effort toward therapeutic intervention for CD. There has been substantial endeavor to develop various therapies and treatment regimen toward CD management with differential success rates. The proteolytic degradation of protease-resistant domains in gluten require specific endoproteases which could be a possible therapeutic target for CD.

Gluten Protein

Gluten is obtained after removal of water-soluble components along with starch particles from cereals. It is an insoluble protein and the major protein constituent of wheat, rye, and barley (Table 1). Wheat has 8% to 17% protein, 5% to 10% of lipid, starch, and other nonstarch carbohydrates (Rajpoot and Makharia 2013). Of the total wheat protein content, 78% to 85% belong to the gluten family. This is comprised of numerous subcomponents, which are either monomers (single chain polypeptides) or polymers (multiple polypeptide chains linked by disulphide [SS] bonds; Wrigley and Bietz 1988). Gluten has a higher percentage of proline and glutamine, along with a lower percentage of charged amino acids. Native molecular weight (MW) of gluten is between 10 to more than 30 kDa (Waga 2004; Wieser 2007).

Classification of Gluten

Based on relative solubility, gluten is classified into *Gliadins* (prolamins I): soluble in 70% ethanol or aqueous alcohol, and *Glutenins* (prolamins II): soluble only in acid solutions (Osborne 1907; Rajpoot and Makharia 2013). They both have an abundance of proline and glutamine amino acid, but differ from each other in structure. Gliadins are monomeric polypeptides of 28 to 55 kDa with a weak hydrogen bonding, while glutenin comprises of both low (32 to 35 kDa) and high MW (67 to 88 kDa) subunits within polymeric complexes, connected by intermolecular

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Table 1–Types of protein present in cereals.

Cereals	Protein nomenclature
Wheat	Gliadin
Barley	Hordein
Rye	Secalin
Oat	Avenin
Maize	Zein
Rice	Glutelin

SS bonds (Kaczkowski and Bernacka-Mieleszko 1980; Rajpoot and Makharia 2013).

Gliadins

Gliadins provide viscosity and extensibility to the gluten (Shewry and others 2002; Anjum and others 2007; Wieser 2007). These are subclassified into 4 types based on their primary structure namely; alpha (α), beta (β), gamma (γ), and omega (ω). It is fractioned into more than 100 subcomponents using RP-HPLC (reversed phase high performance liquid chromatography). Gliadins are further classified into 4 subtypes based on MW and amino acid sequence composition; viz. α/β, γ, ω1,2, and ω5 (Table 2; Wieser 1996). Of these, ω-gliadins contain a higher percentage of glutamine, proline, and phenylalanine. The ω5 gliadins have highest MW (approximately 50 kDa), while ω1,2 gliadins have lower (approximately 40 kDa). Alpha (α)/beta (β) and gamma (γ)-gliadins have MW between 28 and 35 kDa, but have lower percentage of glutamine and proline, compared to ω-gliadins. Repetitive sequences of glutamine and proline (PQQPFPQQ) are present in all these proteins. The QPQPFPQQPYP dodecapeptides are repeatable sequences of α/β gliadins and are repeated 5 times, while γ-gliadins contain 16 repeated sequence of QPQQPFP.

Glutenins

Glutenins provides elasticity to the gluten. Its subcomponents are connected by interchain and SS bonds have MW 2×10^6 to 10×10^6 Da (Wieser 1996). Glutenin macropolymer is the largest protein, which plays a vital role in conferring dough properties to wheat flour. Glutenin subunits (GS) comprise of low (LMW-GS) and high MW (HMW-GS) fractions. LMW-GS represents approximately 20% of gluten whereas HMW-GS accounts for approximately 10% of dry weight. Based on the amino acids composition and MW, LMW-GS are similar to α/β- and γ-gliadins (Table 2; Waga 2004). Glutenin possesses 2 sites, N-terminal site with QQQPPFS (glutamine- and proline-rich repetitive units) and C-terminal site of 42 residues, interlinked with intrachain SS bonds (Wieser 2007).

Structure of Gluten

Gluten is characterized based on its structure and amino acid sequence. Gliadins and glutenins have specific but repetitive amino acids, which generate oligopeptides of unique size and sequence arrangement (Tatham 1995). The α-gliadins have 2 domains: amino terminus (domain-a) with repeat sequence and a carboxyl terminus (domain-b) having specific cysteine residues (Shewry and Tatham 1997). Glutamines are abundant in both domains of α-gliadin as polyglutamine sites (Anderson and others 1991). Similar structures have been reported for β-, γ-gliadins as well as low MW glutenins. A signature peptide of Pro-Gln-Gln-Pro-Phe-Pro and Pro-Gln-Gln-Pro-Tyr have been identified within α- and β-gliadin, respectively. Gamma (γ)-gliadins have a repeat peptide of Pro-Gln-Gln-Pro-Phe-Pro-Gln (Sygiyama and others 1986). Omega (ω)-gliadin contain similar repeat peptide as γ-gliadin albeit small and distinct C- and N-terminal sequences. In addition to this, ω-gliadin contain additional glutamine at the end of the peptides (Pro-Gln-Gln-Pro-Phe-Pro-Gln-Gln). The ω-gliadin is sulphur (S^-) deficient whereas other gliadin fractions of α, β, and γ are rich in sulphur (S^+; Kaczkowski 2002).

