Chapter 2
Definition of Celiac Disease and Gluten Sensitivity

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Introduction

As an important component of wheat, rye, and barley, gluten can be found in a large variety of foods consumed throughout the world. However, the introduction of gluten-containing grains in the human diet about 10,000 years ago created the conditions for human disease related to gluten exposure. These reactions to gluten represent a heterogeneous set of conditions, including celiac disease (CD), non-celiac gluten sensitivity, and wheat allergy, which combined affect about 10 % of the general population [1].

The immune-reactive component of gluten is gliadin, a complex glycoprotein rich in proline and glutamine. Because of this structure, intestinal enzymes cannot entirely degrade the protein. We do know that undigested or partly digested gliadin can affect a wide range of human cells. The effects of gliadin on the myelocytic leukemia cell line, K562, and various intestinal cell lines are, respectively, its agglutinating activity [2], its capacity to induce rearrangement of the epithelial actin cytoskeleton by redistribution of F-actin [3], and its cytotoxic activities including inhibition of cell growth, induction of apoptosis, and alteration of redox equilibrium [4, 5].

There are three variants of gliadin, the alpha-, gamma-, and omega-variant, with the alpha-gliadin variant being the most prevalent. A 13-mer and a 33-mer alpha-gliadin motif have been reported to exert a cytotoxic effect on intestinal epithelial cells [6] and to be capable of activating gut-derived T-cell lines from CD patients [7],

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respectively. Furthermore, two 20-mer intestinal permeating and an immunomodulatory 17-mer alpha-gliadin peptide have recently been identified [8, 9].

CD, non-celiac gluten sensitivity, and wheat allergy represent distinct pathophysiological reactions to gluten ingestion, with differing clinical presentations, serological markers, and long-term treatments. Though current research strives to clarify the boundaries between these entities, their differences can be difficult to distinguish. This chapter provides an overview of the ever-evolving definitions of gluten-related disorders.

Celiac Disease

CD, an autoimmune-mediated enteropathy triggered by gluten ingestion in genetically predisposed individuals, is one of the most common chronic digestive disorders, showing an overall prevalence worldwide of 1 % with large variations between countries [10]. The disease prevalence is even higher amongst first-degree relatives of CD patients (8–15 %) [11, 12] and other at-risk groups, such as patients with other genetic diseases like type 1 diabetes mellitus, Hashimoto's thyroiditis, Down syndrome, or IgA deficiency [13–17]. Importantly and contrary to previous assumption, CD is not confined to Europe; rather it is present worldwide [18] and it is increasing over time [19].

The genetic predisposition to CD is strong but complex (see Chap. 5 on HLA genetics). Human leukocyte antigen (HLA) haplotypes DQ2 and DQ8 are found in at least 95 % of patients with CD [20]. While the presence of these alleles provides a strong negative predictive value, their positive predictive value is low. Indeed, although 30 % of the general population carries the HLA-DQ2 allele [20], the prevalence of CD is currently 1 % [10]. As much as 65 % of the genetic component of CD may be caused by a complex, still undefined, mosaic of over 40 non-HLA genes, each adding a small contribution to the risk of CD development [20, 21].

Clinical Presentation

The clinical presentation of CD is highly variable, including typical (gastrointestinal symptoms), atypical (extra-intestinal symptoms), latent (no intestinal damage despite ingesting gluten, but later develops villous change; retrospective diagnosis), and silent (asymptomatic, discovered via screening) forms [22, 23]. The presenting symptoms may vary from diarrhea, constipation, vomiting, malnutrition, or failure to thrive to chronic fatigue, joint pain, anemia, osteoporosis, or migraines. Many times, the onset of symptoms occurs during the first 24 months of life, usually some months after the introduction of gluten-containing cereals in the infant's diet. A recent study highlights the importance of timing with regard to gluten introduction into the diet in genetically susceptible infants. Those infants to whom gluten

was introduced in the diet at 6 months developed CD more frequently than those infants to whom gluten introduction was delayed until 12 months of age [24]. However, it is important to note that initial signs and symptoms of CD can occur at any age, including adults and the elderly [19, 25]. Unlike the relatively rapid reaction seen in wheat allergy, the signs and symptoms of CD usually do not manifest until weeks to years after exposure.

