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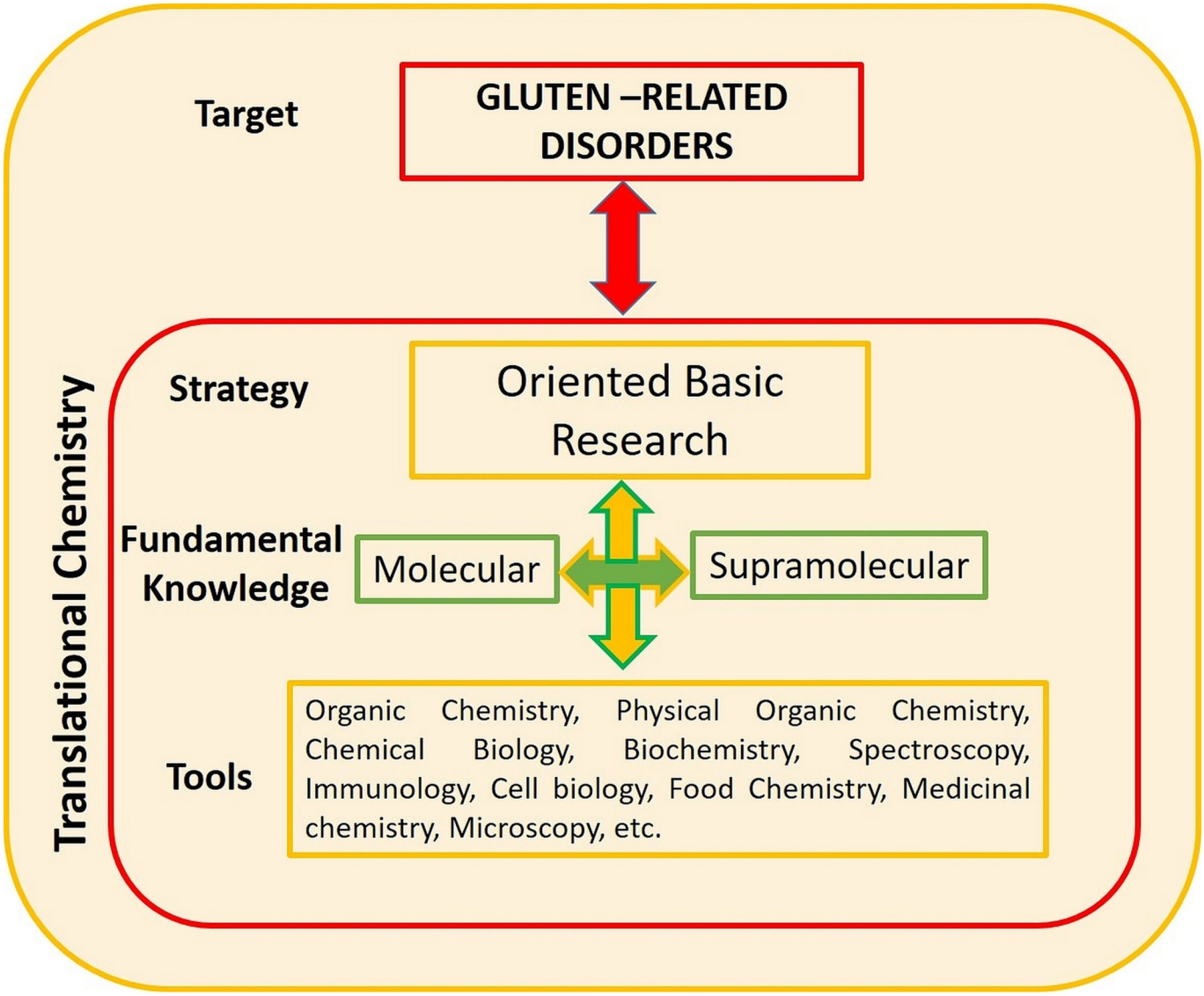
Translational Chemistry Meets Gluten-Related Disorders

Karen M. Lammers,[b] Maria G. Herrera,[c] and Veronica I. Dodero*[a]



Gluten-related disorders are a complex group of diseases that involve the activation of the immune system triggered by the ingestion of gluten. Among these, celiac disease, with a prevalence of 1%, is the most investigated, but recently, a new pathology, named nonceliac gluten sensitivity, was reported with a general prevalence of 7%. Finally, there other less-prevalent gluten-related diseases such as wheat allergy, gluten ataxia, and dermatitis herpetiformis (with an overall prevalence of less than 0.1%). As mentioned, the common molecular trigger is gluten, a complex mixture of storage proteins present in wheat, barley, and a variety of oats that are not fully degraded by humans. The most-studied protein related to disease is gliadin, present in wheat, which possesses in its sequence many pathological fragments. Despite a lot of effort to treat these disorders, the only effective method is a long-life gluten-free diet. This Review summarizes the actual knowledge of gluten-related disorders from a translational chemistry point of view. We discuss what is currently known from the literature about the interaction of gluten with the gut and the critical host responses it evokes and, finally, connect them to our current and novel molecular understanding of the supramolecular organization of gliadin and the 33-mer gliadin peptide fragment under physiological conditions.

1. Introduction

Translational research combines basic and clinical science to promote knowledge in prevention, diagnosis, and therapy of human disease. The final goal is the promotion of quality of life and society well-being.[1] Likewise, a translational chemistry approach would provide the initial foundation to understand human diseases at the molecular level. Using chemical tools,[2] chemists can connect molecular information and biological function or dysfunction. This methodology has been used in the areas of medicinal chemistry, chemical biology, and biochemistry. Additionally, it is possible to link molecular information and to establish physicochemical models to understand complex systems, such as those observed in human diseases. This last strategy is commonly used in physical organic chemistry research, and as Prof. George Whitesides recently defined, "it is not really about physical chemistry, organic chemistry, or even chemistry. It is a strategy for the design of programs in experimental scientific research, which offers a general, and remarkably versatile, method for tackling complex problems."[3] From the perspective of the aforementioned chemical disciplines and the idea to translate this knowledge into biomedical research, we embarked recently on translational chemistry research to shed light on the complicated immunological disorders related to gluten. Herein, we review these diseases from a clinical point of view to the up-to-now molecular and supramolecular understanding.