Immunogenicity of Gluten

Majority of the studies have highlighted gliadin fraction of wheat as a causal agent for the onset of CD. However, gliadin is composed of 4 subcomponents, among which only α-gliadin is associated with CD (van Herpen and others 2006). Immunogenic properties of gluten are localized within a small stretch of proline- and glutamine-rich amino acid sequences. Allergic response could either be induced by pentapeptide (Gln-Gln-Gln-Pro-Pro) or tetapeptide (Gln-Gln-Gln-Pro and Pro-Ser-Gln-Gln) which in turn may lead to CD. Pentapeptide reacts with IgE antibodies in serum of patients and induce an allergic response (Watanabe and others 1995). Generally, gluten is catabolized into small peptides, of which a small fraction is allergenic. These fractions have been separated by chromatography and confirmed by ELISA (Tanabe and others 1996). An another study have highlighted the functional role of peptides toward intestinal mucosa enterocyte enlargement (De Ritis and others 2008). A 266-residue α-gliadin was fractionated into 3 peptides: CB1, CB2, and CB3. CB1 and CB2 affect growth of enterocytes while CB3 is inactive. Digestion of CB1 with chymotrypsin produces 3 small components (Ch1.1, Ch1.2, and Ch1.3), of which only Ch1.1 is immunogenically active. Ch1.1 peptide can be further digested with chymotrypsin to produce Ch1.1.1 and Ch1.1.2, termed as "toxic" peptides (Waga 2004). All Gln-Gln-Gln-Pro and Pro-Ser-Gln-Gln containing peptides were identified within patients with CD. Highly toxic tetrapeptide were observed at the N-terminal site of α-gliadin (residues 1 to 55) whereas absence of toxicity was observed at C-terminal. The role of these penta- and tetrapeptide in causing allergy and CD have also been demonstrated by other studies (Wieser 1996; Maruyama and others 1998).

Gluten and Onset of CD

CD is a gluten or prolamines sensitive, chronic, lifelong, and systemic autoimmune disorder. It is triggered by the seed storage protein, which is mainly present in wheat (gliadin), barley (hordein), and rye (secalin). Gliadin causes activation of proinflammatory cytokines through Th1/Th17 adaptive immune system within CD patients. It has been reported that the interaction of peripheral blood mononuclear cells of CD patients with gliadin produces interleukin 1β (IL1β) and IL18 (Palova and others 2013). Recently, interleukin 15 (IL15) was also found to be upregulated in the epithelium and lamina propria of the CD patients (Abadie and Jabri 2014). Th1 response increases interferon gamma (IFNγ) and IL15, leading to intraepithelial lymphocyte toxicity and onset of CD (Figure 1; Moraes and others 2014; Serena and others 2015). Gliadin peptides also react with CXCR3 receptors, expressed on the apical side of the epithelium, leading to deamination by transglutaminase 2 (TG2) at the lamina propria (Serena and others 2015). TG2 deaminates glutamine and introduces negative change onto gliadin peptides, which could bind to human leukocyte antigen (HLA) antigens, encoded by HLA class II genes, *HLA-DQ2* or *HLA-DQ8*. These deaminated peptides also reacts with intestinal submucosal dendritic cells, gluten specific CD4+ Th1 T cells and macrophages. It has also been observed that these deaminated peptides can stimulate tumor necrosis factor

Table 2–Characterization of various subcomponents of gluten (Wiser 2007).

Type		α/β-gliadins	γ − gliadins	ω 1,2-gliadins	ω 5-gliadins	x-HMW-GS	y-HMW-GS	LMW-GS
Proportions[a] (%)		28 to 33	23 to 31	4 to 7	3 to 6	4 to 9	3 to 4	19 to 25
Composition of partial amino acid (%)	Glutamine	37	35	44	56	37	36	38
	Proline	16	17	26	20	13	11	13
	Phenylalanine	4	5	8	9	0	0	4
	Tyrosine	3	1	1	1	6	5	1
	Glycine	2	3	1	1	19	18	3

[a]As per total gluten proteins, HMW, high molecular weight; LMW, low molecular weight.