Diagnosis by a "four out of five rule" has been proposed to account for the variability in CD presentation [26]. Under this rule, patients must meet at least four of the following five criteria to be diagnosed with CD:

- Typical symptoms seen in CD
- Positive serological markers such as serum anti-transglutaminase (TTG) antibodies or antigliadin antibodies
- Small intestine biopsy showing absent or blunted villi (Marsh II–III a–c), and increased numbers of CD3+ intraepithelial cells
- Positive genetic screening for HLA-DQ2 or -DQ8
- Improvement of symptoms with a gluten-free diet

Treatment for CD is the lifelong implementation of a gluten-free diet, in which all gluten-containing foods are eliminated from the diet. Compliance with a strict gluten-free diet reverses small intestinal changes in the vast majority of patients and reduces the risk of complications from CD (osteoporosis, lymphoma, infertility). However, this change in diet can be difficult to implement and maintain, not only because gluten-rich products are an important part of the Western diet, but also because of "hidden" gluten in processed foods [27, 28]. Adding to the challenge, designated gluten-free foods are often more expensive than their gluten-containing counterparts. Moreover, eating gluten-free can be exclusionary, as it makes it difficult to eat at restaurants for fear of cross-contamination. Given the negative impact of the gluten-free diet on the quality of life of affected individuals, there is currently a strong interest on possible alternative strategies of treatment or prevention [29, 30].

Non-celiac Gluten Sensitivity

Non-celiac gluten sensitivity is the least clearly defined of the gluten-related disorders as it has only become widely recognized in recent years [1, 31–33]. When the reaction to gluten is not mediated by an allergic or autoimmune response, gluten sensitivity may be considered [1, 34, 35]. The lack of clear diagnostic criteria may have led to non-celiac gluten sensitivity being undiagnosed and underdiagnosed by physicians for many years. The prevalence of non-celiac gluten sensitivity is estimated to be between 3 and 6 % [1, 36]. The genetic component of gluten sensitivity is not yet completely understood. Only 50 % of non-celiac gluten sensitivity patients express the HLA-DQ2 or HLA-DQ8 haplotype, indicating that these genes are not necessary or sufficient to develop gluten sensitivity [1].

Clinical Presentation

Non-celiac gluten-sensitive patients usually present with the same variety of symptoms (diarrhea, stomach pain, etc.) and prevalence of extra-intestinal symptoms (headache, "foggy brain," fatigue, rash, joint pain, depression, anxiety, etc.) as seen in CD [1]. Due to the absence of distinct pathology on biopsy, and lack of identifiable serological markers (e.g., negative CD serology but with possible presence of anti-gliadin antibodies [1, 37]), gluten sensitivity is currently a diagnosis of exclusion. As such, non-celiac gluten-sensitive patients must meet the following criteria for diagnosis:

- CD, IgE-mediated wheat allergy, and other clinically overlapping diseases (type 1 diabetes mellitus, inflammatory bowel diseases, *Helicobacter pylori* infection) have been excluded
- Negative skin prick test for wheat
- Negative autoantibody serology (EMA-IgA and TTG-IgA)
- Small intestine biopsy demonstrates normal mucosa (Marsh 0) or increased intraepithelial lymphocytes (Marsh I)
- Symptoms are triggered by gluten exposure
- Improvement of symptoms within a few days of a gluten-free diet

Gluten and the Irritable Bowel Syndrome Connection

Whether the prevalence of the irritable bowel syndrome (IBS) is higher in CD has been a point of controversy. A meta-analysis of five case–control studies found a fourfold increase of CD among patients with IBS meeting the Rome II criteria compared with controls (OR 4.34 [95 % CI 1.78–10.6]) [38]. However, a subsequent study found a similar prevalence of CD in non-constipated IBS patients when compared to controls [39].

Gluten-free diets are recommended with increasing frequency for IBS symptoms in the absence of CD. Patients who do not have CD, but possess a consistent genotype of HLA-DQ2/8, have also reported benefit from a gluten-free diet. There are several reports linking gluten ingestion with worsening of IBS symptoms and gluten restriction with improvement of IBS [32, 40]. A subgroup of patients with IBS, that is, patients with diarrhea-predominant irritable bowel syndrome (IBS-D), can benefit from a gluten-free diet. Vazquez-Roque et al. report on a randomized, controlled trial designed to explore whether a gluten-free diet benefits patients with IBS-D [41]. Subjects on a gluten-free diet exhibited lower stool frequency than those on a gluten-containing diet ($P = 0.04$; 95 % confidence interval [CI], −0.652 to −0.015). In addition, the impact on stool frequency of a gluten-free diet was greater for patients who were HLA-DQ2/8 positive. Gluten ingestion was shown to increase the small intestinal permeability in these patients, and especially those patients who carry the HLA-DQ2/DQ8 haplotype. The implementation of a gluten-free diet in this subgroup of patients restored the intestinal barrier function. Interestingly,