[a] *Prof. V. I. Dodero*
Department of Chemistry
Organic Chemistry III, Bielefeld University
Universitätsstraße 25, 33615 Bielefeld (Germany)
E-mail: veronica.dodero@uni-bielefeld.de

[b] *Dr. K. M. Lammers*
Laboratory Immunogenetics
Department of Medical Microbiology and Infection Control
VU University Medical Center
1081 Amsterdam (Netherlands)

[c] *Dr. M. G. Herrera*
Faculty of Pharmacy and Biochemistry
Institute of biological chemistry and Physicochemical
CONICET-University of Buenos Aires
Junín 956, C1113AAD, Buenos Aires (Argentina)

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2. Gluten-Related Disorders: The Clinical Point of View

Gluten-related disorders are complex immune-mediated diseases that affect around 1–7% of the general population.[4] They are named according to clinical symptoms and type of immunological response, for example, wheat allergy, celiac disease (CD), gluten ataxia, dermatitis herpetiformis, and nonceliac gluten sensitivity (NCGS) (Figure 1). It is assumed that there must be a particular susceptibility in an individual that could trigger these kinds of diseases, but parameters have not yet been well clarified, except for CD,[5] for which HLA-DQ genes are of invaluable importance in the diagnosis given that virtually all CD patients (>97%) carry the coding variants for HLA-DQ2 and/or HLA-DQ8 molecules.[6] However, although carriage of the HLA-DQ2/DQ8 haplotype is an essential factor in the pathogenesis of CD, it has low predictive value.[5] This is illustrated by the fact that whereas 30–40% of the general population carries this genotype, the prevalence of CD is about 1% worldwide.[7] Genome-wide association studies (GWASs) have shown that there are also non-HLA genes involved in the disease.[8] Up to now, diagnostic tools for diagnosis exist only for CD and dermatitis herpetiformis. In the case of CD, these strategies are genetic markers (HLA-DQ2/DQ8), specific serological antibodies (anti-transglutaminase antibodies), histopathological analysis of the duodenal–jejunal biopsy specimens, and the clinical response to the implementation of a gluten-free diet (GFD).[9]

CD is the most common food intolerance in western society.[11] It is an autoimmune enteropathy that may present not only with intestinal manifestations but also systemic ones, such as anemia, loss of weight, short stature, osteoporosis, and peripheral neuropathy.[12] Other specific conditions associated with CD are, for example, Addison's disease, non-Hodgkin lymphoma, neurological disorders, and type I diabetes mellitus.[13] As CD cannot be cured pharmacologically,[14] the only effective

treatment for gluten-related disorders is a life-long abolishment of gluten from the diet.

A strict gluten-free diet (GFD) improves the clinical symptoms, diminishes the titers of the CD-specific antibodies, and finally heals the intestinal mucosa and restores health. In daily life, adhering to a GFD appears to be difficult and has a huge impact on the quality of life of the patient.[15] The two main long-term complications of not following a GFD in CD are osteoporosis and malignancy. Also, prolonged gluten consumption in nondiagnosed individuals may contribute to the refractory forms of CD, for which there is no response to a GFD and, therefore, intestinal inflammation is maintained. There are two forms of refractory CD: type I, which responds to immunomodulators, and type II, for which there is no effective treatment, and this type is associated with an increased incidence of T-cell lymphoma.[16]

In these days, there is a broad field of research dedicated to finding new alternatives that reduce the immunogenicity of gluten in cereals to prevent people from triggering an immune response.[17] The most-advanced studies are devoted to prolyl endopeptidases degrading toxic gluten peptides (ALV003, AN-PEP)[18] For AN-PEP[19] and larazotide acetate (AT-1001, human zonulin inhibitor),[20] clinical trials have been conducted. However, they will not become an alternative to a gluten-free diet but rather a supplement to it, which will enable patients to ease nutritional restrictions. Another potentially promising approach is nutritional therapy. Various dietary components including long-chain ω-3 fatty acids, plant flavonoids, and carotenoids have been demonstrated to modulate oxidative stress, gene expression, and production of inflammatory mediators. Therefore, their adoption could preserve intestinal barrier integrity and, hence, could play a protective role against toxicity of gliadin peptides.[21] The other gluten-related disorders are much less understood from a molecular perspective. Actually, in the case of nonceliac gluten sensitivity the diagnosis can

Karen Manon Lammers followed the "Free Doctoral Exam" in medicine with specialization in hematology, hemostasis, thrombosis, and inflammation and received a Master's degree from the University of Amsterdam (The Netherlands). She performed her Ph.D. research at the University of Bologna in Bologna (Italy) and the VU University Medical Center in Amsterdam (The Netherlands) and received her Ph.D. degree in 2004 after defending her thesis at VU University Medical Center entitled "Pouchitis, A Model To Study the Pathogenesis of Intestinal Inflammation; Immunomodulation by Probiotics and Immunogenetic Studies". From 2006 on, she worked in the USA on the innate immune response in celiac disease at the University of Maryland, School of Medicine, in Baltimore and at Massachusetts General Hospital/Harvard Medical School in Boston as a Postdoctoral Fellow and later as an Assistant Professor. Since 2016, she has worked as a senior researcher at TubaScan in Amsterdam (The Netherlands), where she conducts translational biomarker research for celiac disease and participates in an interdisciplinary collaboration on the 33-mer gliadin peptide with Prof. Veronica Dodero, University of Bielefeld (Germany). She maintains a Visiting Scientist position at Massachusetts General Hospital. Her main interests are inflammation and infection, immunogenetics, and integrative health.

María Georgina Herrera studied biochemistry at the University of the South (Argentina) and received her degree in 2011. In 2011, she started Ph.D. research at the University of the South (Argentina) under the direction of Prof. Veronica Dodero. She received her Ph.D. degree in 2016 defending her thesis entitled "Elucidation of the Auto-organization of the Protein Gliadin and Its Immunogenic 33-mer Peptide". Since 2016, she has been conducting postdoctoral studies at the University of Buenos Aires (Argentina) studying proteins related to Friedreich Ataxia disease. She maintains high and interdisciplinary collaboration with Prof. Veronica Dodero, studying the structural features of gliadin-related disorders. Her main interests are structural biology, mitochondrial metabolism, aggregopathies, and inflammation.