(TNF), IL17, and proinflammatory cytokines (IFN), due to adaptive immune response (Figure 1). Such immunological changes enhance the permeability of the intestine, thereby causing harm to the intestinal mucosa (Serena and others 2015). Various epitopes are known for their active role in enhanced gut permeability, immunomodulatory actions, and cytotoxicity (De Nitto and others 2009; Lammers and others 2011; Serena and others 2015). α- and ω-gliadins also posses similar epitopes, however, α-gliadin-derived 33-mer (57 to 89 peptide) is immunologically important. Overall, CD is a T-cell–mediated condition in which gliadin family of peptides activate immune cells of the lamina propria of the small intestine and react with T-cell lymphocytes to induce adaptive Th1 response (Ortega and others 2013; Mazzarella 2015).

CD: Leaky Gut Syndrome or Autoimmune Disease?

Onset of gluten sensitive CD is preceded by an interaction between gliadin peptides and immune cells. However, for this to happen, gliadins have to be in close proximity of immune cells by crossing the epithelial barrier. Toward this, macromolecule transport occurs either through transcytosis or by paracellular transport. The successful transport of antigens and its interaction with the immune system is very low (Garrote and others 2008; Fasano 2011). Thus, paracellular transport is another possible route to cross the epithelial barrier and initiate dietary/microbial antigen-derived immune response. Paracellular transport of antigens strongly correlates with enhanced intestinal permeability during onset of CD. Increased intestinal permeability (leaky gut syndrome) is the