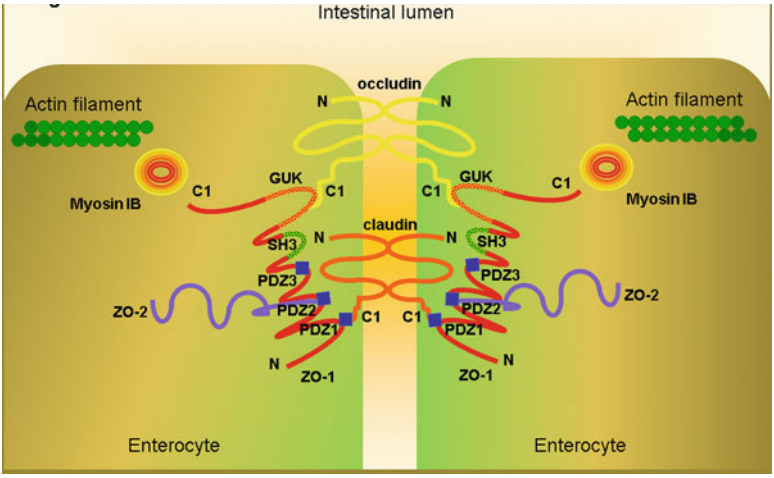

Fig. 2.1 Schematic drawing of the tight junction (TJ) complex. Intestinal epithelial permeability is regulated by the intercellular tight junction protein complex that consists of many components including zonula occludin (ZO)-1, occludin, claudins, and junctional adhesion molecules. These TJ proteins maintain cell–cell adhesion in epithelial monolayers. The overall balance of TJ protein expression is thought to define the regulation of the paracellular path by the TJ complex

decreased expression of tight junction proteins zonula occludens (ZO)-1, claudin-1, and occludin correlated with the increased permeability [41]. Overall there appears to be a connection of gluten ingestion to worsening gastrointestinal symptomatology and improvement upon withdrawal at least in IBS-D.

Pathogenesis

Barrier Function in Celiac Disease and Gluten Sensitivity

Intestinal epithelial permeability is regulated by intercellular tight junction protein complex that consists of many components such as ZO-1, occludin, claudins, and junctional adhesion molecules [42, 43]. These tight junction proteins maintain cell–cell adhesion in epithelial monolayers [44, 45] and the overall balance of tight junction (TJ) protein expression is thought to define the regulation of the paracellular path by the TJ complex (Fig. 2.1).

Zonulin, now identified and characterized as pre-haptoglobin-2 [46], is the human analogue of Zonula occludin toxin derived from *Vibrio cholera* [47]. It is released by the small intestinal mucosa after challenge with gliadin or bacteria [48] and modulates the paracellular intestinal permeability by a PAR2-dependent transactivation of epithelial growth factor receptor and subsequent phosphorylation of TJ proteins [46].

In addition to an environmental factor and genetic predisposition, an impairment of the intestinal barrier function is thought to be an early biological event that precedes the onset of several autoimmune diseases [42, 49]. While under normal physiological circumstances the intestinal epithelium is impermeable to macromolecules, in CD the epithelial barrier function is compromised. In the active phase of the disease, serum titers of zonulin are increased and, consequently, intestinal permeability is augmented [42, 50]. Ex vivo experiments designed to measure the intestinal permeability show that there is an altered junctional structure between epithelial cells [51]. In line with these data, genomic studies have also reported an involvement of genes that control intestinal permeability, including *PARD3*, *MAGI2*, and *MYO9B*, in CD [52–54].

In contrast, the barrier function seems to be conserved in non-celiac gluten sensitivity. Small intestinal permeability, measured with a LA/MA double sugar probe, was significantly lower in gluten-sensitive patients compared to that in CD patients as well as control subjects [31]. In addition to differences between CD and non-celiac gluten sensitivity with regard to intestinal permeability, there are also differences in mucosal TJ protein gene transcripts between the two conditions. The mucosa of subjects affected by gluten sensitivity expresses significantly higher levels of transcripts for claudin-4, a protein involved in TJ-dependent enhancement of the barrier function, relative to that of CD or in healthy individuals [31]. These findings suggest that the distinct clinical and serological features between celiac and gluten-sensitive patients are associated with marked differences in intestinal barrier function and with apparent differences in the expression of *CLDN4* gene expression.