Veronica I. Dodero performed her Ph.D. work at Universidad Nacional del Sur (UNS, Argentina) and the University of Dortmund (Germany) in organometallic chemistry with DAAD and CONICET (the National Scientific and Technical Research Council) fellowships. Additionally, she obtained the Aaron and Fanny Fidelef Nijamkim's Award for the best Ph.D. student in chemistry. In 2003, she started a postdoctoral position in the Organic Chemistry Department of the University of Santiago de Compostela (Spain) with Prof. Dr. J. L. Mascarenas in the area of chemical biology. In 2007, she started a second postdoctoral position in Madrid (Spain) in the field of medicinal chemistry, expanding her portfolio of synthetic expertise. In 2008, she started her first independent research position as a CONICET Researcher in Argentina, and in 2009, she was awarded a professorship position in organic chemistry at UNS. Since 2015, she has been working as an experienced researcher with a Georg Forster fellowship of the Alexander von Humboldt Foundation (HERMES) in the group of Prof. N. Sewald at Bielefeld University. Her research topics are related to using chemical tools to understand/control self-assembly in water. Her research projects combine synthetic organic and peptide chemistry, structural and physicochemical characterization, microscopy, and biophysical and cellular biology techniques. Using a translational chemistry approach, she is leading a transdisciplinary project to shed light on gluten-related disorders.

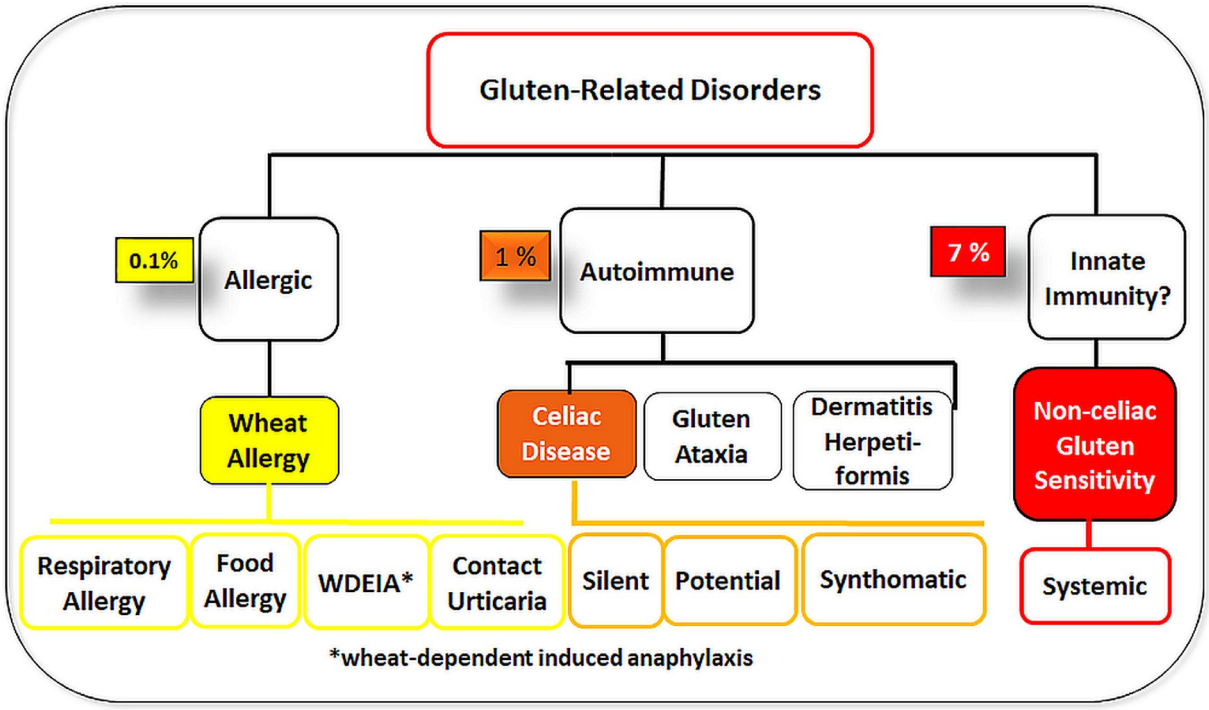

Figure 1. Pathogenesis of gluten-related disorders. The percentages correspond to the prevalence of each disease in the general population (adapted from Sapone et al.[10] and Fasano et al.[4]).

only be made by exclusion of gluten from the diet (refer to more specialized Review articles[4,9]). Taking into account the increased spectrum of gluten-related disorders and the estimated high and increasing prevalence of these diseases,[4,14] it is highly desirable to come to a full understanding of these pathologies from a molecular point of view to provide better diagnosis and appropriate treatment.

3. What is Gluten? A General Description

All aforementioned gluten-related disorders have a common trigger, that is, the contact or consumption of gluten. The term "gluten", as described in the dictionary, is "the tough, viscid, nitrogenous substance remaining when the flour of wheat or other grain is washed to remove the starch and it gives to dough its tough elastic character".[22] From a chemical point of view, gluten is composed mainly of proteins with a high content of the amino acids glutamine and proline in their sequence, generally referred to as prolamins. In the seed, prolamins act as a storage source of nitrogen for germination.[23] The major prolamin from wheat is gliadin, but homologous proteins are found in barley (hordeins), rye (secalins), and oat (avenins). The prolamin proteins of these cereals undergo incomplete enzymatic degradation during in vivo digestion, which produces peptides that result in toxicity in predisposed individuals. In wheat gluten, there are two main types of proteins: one is the alcohol-insoluble fraction, which is traditionally called glutenins, and the alcohol-soluble fraction called is gliadins.[24]

4. The Structure of Gliadin Proteins

The most-studied protein related to gluten-consumption diseases is gliadin, the prolamin from wheat.[25] It is composed of different isoforms that are classified as α (25–35 kDa), β (30–35 kDa), γ (35–40 kDa), and ω (55–75 kDa) depending on their electrophoretic mobility.[26] These proteins share a similar primary structure consisting of an N-terminal domain; a hydrophobic central domain that is rich in proline, glutamine, and phenylalanine; and a nonrepeating region including cysteine (Figure 2). The α- and β-gliadins have between 250 and 300 amino-acid residues with a very similar structure and sequence, so they are usually grouped. Also, α/β- and γ-gliadins contain six and eight cysteine residues, respectively, that are located in a conserved position. The ω-gliadin possesses 350 amino acids and is globally more polar than the rest of the isoforms. It does not contain the amino acid cysteine in its composition, and for that reason, it is not able to present a cross-linking through disulfide bridges.[27] Similar to other gliadins, ω-gliadin is also characterized by high contents of proline and glutamine, which are located in a repetitive and characteristic sequence PQQPFPQQ.[28] All gliadins possess few positively charged amino acids. Although this group of proteins has been studied extensively, elucidation of their secondary and tertiary structures is scarce. Whereas under normal environmental conditions crystallization of the proteins fails, in a microgravimetric environment it is possible to elucidate their structure.[29] The main reason for the lack of structural information is the low solubility of gliadin in water, its high molecular weight, and its high proline content.[25c] These intrinsic characteristics make it difficult to obtain structural information