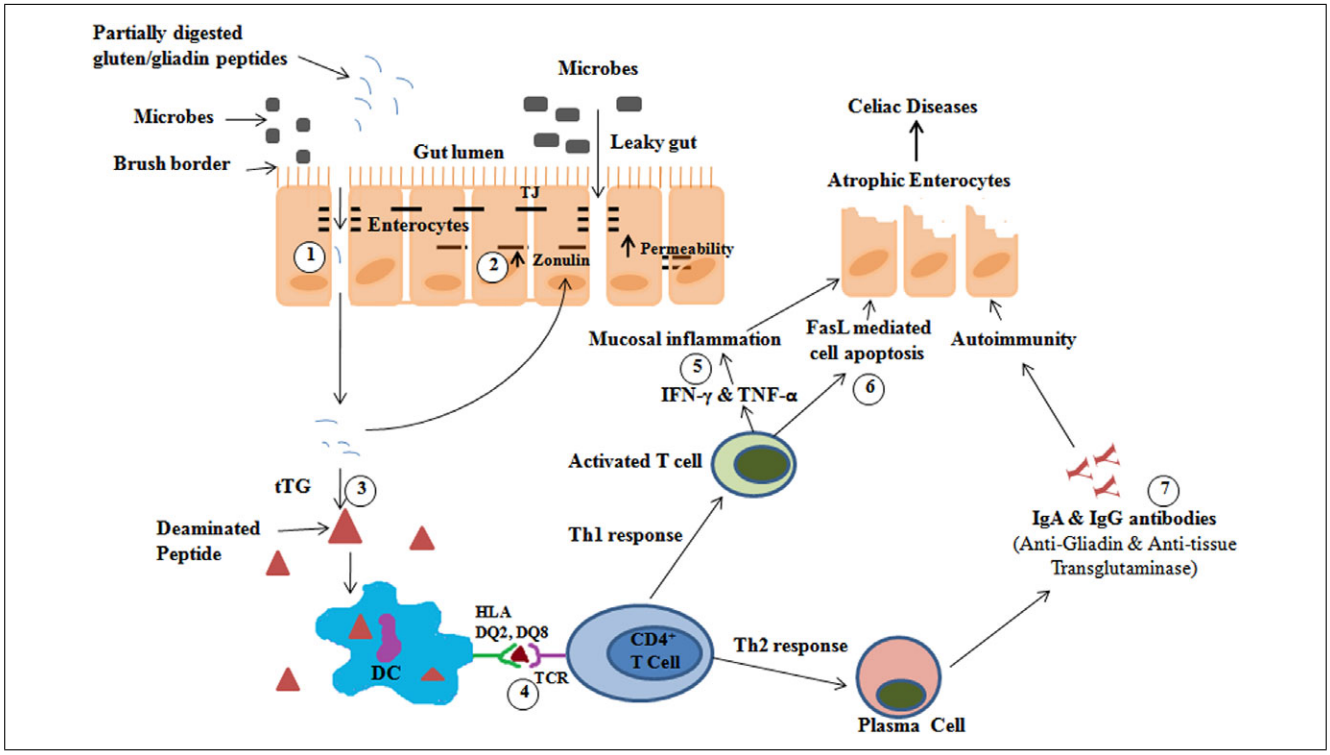

Figure 1–Mechanism of celiac disease and acting sites of probiotic for improvement of the celiac disease symptoms. In celiac disease, partially digested gliadin peptides may cross the intestinal epithelium through paracellular route (A). Paracellular transport occurs as a consequence of an increased enterocytic permeability with increased zonulin secretion (B). Tissue transglutaminase catalyzed deaminated gliadin peptides (C) activates adaptive immune response through activation of $CD4^+$ T cells (D), which in turn stimulate cytotoxic T cells and fibroblasts to produce a particular matrix metalloproteinase pattern which is responsible for degradation of both extracellular matrix and basement membrane (E). Activated T cells triggers enterocyte apoptosis by producing molecules like Fas ligand and granzyme (F). An autoimmune response could also be triggered by activated $CD4^+$ T cells by inducing lymphocyte B differentiation into plasma cells producing specific antigladin and antitissue transglutaminase antibodies (G). DC, dendritic cell; HLA, human leucocyte antigen; TCR, T-cell receptor; TJ, tight junction; tTG, tissue transglutaminase.

preceding stage of CD (Fasano 2012). Gliadins are found to elevate expression of the zonulin protein in epithelial IEC-6 and Caco-2 cells (Thomas and others 2006; Lammers and others 2008; Fasano 2012). Gliadin increases zonulin secretion in both celiac and non-CD individuals (Drago and others 2006). Zonulin can induce a rearrangement of actin filaments to destabilize integrity of junctional complexes (tight, adherent, and gap junctions, along with desmosomes) to increase intestinal permeability (Drago and others 2006; Fasano 2012). Thus, it seems that gliadin boosts intestinal permeability to trigger paracellular transport of antigens resulting in immunological inflammatory response. This is followed by a cascading process in which intestinal inflammation further increases intestinal permeability, leading to onset of CD (Drago and others 2006; Lammers and others 2008; Fasano 2012). In other words, leaky gut contributes to the autoimmune response in CD.

Physiological Symptoms and Diagnosis of CD

Three major symptoms of CD include: (i) anemia, caused due to iron deficiency, (ii) abdominal problems of diarrhea and constipation, and (iii) feeling tired and lazy. Common physiological symptoms of CD in adult patients are diarrhoea, weakness, weight loss, vomiting, loose stool, and distended abdomen. Child with CD show thrive, failure, defects in dental enamel, and short stature (Donald and Antonioli 2003). Other general symptoms of type 1 diabetes, hypothyrosis, osteoporosis, steatorrhea, arthralgias, skin rash (dermatitis herpetiformis), and postprandial abdominal pain have also been reported in CD (Lundin and others 2015; Castillo and others 2015). It is also advisable to screen immediate family members, inclusive of siblings, mother ,and father to help understand the family history (if any) for better disease prognosis.

Elevated serum levels of aminotransferase is also used for CD diagnosis, in absence of etiology (Rubio-Tapia and others 2013; Lundin and Solid 2014; Seran and others 2015). Primary screening for CD is carried out with serological testing using celiac specific antibodies. Positive cases from primary screening undergo duodenal mucosal biopsy for confirmation of CD. Mazumdar and others (2010) have demonstrated specific histological changes in intestinal tissue of CD (Figure 2). Such information is useful for qualitative assessment of CD as well as prognosis (Mazumdar and others 2010). Majority of CD diagnosis is done using IgA antibodies for the enzyme TG2, antitissue transglutaminase and IgG antideaminated gliadin peptide (DGP) (Rubio-Tapia and others 2013). These antibodies are used as CD serological markers. During diagnosis of CD using patient serum, IgA is deposited against TG2 inside the mucosa, which is stained (Mazumdar and others 2010). Although, IgG is a specific and sensitive diagnostic method. CD is also positively associated with genetic polymorphism of HLA-DQ2 or DQ8 locus. HLA-DQ2 heterodimer is reported in CD carriers and have a susceptibility to CD. HLA-DQ2 typing can be used to rule out the possibility of CD, where the diagnosis is equivocal. Given the variable clinical manifestation and the heterogeneous histology, a standard diagnostic pipeline for the diagnosis of CD is very important (Bhatnagar and Tandon 2006; Lundin and others 2015; Rubio-Tapia and others 2013). However, for more than 2-year-old CD patients, combined test is recommended, including IgA TTG and DGP (IgA and IgG). Confirmatory diagnosis of CD should ideally include medical history complemented with serology, physical examination, upper endoscopy, and duodenum biopsy. Upper endoscopy and small bowel biopsy are key physiological domains for the diagnosis of CD patients. More than 2 distal duodenum biopsies are recommended to confirm CD (Rubio-Tapia and others 2013).