Immune Response of Celiac Disease and Non-celiac Gluten Sensitivity

When the integrity of the intestinal tight junction complex is compromised, an immune response to environmental antigens develops and in genetically predisposed individuals may result in the pathogenesis of CD. CD is considered a classical Th1-mediated disorder because of the increased mucosal gene expression of interferon (IFN)-γ, but not IL-4, in the active phase of the disease [55, 56]. The adaptive immune response in celiac disease is triggered by tissue transglutaminase (TTG)-deamidated gluten peptides that bind with high affinity to HLA-DQ2 or -DQ8 [57]. This involves the mucosal recruitment and activation of Th1 cell clones and production of the Th1 cytokine, IFN-γ (Fig. 2.2).

Another characteristic of CD is the increased numbers of CD3+ intraepithelial lymphocytes. Following the identification of the Th17 T-cell subset [58], and the growing appreciation that these cells are centrally involved in the pathogenesis of autoimmune disorders, recent reports have confirmed the enhanced expression of Th17-active cytokines, IL-1β and IL-23, and the Th17-associated cytokine, IL-17A, in active CD [59–61]. The villous atrophy observed in active CD might be, at least in part, a result of NKG2D (natural killer group 2, member D)-mediated epithelial cell death by intraepithelial cytotoxic T lymphocytes [62]. Reports on regulatory

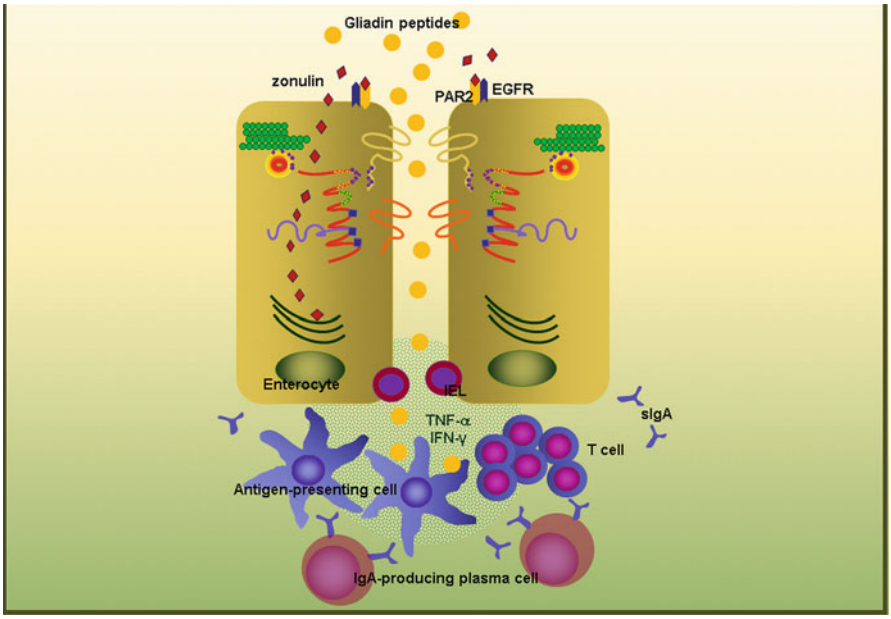

Fig. 2.2 The immune response in the autoimmune enteropathy, celiac disease (CD). In response to undigested gliadin peptides, enterocytes release zonulin that via a PAR2-mediated transactivation of EGFR induces phosphorylation of a major tight junction protein, zonula occludens (ZO)-1. This results in disassembly of the tight junction complex and, hence, increase in intestinal permeability. This allows the gliadin peptides to enter the lamina propria and an immune response is mounted against the gliadin peptides. In response to the accumulation of gliadin peptides in the lamina propria, enterocytes produce IL-15 that recruits intraepithelial lymphocytes (IEL). Histology of active CD shows an increased number of IEL. Tissue transglutaminase (tTG) deamidates the gliadin peptides. The peptides then bind with high affinity to the HLA-DQ2/DQ8 receptor on antigen-presenting cells and are presented to T helper (Th) cells. CD is a Th1-mediated autoimmune disease. The activated Th1 cells secrete inflammatory mediators that attract and activate other immune cells. One key cytokine in this Th1-mediated inflammation is interferon-gamma. The Th1 cells activate natural killer cells to attack enterocytes. B cells mature in IgA antibody producing plasma cells. Hallmark of established CD is the presence of IgA autoantibodies, the anti-TTG, and anti-endomysial (EMA) antibodies in the serum

T cells do suggest that these cells are present in sufficient number in the intestinal tissue, but exert an impaired suppressor function [63, 64].