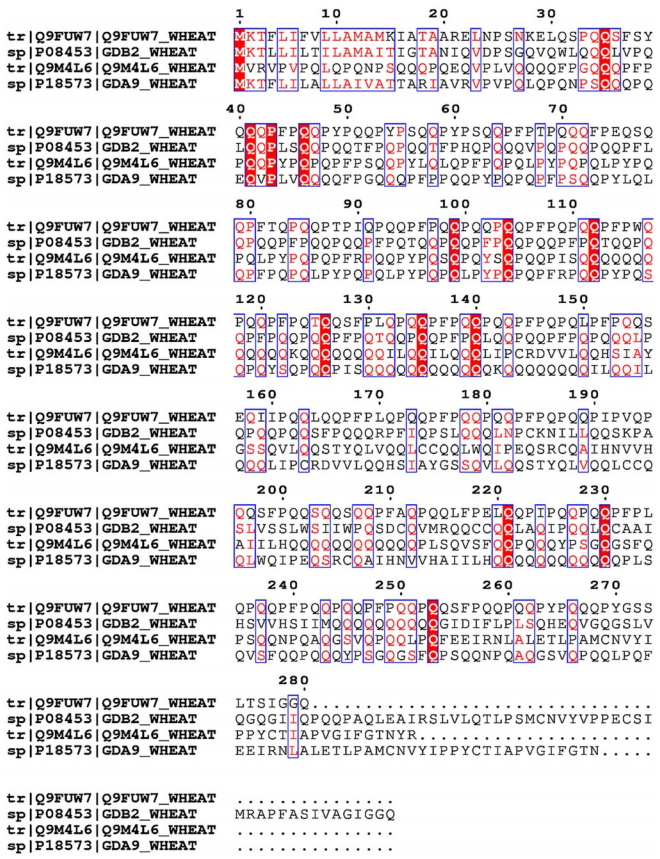

Figure 2. Sequence alignment of representative sequences of α- (Q9FUW7), α/β- (P08453), γ-(Q9M4L6) and ω-gliadins (P18573) by using ESPript 3.0.[33] The conserved residues are in white with a red background. Residues in red are similar within a group, and residues framed in blue are similar across groups.

through high-resolution techniques such as nuclear magnetic resonance or X-ray diffraction. That is why structural information for this protein has been obtained mainly from spectroscopic techniques.[30] Gliadin can be solubilized in water by using mixtures of salts and alcohols. Using Raman spectroscopy, Blanch et al.[31] have shown that α-gliadins possess a high α-structure content, and to a lesser extent, β sheet and polyproline II (PPII) structures at pH 3.5. In the case of ω-gliadins, a well-defined PPII structure has been determined with some β turns at pH 2.6. Moreover, Tatham and Shewry have shown by circular dichroism that an ethanol solution (70%, *v/v*) of α-, β-, and γ-gliadins presents with 36–37% α helix, 11–12% β sheet, and 52–53% random structure and β turns. They also describe that α-, β-, and γ-gliadins are stabilized by hydrogen bonds and disulfur bridges, whereas ω-gliadins are stabilized by hydrophobic interactions.[32]

5. The Self-Assembly Properties of Gliadin: A Supramolecular View

Previous studies have pointed out that gliadin can self-assemble under different conditions in aqueous media. By desolvation, gliadin generates spherical aggregates with diameters in the range of 100 to 500 nm. This method consists of treating a protein with a precipitant solvent and has been employed to obtain nanoparticles of gliadin as a colloidal system for drug delivery.[34] Additionally, gliadin self-organizes in aqueous media to form metastable supramolecular structures. Kasarda et al. have demonstrated that α-gliadin reversibly aggregates into fibrils at pH 5.0 in acetic acid and low ionic strength, and this process can be modulated by lowering the pH.[35] Moreover, Sato et al. have shown that a solution of gliadin in distilled water below 10 wt% with high ionic strength is composed mainly of ellipsoid monomers that are 11.3 nm in length and 2.5 nm in width, with small amounts of dimers and oligomers. Above 15 wt%, gliadins form gel-like hydrated solids, and at higher concentrations, larger aggregates are present.[36] More recently, it has been reported that if a mixture of commercial gliadins is dissolved and homogenized in water at pH 3.0 under low ionic strength, α- and β-gliadins self-organize (Figure 3). The self-assembly process is spontaneous, and nano-

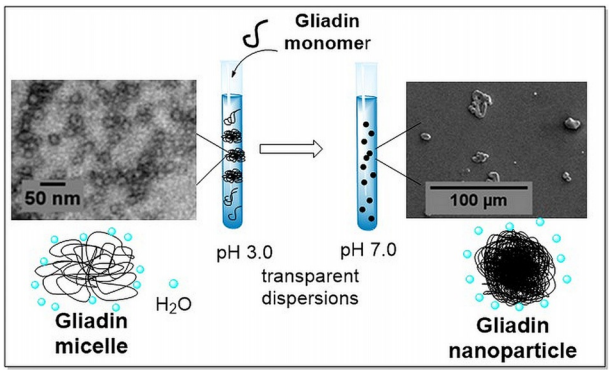

Figure 3. Supramolecular organization of gliadin in aqueous medium reported by Herrera et al.[37] Although both solutions are clear and transparent, gliadin molecules are not randomly dispersed in water; instead, they self-assemble to form micellar oligomers or nanoparticles depending on the pH value.