Challenges of CD Diagnosis

Although initially considered a pediatric condition, present data suggests that 25% of CD cases are in the age group of 60 years. In addition, the median age of diagnosis is increasing, which has attracted attention to understand the CD pathophysiology in greater detail. The urgency to correctly diagnose and device effective interventions is driven by recent evidences of incidence of certain types of cancers among CD patients. This includes non-Hodgkin lymphoma, enteropathy-associated T-cell lymphoma, small intestinal adenocarcinoma, and esophageal and oropharyngeal squamous carcinoma. Although, the positive part is that adherence to gluten-free diet (GFD) has shown to decrease the chances of malignancies (Briani and others 2008). The diagnosis of CD is compounded by a wider range of villous architecture: from near-normal to total villous atrophy, with many intermediate stages. There are also incidences of incongruence between biopsy, serology, and clinical symptoms of CD. In such complex situations, revisiting the diagnosis along with HLA typing may be considered. Because nearly all celiac patients (and approximately 25% to 40% of the general population) carry the HLA-DQ2 and/or HLA-DQ8 alleles, the absence of both markers has a very high negative predictive value. As an additional means to confirm CD, improvement in response to GFD is a good measure (Marsh 1992). However, it is pertinent to mention that there are situations when adherence to GFD does not induce improvement in CD patients. In such situations, it may be advised to re-evaluate the CD patients by biopsy as well as to ascertain that GFD is not contaminated with traces of gluten-containing cereals (Thompson 2004). Thus, given the variability in terms of diagnosis, treatment, pathogenesis, and prognosis, it is extremely important to ascertain and adhere to gold standards at every stage of the disease.

Cause of CD

Environmental and genetic factors influence the etiology of CD. The major genetic predisposing factor for CD is human leukocyte antigen (HLA polymorphism, harboring nearly 40% of genetic variation. CD is confirmed by MHC class II HLA DQ2 and DQ8. Large number of celiac patients (approximately 95%) carry variants of DQ2 encoded by alleles DQA1∗05/DQB*02 and remaining, approximately 5%, carry DQ8 encoded by DQA1*03/DQB1*03:02 alleles. On the basis of genome-wide association studies and dense genetic mapping, 57 non-HLA loci have also been identified which help explain nearly 18% of the genetic variation associated with CD. Interestingly, many studies in recent times have reported an association of gut microbiota dysbiosis with CD. It is characterized by an abundance of *Proteobacteria* and *Bacteroidetes* with lower levels of *Firmicutes* during disease state. Parallely, studies have highlighted the abundance of *Firmicutes* and lower amount of *Bacteroides* before the onset of CD in genetically predisposed populations (Serena and others 2015). Enrichment of *Mycobacterium* and *Methylobacterium* spp. in CD adults whereas *Haemophilus* and *Neisseria* spp within children have also been reported. Moraes and others (2014) had studied the role of Gram-negative bacteria in gluten tolerance in genetically susceptible individuals.

Therapeutic Management of CD

At present, lifelong GFD is the only efficient therapy for CD. Even a small quantity of gluten (50 mg/d) can be immunogenic, so all food items and dietary supplements with gluten and its derivatives must be withdrawn from the celiac patient diet. The GFD standards are monitored by the Codex Alimentarious Commission

of the World Health Organization (Geneva, Switzerland) and by the Food and Agricultural Organization (Rome, Italy), amended in 1983. As per this, GFD should not contain more than 20 ppm of gluten and also devoid of prolamines from wheat or *Triticum* species like wheat, barley, rye, and oats (Rajpoot and Makharia 2013). But it is very challenging to maintain lifelong GFD, especially in developing countries like India (Makharia 2014). Some other cofactors affecting adherence to GFD are blandness, minor nutritional content, higher cost of GFD meal as well as ease of availability. Thus, patient-friendly alternative therapy of CD needs attention. Induction of immune tolerance for CD includes usage of probiotics, gluten vaccination, gluten tolerance and immunomodulation, tissue transglutaminase inhibitors, HLA-DQ2 or HLA-DQ8 blockers, genetically modified gluten, and glutenase-supplement diet (Castillo and others 2015).

Evolving Therapies for CD Management

Various studies have shown the intervention of probiotics (De Angelis and others 2006), gluten vaccination (Keijzer and others 2013), gluten tolerance and immunomodulation (Veeraraghavan and others 2015), tissue transglutaminase inhibitors (Sollid and Khosla 2011; Makharia 2014), HLA-DQ2 or HLA-DQ8 blockers (Kim and others 2004; Xia and others 2006; Kaporchan and others 2008; Xia and others 2008), genetically modified gluten (Schuppan and others 2009; Sollid and Khosla 2011; Stoven and others 2012), and glutenase supplement diet (Caputo and other 2010) could subjugate the onset of CD. These possible therapies for CD management are under development and requires further research.

Probiotics

Probiotics contain specific microbial species which have a positive impact on human health. Most of the probiotics use *Bifidobacteria* and *Lactobacilli* genera of microbial strains. *Bifidobacterium lactis* (HN019 strain) can increase nonspecific immune functions, like activation of macrophages, natural killer cells and antigen-specific cytotoxic T lymphocytes. It has been demonstrated that human cohort fed with 450 gm of yoghurt/d for 4 mo showed a significant increase in γ-interferon (Halpern and others 1991; Fooks and others 1999). Maneuvering the relative abundance of microbial community in celiac patients by probiotics is one of the better options for CD therapy (Table 3).