The pathogenesis of non-celiac gluten sensitivity is not yet understood, but the results we have obtained so far suggest that there is a predominant involvement of the innate immune response rather than the adaptive immune response. Thus far we have observed that in contrast to CD, in non-celiac gluten sensitivity the mucosal expression of IFN-γ, IL-17A, IL-6, and IL-21, cytokines that have an established role in the pathophysiology of Th1 and Th17 responses, is not increased [31, 61]. In addition, we observed a significant reduction in the expression of FoxP3 (forkhead box P3), a T-regulatory cell marker, relative to controls and CD patients.

Although the mucosa in non-celiac gluten sensitivity contained a moderately increased number of CD3+ intraepithelial cells, these numbers were significantly lower than in active CD patients [31]. In the context of relatively conserved villous architecture, these data suggest a more limited involvement of the adaptive immune system in non-celiac gluten sensitivity and may explain why this condition is not accompanied by significant autoimmune phenomena.

Wheat Allergy

Wheat allergy is defined as a true allergic response to wheat that affects the gastrointestinal tract, the respiratory tract, or the skin. IgE plays a central role [1, 65]. In different studies, the prevalence of wheat allergy ranges from 0.5 [66] to 9 % [67] and may be age dependent. There is controversy as to whether sensitization to wheat decreases over time [67, 68]. Amongst food allergies, wheat is identified by the Food and Drug Administration as one of the eight most common allergens, along with milk, eggs, fish, shellfish, tree nuts, peanuts, and soybeans. Together, these foods are responsible for 90 % of all food allergies (Public Law 108-282, Title II, Food Allergen Labeling and Consumer Protection Act of 2004. U.S. Food and Drug Administration, Revised 2004[1]). Positive correlation of food allergy in parents and their children suggests that there is a genetic predisposition for food allergies [69].

Clinical Presentations

Wheat allergy patients typically describe skin, respiratory, or gastrointestinal symptoms, which occur within minutes to hours after wheat ingestion. Symptoms are varied and may include stomach pain, bloating, vomiting, diarrhea, hives, atopic dermatitis, urticaria, rhinitis, and in severe cases, anaphylaxis or death. If wheat allergy is suspected, diagnosis is usually made by elevated IgE serum assay or a positive skin prick test for wheat. However, since the positive predictive value of these tests is only 75 %, in some cases, a food challenge may be necessary for diagnosis [1]. Treatment includes dietary avoidance of wheat and all wheat by-products. Since some studies suggest that wheat allergy may be outgrown, a periodic food challenge regardless of IgE levels to determine if wheat can be tolerated has been suggested [65]. Other studies suggest that less allergenic strains of wheat that are better tolerated by wheat allergy patients may exist [30, 70].

[1] Publication is available at: http://www.fda.gov/food/labelingnutrition/FoodAllergensLabeling/GuidanceComplianceRegulatoryInformation/ucm106187.htm.

Pathogenesis

Most of the studies have been performed on Bakers' asthma, but similarities with the other food allergy conditions, atopic dermatitis, urticaria, and anaphylaxis exist [1]. Wheat allergy is an IgE-mediated allergic reaction and IgE-specific antibodies to alpha-, beta-, gamma-, and omega-gliadins are detected. The adaptive immune reaction to gluten in this condition is mediated by T lymphocyte-driven activation in the gastrointestinal mucosa and repeated sequences in the gluten peptides, for example, Ser-Gln-Gln-Gln-(Gln-)Pro-Pro-Phe, which may induce cross-linking of IgE antibodies and trigger the release of chemical mediators from mast cells in the blood of patients with wheat allergy [71].

Conclusion

Contrary to our previous belief that clinical reaction to gluten was limited to CD, we now appreciate that gluten can instigate different reactions, including wheat allergy and non-celiac gluten sensitivity. While clinically these three conditions overlap and, therefore, make the differential diagnosis much more difficult, the mechanism underlying these conditions is very different. The lack of specific biomarkers and the poor definition of non-celiac gluten sensitivity have created great confusion among healthcare professionals. Progress made during the last few years will hopefully ease this confusion, particularly when a validated biomarker for the diagnosis will become available.

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