spherical structures are stabilized in the transparent water solution. If the pH is changed to 7.0, phase separation is observed, whereas the α-gliadins remain in solution as nanoparticle aggregates. Although no differences are detected in the secondary structure at either pH value, differential exposition of tyrosine and tryptophan is observed, which can be caused by a tertiary structure change. To evaluate structural changes, such as the exposure of hydrophobic sites, Nile Red (NR) fluorescence can be employed. By using this technique, this fluorophore increases the fluorescence of α-gliadin and the maximum emission is blueshifted at pH 3 (relative to that of pure α-gliadin), but not at pH 7.0. This behavior is indicative of NR binding to gliadin and suggests that hydrophobic sites are accessible at pH 3.0. These results further indicate that at pH 3.0 the system is self-organized as micellar nanostructures, whereas at pH 7.0 the structures have a lower surface charge (from +13 mV at pH 3.0 to +4 mV at pH 7.0) and colloidal nano- and microparticles are detected.[37]

The formation of these nanostructures in the digestive system may block the degradation sites of the digestive en-

zymes. By simulation, the α-2 gliadin protein is anticipated to be cleaved by pepsin in 48 positions and by chymotrypsin in 35 locations, but in reality, this does not happen. In that case, the supramolecular behavior of gliadin under physiological conditions at pH 3.0 and 7.0 offers an essential clue to the understanding of why pepsin or chymotrypsin in vivo does not fully degrade this protein. Of course, once in the small intestine the high amount of proline plays a crucial role in endopeptidase activity, but the high content of proline by itself does not explain the proteolytic resistance observed.

6. The Interaction of α-Gliadin and Its Proteolytic-Resistant Peptides with the Intestinal Mucosa

Among gliadins, the α-gliadin isoform and its digest (proteolytic-resistant fraction after pepsin and trypsin digestion) are frequently used to study the gliadin-mediated effects on primary cultures and cell lines.[38] The rationale behind this choice is that the gliadin digest most closely mimics the in vivo situation. Due to gliadin's incomplete degradation, a vast array of mostly unknown small and large peptides comes into contact with the gut epithelium and after transcellular and/or paracellular translocation with the lamina propria, they can be recognized by the mucosal immune system.

6.1. The Gut-Associated Lymphoid Tissue

The mucosal immune system has the relevant task to distinguish which of the many luminal antigens that it encounters are benign to the host, for instance, food proteins and commensal flora, and which of them form a danger to the host, for instance, invading pathogens. Under normal conditions, a milieu of immune tolerance reigns at the mucosal site, but the mucosal immune system must possess a readiness to mount an adequate proinflammatory immune response to danger signals to protect the host. Different cell types take part in these elaborate dynamics.[39] Intestinal epithelial cells (Figure 4a) are the first level of control, as they "sense" the nature of the luminal environment (Figure 4b) and send crucial information to underlying immune cells, for example, by the production of cytokines and expression of membrane receptors (Figure 4c–e). The gut-associated lymphoid tissue from the small intestine consists of effector sites, that is, scattered lymphocytes in the lamina propria (Figure 4f) and epithelium and organized tissues such as Peyer's patches and mesenteric lymph nodes (Figure 4g,h). In particular, the mesenteric lymph nodes are crucial for the induction of tolerance and immunity. Generally, the antigen is taken up by dendritic cells and is presented in the context of HLA class II to $CD4^+$ T cells.[40] Possible places at which antigen uptake can occur are Peyer's patches (Figure 4g) and, if the antigen has gone through the epithelial monolayer, the villous lamina propria (Figure 4f). The antigen-loaded dendritic cells within the Peyer's patches can present to $CD4^+$ T cells in loco or can migrate from the Peyer's patches via afferent lymphatics to the mesenteric lymph nodes and present there to $CD4^+$ T cells. Likewise, villous lamina propria dendritic cells can reach the mesenteric lymph node via afferent lymph vessels, and there they present the antigen to $CD4^+$ T cells (Figure 4g–i). Wherever the site of antigen uptake may be, all antigen-primed lymphocytes obtain the intestinal homing molecule, α4β7, and leave the mesenteric lymph node via efferent lymph vessels and re-enter the intestinal mucosa at which they take their particular place. For example, B cell blasts mature into IgA-producing plasma cells and remain in the lamina propria (Figure 4k), $CD4^+$ T cells are distributed over the villus lamina propria (Figure 4j), whereas most $CD8^+$ T cells migrate to the epithelium. The T cells can provide help as effector cells to B cell plasmablasts to produce IgA, and they can be antigen-specific memory $CD4^+$ and $CD8^+$ T cells or regulatory T cells. $CD8^+$ T cells also can exert strong cytotoxic activity, usually to antigens that are present in the context of HLA class I molecules.[40b]

Antigen-primed $CD4^+$ T cells are involved in local immune regulation and produce large amounts of interferon (IFN)-γ, interleukin (IL)-4, and IL-10.[41] The coexistence of these different cytokines is a feature of normal homeostasis, and disturbances in their secretion reflect pathogenesis of gastrointestinal disorders, such as the high mucosal titers of IFN-γ in the active phase of CD.[42] The production of IL-10 is essential in the maintenance of tolerance (Figure 4e,i,j), whereas in immunity it is crucial in the regulation of inflammation.[41b] In addition to the environmental factors derived from the intestinal lumen, such as food proteins or bacterial products, genetically determined (host) factors may impact the immunological outcome. As professional antigen-presenting cells, the dendritic cells integrate these genetic and environmental factors and shape the T lymphocytes, to which they present the antigen, to maintain intestinal homeostasis or to induce immunity. Other features of intestinal tolerance are the abundant production of the cytokines TGF-β and IL-10 by tissue macrophages, mesenchymal cells, and epithelial cells and, furthermore, the production of the enzyme cyclo-oxygenase 2 (COX-2) by epithelial cells (Figure 4c). COX-2 is an essential mediator in the synthesis of prostaglandin-2 (PGE2) from arachidonic acid, and it fulfills a crucial role in immune tolerance. PGE2 increases IL-10 production, decreases proinflammatory mediators such as HLA class II, IL-12, tumor necrosis factor (TNF)-α, and primes dendritic cells to shape T cells to tolerogenic and regulatory T cells (Figure 4e,i). Inhibition of COX-2 in a murine model results in features characteristic of celiac disease possibly due to T-lymphocyte stimulation in an environment deprived of COX-2-dependent arachidonic acid metabolites.[43]