Table 3–Mechanism and importance of probiotics in celiac disease.

Mechanism	Possible probiotics
Enzymatic gluten degradation or preingestion fermentation	VSL#3 long-lasting fermentation by *Lactobacilli* and fungal proteases
Maintenance of barrier of gastrointestinal tract	*Bifidobacterium* and *Lactobacilli* play a fundamental role

De Angelis and others (2006) reported the potential benefits of a probiotic cocktail with 8 strains (VSL#3), *Bifidobacterium breve, Bifidobacterium infantis, acidophilus, Lactobacillus plantarum, Lactobacillus casei, Lactobacillus delbrueckii* sub sp. *bulgaricus, Streptococcus thermophilus* and *Bifidobacterium longum*, which decreased wheat-induced discomfort. It was also observed that individual probiotic strains were inadequate (VSL#3) to break down gliadin compared to the efficiency with pool of 8 strains. This probiotic VSL#3 preparation also showed beneficial promise toward treatment of CD. Few other probiotic combinations are also used for the treatment of CD which include Florisia (*Lactobacillus brevis, Lactobacillus plantarum, Lactobacillus salivarius,* and subsp. *salicinius*), Oxadrop (*Lactobacillus acidophilus, B. infantis, L. brevis,* and *S. thermophilus*), and Yovis (*B. infantis, B. breve, L. acidophilus, B. longum, L. plantarum, L. casei, L. delbrueckii* subsp. *bulgaricus, Thermophilus, Streptococcus salivarius* subsp. and *Enterococcus faecium*; De Angelis and others 2006). Lindfors and coworkers have reported that *B. lactis* exerted a defensive effect on the epithelial cells against cellular damage induced by gliadin incubation (Lindfors and others 2008).

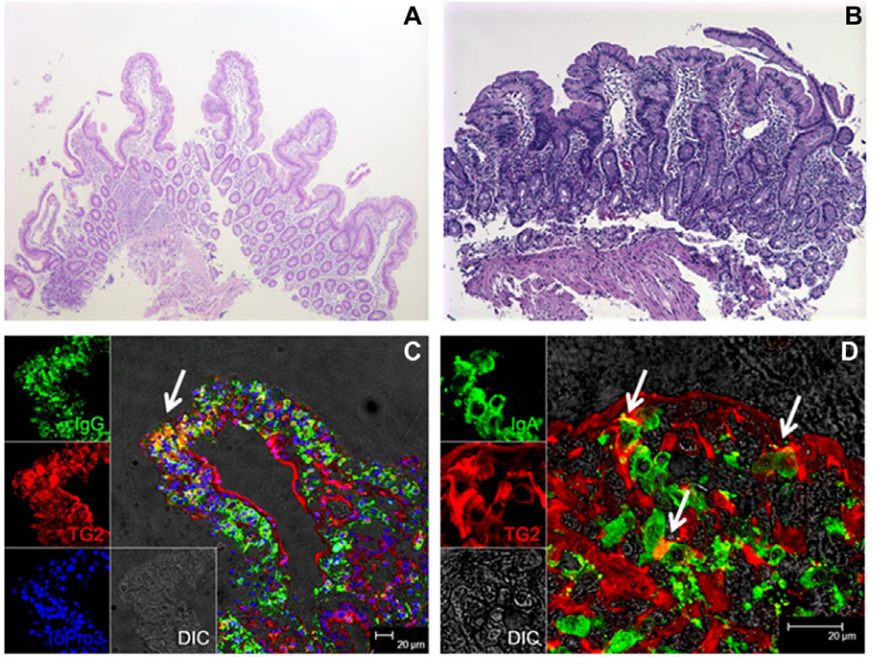

Figure 2–Histopathological changes in normal and gluten-sensitive duodenum. Duodenal biopsy from healthy (A) and gluten-sensitive macaque outlined by short and flattened villi (B). Immunofluorescent analysis of duodenal tissue section from gluten-sensitive macaque identified the presence of single-positive TG2+ (red), IgG+ /IgA+ (green) cells, as well as TG2+IgG+ (C) and TG2+IgA+ (D) double-positive cells. Original images are adopted from Mazumdar and others (2010).

Gluten vaccination

For CD patients, desensitizing or therapeutic vaccine called Nexvax2 was developed by Immusan T, Cambridge, Mass., U.S.A. The peptide-based immunotherapy vaccine was able to shift the T-cell response from proinflammatory to regulatory role, by inducing immune tolerance for gluten and facilitating digestion of gluten containing food (Keijzer and others 2013). Subcutaneous injection of these peptides in HLA-DQ2 transgenic mice, suppressed the $CD4^+$ T-cell proliferation, reduced IFN-γ and IL-2 production, and at the same time improved gluten tolerance and enhanced Tregs expression (Veeraraghavan and others 2015).