In case pathogens are encountered, local inflammation is induced by Toll-like receptor (TLR)-mediated production of inflammatory mediators, such as IL-1, IL-6, and IL-8 by macrophages, mesenchymal cells, and epithelial cells (Figure 5a). This inflammatory environment shapes the dendritic cells (DCs). As a result, the DCs undergo complete maturation after having taken up the antigen in the Peyer's patches or lamina propria (Figure 5b) and start to produce the proinflammatory cytokine IL-12. Under these circumstances, antigen presentation to naïve $CD4^+$ T cells leads to their differentiation into gut-homing Th1 cells, which produce IFN-γ and cause further

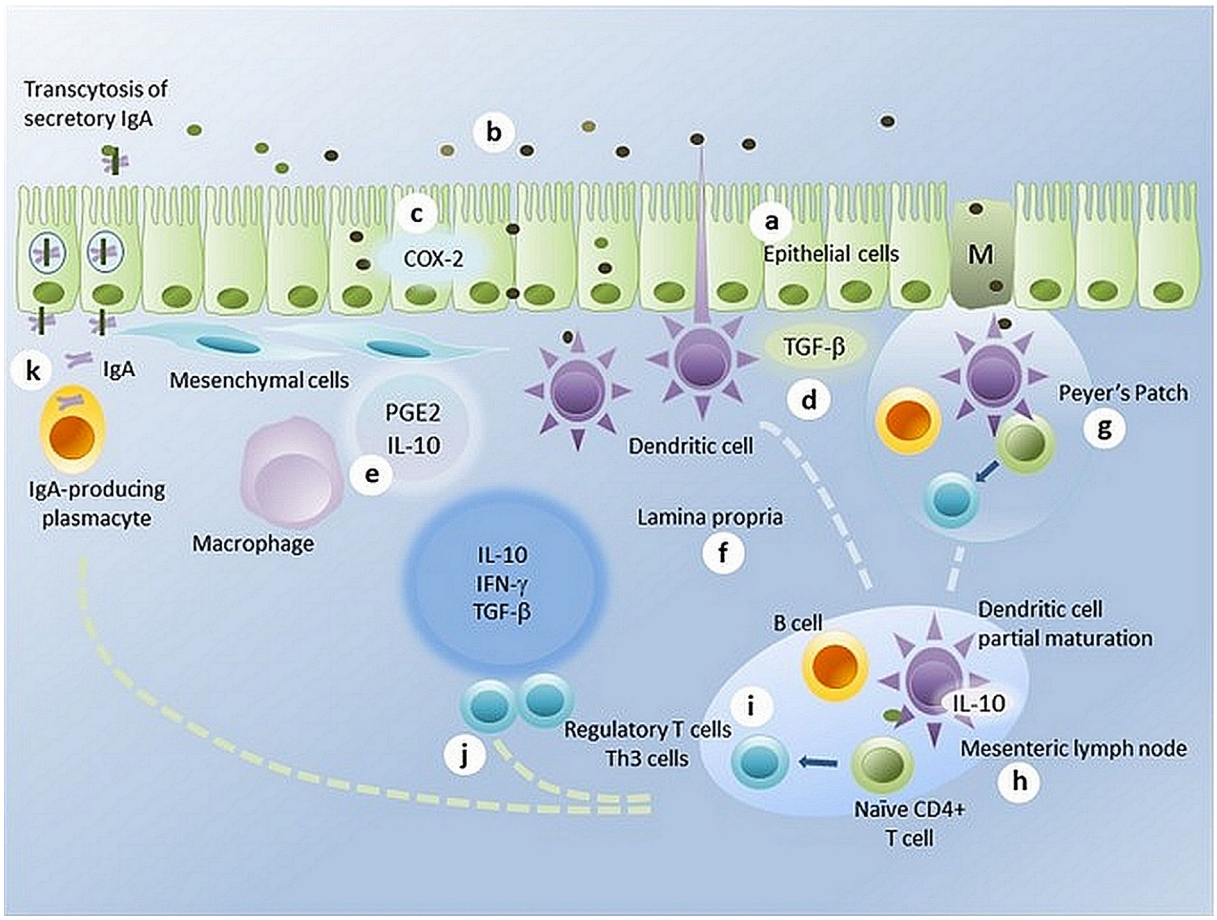

Figure 4. Intestinal homeostasis. Under normal conditions, the intestinal environment is an environment that favors tolerance to allow commensal bacteria and dietary antigens and maintain intestinal homeostasis. Intestinal epithelial cells (a) form a tight barrier between *milieu exterieur* and *milieu interieur*. They sense the nature of the luminal antigens and send information to underlying immune cells, for instance, by the production of tolerogenic cytokines (c–e). Food antigens and products from the microflora (b) are taken up by dendritic cells (DCs) in the lamina propria (f) or Peyer's patches (g). In the absence of inflammation, DCs then undergo partial maturation under the influence of prostaglandin 2 (PGE2) and IL-10, produced by macrophages and mesenchymal cells (e), and transforming growth factor (TGF)-β and COX-2 produced by epithelial cells (c, d). DCs produce the anti-inflammatory cytokine, IL-10, and present the antigens to naïve $CD4^+$ T cells in the Peyer's patches or mesenteric lymph nodes (h), in which they differentiate into regulatory T cells under the influence of IL-10 (i). From the mesenteric lymph node, recirculation and homing to the mucosa of T cells (j) and plasmablasts occur (k). These events lead to local IgA production and immune tolerance.

inflammation (Figure 5 c). Likewise, plasmablasts home to the intestinal mucosa and mature into IgA-secreting plasma cells (Figure 5 d). These events lead to local immunity and IgA production.

6.2. The (Patho-)Physiological and Immunogenic Properties of the Gliadin Digest

Gliadin digest induces physiological changes and immunological responses, many of which are observed in both health and disease, albeit the measure of these effects of gliadin is much higher and prolonged in disease. In this scenario, specific and characteristic for the active phase of celiac disease are the presence of gliadin-restricted Th1/Th17 T-cell-mediated immune responses (Figure 5 c, e),[42,44] an increase in intraepithelial lymphocytes (Figure 5 f), and villus blunting (Figure 5 g).

In the gut lumen, the gliadin digest first interacts with the intestinal epithelium. It is still a matter of debate how gliadin and its digestive fragments pass the enterocyte monolayer, but available information suggests the involvement of both transcellular (Figure 5 g, h) and paracellular pathways (Figure 5 i). Some evidence for endocytosis of gliadin and its digest is shown by the finding that gliadin fragments induce remodulation of the actin cytoskeleton, which interferes with the brush-border membrane trafficking of vesicles.[45] Another school of thought includes the possibility of paracellular transport over the intestinal epithelium.[46] In this respect, it has been shown that the gliadin digest binds to the apically expressed chemokine receptor (CXCR3).[47] Gliadin's interaction with CXCR3 induces the secretion of zonulin, a physiological modulator molecule of intestinal permeability, which in turn binds, through transactivation of the epithelial growth factor receptor (EGFR), to protease-activated receptor 2 (PAR2).[48] These interactions

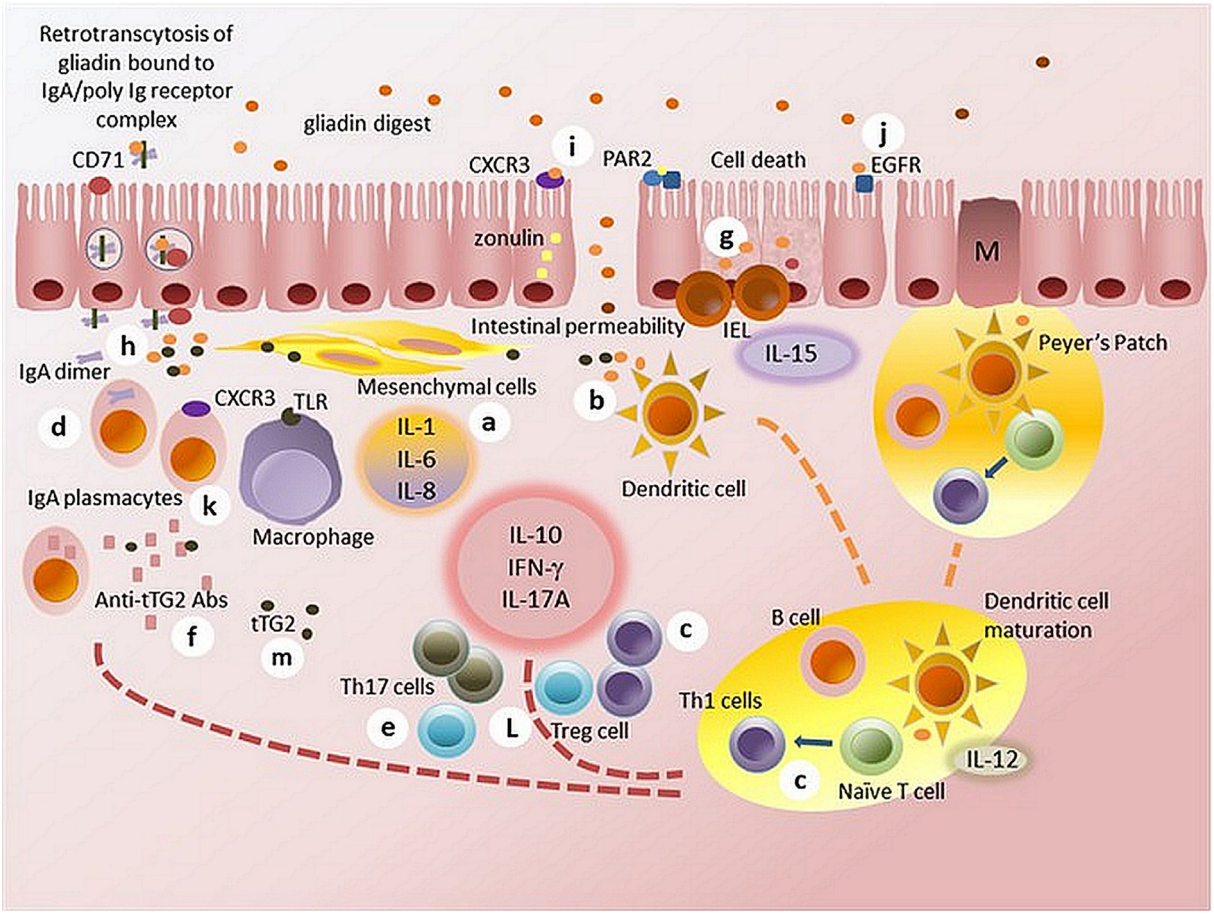

Figure 5. Immunity in celiac disease. Whereas under normal conditions an environment of mucosal tolerance exists, there is at the same time a readiness to mount an inflammatory immune response if needed, such as in the case of danger signals (invading pathogens). If pathogens are encountered, local inflammation is induced by the production of inflammatory mediators, such as IL-1, IL-6, and IL-8 by macrophages, mesenchymal cells, and epithelial cells (a). This inflammatory environment shapes the DCs, and as a result, they undergo complete maturation after taking up the antigen in the Peyer's patches or lamina propria (b) and start to produce the proinflammatory cytokine, IL-12. Naïve $CD4^+$ T cells differentiate under the influence of IL-12 into gut-homing Th1 cells, which produce IFN-γ and cause further inflammation (c). Likewise, plasmablasts home to the intestinal mucosa and mature into IgA-secreting plasma cells (d). This event leads to local immunity and IgA production.

lead to reorganization of the cytoskeleton, which results in disassembly of the tight junctions, interepithelial protein complexes that regulate permeability.[44] Barone et al.[49] report an epithelial growth factor (EGF)-like effect of gliadin, including EGFR activation, rearrangement of the actin cytoskeleton, and changes in cell cycle and cell proliferation (Figure 5 j). Using normal mice and MyD88-deficient mice, Thomas et al.[50] have shown that the effects of gliadin digest on intestinal permeability and zonulin release requires MyD88 recruitment. Although the involvement of MyD88 commonly indicates signaling by TLRs, this study shows that the two most plausible—if any—candidates, TLR2 and TLR4, seem not to be implicated in this response. Although it cannot be excluded that other TLRs might be involved in gliadin-induced intestinal permeability, the CXCR3 chemokine receptor has been found to co-localize with MyD88 and appears to possess a Toll/Interleukin receptor (TIR)-like domain, which strongly suggesting its (mere) involvement in gliadin-induced, MyD88-mediated signaling.[47] In cultured duodenal biopsy specimens from healthy individuals and celiac patients, Drago et al.[51] have confirmed that gliadin and its digested fraction induce the release of zonulin and increase intestinal permeability and, furthermore, report disease-specific qualitative and quantitative differences. Thus, whereas the release of zonulin and an increase in permeability are small and short-lived events under healthy conditions, both the release of zonulin and the accompanying breach of barrier function are massive and difficult to restore in celiac tissues. Notably, during the active phase of celiac disease, in addition to increased intestinal permeability zonulin titers are elevated[52] and mucosal expression of CXCR3 is increased (Figure 5 i, k).[47,53]

Figure 5 further depicts inflammatory features that have been described explicitly for CD, the gluten-induced autoimmune enteropathy that is characterized by aberrant Th1/Th17 T-cell-mediated immunity and auto-antibody production leading to severe intestinal damage (Figure 5 e–m). See text for detailed discussion of these features.