Gluten tolerance and immunomodulation

Autoimmune disorders are product of interaction between environmental factors and genetic predisposition, which modulate mechanism/process of immune tolerance and inflammation. Molecules like FOXP3, CD4, CD25 and Tregs are important for tolerance to food proteins. In CD, both T cells and Tregs are involved in the modulation of immune response via inflammatory cytokines like TNFα, IFNγ, IL10, and IL15 (Hmida and others 2012). Maintaining balance between inflammation and tolerance, which can help "reorganize" the immune system and at the same time improve autoimmune disorders may be an ideal therapy for CD (Veeraraghavan and others 2015).

Tissue transglutaminase inhibitors

The multifunctional TG2 protein breaks intermolecular isopeptide bonds between glutamine and lysine that can generate immunogenic peptides (Siegel and Khosla 2007). In CD patients, glutamine residues of gluten peptides are deaminated by TG2 and generate effective T-cell epitope (Sollid and Jabri 2011). TG2 induces crosslinking between gluten peptides and matrix protein, which produces additional autoantigens, which enhance immune response. Thus, it may be useful to develop TG2 inhibitors as potential CD therapeutic targets (Sollid and Khosla 2011; Makharia 2014).

For many years, it was assumed that TG2 inhibition may inhibit gluten peptides through HLA-DQ2 and HLA-DQ8 (Molber and others 2000; Xia and others 2006). Parallely, competitive inhibitor like cystamine which can block T-cell proliferation of gluten reactive T cells was also developed, along with KCC009, an irreversible inhibitor which increased TG2 affinity (Choi and others 2005). These inhibitors represent effective oral options capable of TG2 inhibition in small intestine with low toxicity profile and short serum half life. Thus, it was concluded that TG2 inhibition can be effective both for *in vitro* and *ex vivo* innate immune response and to prevent gliadin-induced adaptive immune response (Molberg and others 2001; Lebreton and others 2012; Rauhavirta and others 2013; Veeraraghavan and others 2015).

HLA-DQ2 or HLA-DQ8 blockers

In celiac patients with HLA-DQ2 and HLA-DQ8 haplotypes, adaptive immune response is modulated by APCs. In such situations, HLA-blocking compounds may be effective for immune activation (Kim and others 2004; Xia and others 2006; Kaporchan and others 2008; Xia and others 2008). Many research groups have designed blockers for specific HLA-DQ2 haplotype. Gluten peptide series was designed by Kapocharan and others, in which azidoprolines replaced the proline residues and these modified gluten peptides have 100- to 200-fold enhanced binding affinity compared to natural gluten peptide for HLA-DQ2 (Kapocharan and others 2008; Kapocharan and others 2010). Few nonimmunogenic compounds that reduce gluten-mediated immune responses were also discovered. Juse and others, prepared high-affinity HLA-DQ2 binders on the basis of positional scanning of nonpeptide library to estimate most favorable amino acid substitution leading to improved binding (Juse and others 2010). Crystallized structure of HLA-DQ2 was used to create gluten peptide analogs customized with an aldehyde group (Siegel and others 2006). These customized peptides have high affinity as well as strong binding potential for HLA-DQ2 ligands and reversible TG2 inhibitors (Siegel and others 2007). However, this therapeutic approach is limited by the ability of modified peptides to reach lamina propria of the small intestine as well as competing with immunogenic gluten peptides (Veeraraghavan and others 2015).

Genetically modified gluten

Genetically modified grains are one of the possible ways to overcome the immunogenic gluten in genetically susceptible individuals (Schuppan and others 2009; Sollid and Khosla 2011; Stoven and others 2012). Abundant amount of immunogenic peptides are present in gluten that induce immune response (Carroccio and others 2011). In wheat genome, immunogenic peptide coding genes are present across different loci. Thus, it would be challenging to transform wheat strains (Makharia 2014) for these loci. However, RNA interference technology is helpful to overcome the immunogenic gliadin peptides in wheat (Humanes and others 2012). Recent evidences have shown that the ancient wheat varieties are less immunogenic for CD patients (Spaenij-Dekkin 2005). In Chinese spring wheat, specific gliadin genes have been genetically deleted, especially α-gliadin, which decreases the cell-activating epitopes without compromising baking properties of wheat flour dough (van den Broeck and others 2010).

Glutenase supplement diet

Enzyme supplementation may be an alternative therapy for CD patients as an important, effective and safe treatment (Caputo and other 2010). Many bacterial and fungal enzyme supplements are present, which contain endopeptidases or proteases capable of degrading gluten and prolamins (Zamkachari and others 2011). Significantly, glutenase works in the lumen of the small intestine and are not involved in immunological cascade of events in the lamina propria. Thus, it does not cause any side effect on the CD patients. At present, *Aspergillus niger* endopeptidase and ALV003 (combined enzyme product) are available commercially, which have potential to hydrolyze gluten peptides (Kaukinen and Lindfors 2015). ALV003 (Alvine Pharmaceuticals, San Carlos, Calif., U.S.A.) is a 1:1 combination of ALV001 and ALV002. ALV001 is an EP-B2 cysteine endopeptidase, derivative of germinating barley endosperm, while ALV002 is a *Sphingomonas capsulate* bacteria prolyl endopeptidase (PEP; Gass and others 2007). Both endopeptidases were manufactured using recombinant engineering technology and these are active and stable at gastric pH. ALV001 show activity against 33-mer wheat gliadin peptide and α2-gliadin (Bethune and others 2006). The remaining immunogenic oligopeptides are catabolized by ALV002, which were digestible and importantly nontoxic (Gass and others 2007). These endopeptidase were derived from *A. niger* (AN-PEP; DSM, Heerlen, Netherlands). A study has shown that intake of 7 gm of gluten along with AN-PEP for 14 d had reduced symptoms of CD. Safe usage of AN-PEP was confirmed further by no changes in serology or any adverse effects subsequent to its use. AN-PEP may be more effective with lesser amount of gluten, but it is not effective to prevent gluten induced mucosal damage (Veeraraghavan and others 2015). STAN1 is

another microbial enzyme mixture, mainly used as a food supplement (Veeraraghavan and others 2015). In the last few years, oral variant of PEP enzyme has been discovered which show potential for therapeutic usage (Polgar 2002; Fernandez-Feo and others 2013). PEP cleave the gluten-derived peptides and have an ability to digest gluten in the GI tract. Assays, inclusive of mass spectrometry, HPLC, antigluten, T cell, and gluten challenge studies confirm their potential for gluten detoxification (Hausch and others 2002; Shan and others 2002; Shan and others 2004; Marti and others 2005; Gass and others 2005; Shan and others 2005). PEP of *A. niger* have an acidic profile and are activated in the stomach during gluten cleavage (Stepniak and others 2006). Similarly, PEPs with optimum activity at neutral pH in conditions of SC PEP, MX PEP and FM PEP could be effective in detoxifying gluten in the upper part of the small intestine, where a major fraction of the proteins within the body is digested and absorbed (Gass and Khosla 2007). To facilitate this, a specific enteric coating could be done, which will provide protection in gastric environment and can be activated when it reaches duodenum. For MX PEP, a different type of polymer coated formulation has been discovered (Gass and others 2005). Other than these, a gastric PEP should be stable to pepsin, whereas, duodenal PEP must be resistant for presence of membrane enzymes at the intestinal brush border and pancreas. Other research groups have also shown that in the presence of pepsin, *A. niger* PEP is resistant (Stepniak and others 2006), whereas MX PEP and FM PEP showed a mixed response (Shan and others 2004). A 2-enzyme therapy using a combination of a glutamine-specific protease with a duodenally active PEP, is helpful for gluten breakdown under gastric conditions. These types of mixed therapies are capable of rapid digestion of bulk amount of gluten protein. They are also harmless under such gastric conditions, where no single enzyme is entirely effective (Siegel and others 2006).

Conclusions

In this review, we have tried to summarize various aspects of CD ranging from the structure and immunogenicity of the core factor (gluten) inducing the onset of CD, diagnosis, pathophysiology, treatment regimen, and innovative discoveries toward therapeutic interventions. CD also presents itself with a range of clinical symptoms which are quite nonoverlapping between infants and adults. Many a times, this induces challenges toward correct diagnosis of the disease itself. Thus, it is almost essential that combination of diagnostic features including clinical symptoms, intestinal biopsy, genetic predisposition based on HLA typing and prognosis in response to GFD is considered holistically for CD. CD is unique in respect to the functional role of both arm of the immune system, adaptive as well as innate. Although, essentially, CD is an autoimmune disorder in response to partially digested gluten within the GI tract, in genetically predisposed individuals, it also has wide spectrum of extraintestinal complications as well, including cancer. Wide spectrum of degeneracy in the villous architecture also compounds accurate diagnosis.

In this background, GFD is most effective treatment regimen in practice, but the dual challenge of quality and affordability of GFD needs to be addressed for it to be effective at global scale. Thus, more clinical research and trials are required for therapeutic intervention of CD. Encouraging results in the field of therapeutic intervention, especially the combinatorial usage of probiotics, may provide one of the easier option for CD patients.

Conflict of Interest

There is no conflict of interest for any of the authors in this review article.

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