All markers return to baseline expression levels after implementation of a gluten-free diet once the disease goes into remission. Gliadin's interaction with intestinal epithelium produ-

ces stressful effects such as an oxidative imbalance,[38] the production of IL-15 and IFN-γ,[54] and apoptosis if the cells are exposed to gliadin for 48 h.[55] Furthermore, the gliadin fragments can induce both an upregulation of epithelial expression of HLA-E and MICA (major histocompatibility complex class I chain-related gene A) molecules, which are ligands for their respective receptors, CD94 and NKG2D, and selective expansion of subsets of intraepithelial $CD8^+$ cytotoxic T lymphocytes that express CD94 and NKG2D.[56] These pathways are thought to be mechanisms that regulate cytotoxic T-lymphocyte activity in injured cells, thereby minimizing potential damage to adjacent intact cells. Usually, cytotoxic T lymphocytes need co-stimulation with T-cell receptor (TCR) signaling, but recent studies convincingly show that IL-15 can induce changes in the NKG2D signaling pathway that allows for a TCR-independent conversion of $CD8^+$ cytotoxic T lymphocytes into natural killer-like cells. Most of these events are inhibited by neutralizing antibodies to IL-15, which thus confirms the key role of this cytokine as a mediator of intestinal mucosa damage.[57] Although the gliadin digest can induce epithelial IL-15 production under normal conditions,[56,58] IL-15 titers are much higher and intraepithelial lymphocyte numbers are significantly increased in active celiac disease relative to healthy individuals, which suggests a role for aberrant activation of the IL-15/NKG2D signaling pathway in the massive tissue damage observed in active celiac disease (Figure 5g).[59] To understand further the effects of gliadin's digest on innate immune cells, peripheral blood mononuclear cells and primary cells and cell lines from phagocytic lineage have been used in stimulation assays. In peripheral blood mononuclear cells from healthy individuals and CD patients, the gliadin digest induces the production of various cytokines, including TNF-α, IL-10, and IL-8. The latter cytokine appears to be produced in a CXCR3-mediated fashion only in CD patients. Its cellular sources are monocytes and plasmacytoid dendritic cells.[60] In normal murine peritoneal macrophages, gliadin stimulates the production of TNF-α, RANTES, IL-10, and IL-8 and a significant rise in nitric oxide (NO), another mediator of inflammation. These effects are dependent of prior priming of the cells with IFN-γ.[61] Jelínková et al.[62] report similar effects for the human monocyte cell lines, THP1 and U-937, with gliadin-induced production of IL-8 and TNF-α through the activation of the NFκB signaling pathway, and a synergistic effect of IFN-γ has also been shown in human cells. Thomas et al.[50] show in a normal murine model that the gliadin digest, in addition to MyD88-dependent release of zonulin and increased intestinal permeability, also induces the production of proinflammatory cytokines, among which are TNF-α, IL-12, IFN-β, and CXCL10, by macrophages. As signaling through TLRs recruits the adaptor molecule MyD88 and leads to NFκB activation, the study assesses a role for TLR2 and TLR4 in gliadin-induced immune responses but does not find evidence for their involvement. In contrast, Palová-Jelínková et al.[63] have found that TLR4 is involved in gliadin-digest-induced NF-κB activation and IL-1β production by human monocytes. As the effects are not observed after stimulation with synthetic gliadin peptides, the authors do not exclude that another component within the digest, for instance, the amylase tryptase inhibitor for which a TLR4-mediated effect was previously described by Junker et al.,[64] could be responsible for the observed effects. Another effect of gliadin is its neutrophil chemoattractant properties.[65] Upon applying gliadin digest in the intestinal lumen of C57BL/6 Lys-eGFP mice, $CD11b^+Ly6G^+$ cells (a subset consisting of murine macrophages and neutrophils) rapidly migrate from the vessels into the lamina propria. With in vitro chemotaxis assays, migration of isolated murine and human neutrophils to the gliadin digest has been shown. Notably, the gliadin digest and the classical neutrophil chemoattractant, f-Met-Leu-Phe, both induce similar neutrophil migration. Specific inhibition of the f-Met-Leu-Phe receptor, the formyl peptide receptor 1 (FPR1), not only blocks neutrophil migration towards f-Met-Leu-Phe but also towards gliadin, which indicates that gliadin also binds to FPR1.[65] The adaptive immune response specific for celiac disease consists of a Th1-mediated mucosal immune response of gliadin-specific $CD4^+$ T cells with a particular cytokine pattern ($IL\text{-}10^+$, $IL\text{-}2^{low}$, $IL\text{-}4^-$, $IFN\text{-}\gamma^+$).[57] Recently, reports have described the presence of Th17 cytokines in active CD, in particular IL-17A,[44] which suggests the involvement of a Th17-mediated immune response in CD (Figure 5e). Important in the regulation of the Th17-mediated immune response are the $CD4^+CD25^{++}$FoxP3 (forkhead box protein 3)$^+$ regulatory T cells.[66] Although regulatory T cells are increased in number in active CD,[67] their suppressive function seems impaired (Figure 5i).[68] Granzotto et al.[68b] show that regulatory T cells from CD patients are impaired in their ability to suppress the proliferation of $CD4^+CD25^-$ responder cells after CD3/CD28 co-stimulation in comparison to regulatory T cells from healthy individuals (Figure 5c). In their study, Serena et al.[68a] demonstrate overexpression of the alternatively spliced isoform of FoxP3 in CD mucosa. This splice variant cannot properly downregulate the expression of RORγτ, a transcription factor that is essential in Th17 differentiation, possibly favoring skew towards a Th17 immune response (Figure 5e).

Figure 6 provides a summary of the reported effects of the gliadin digest in vivo.

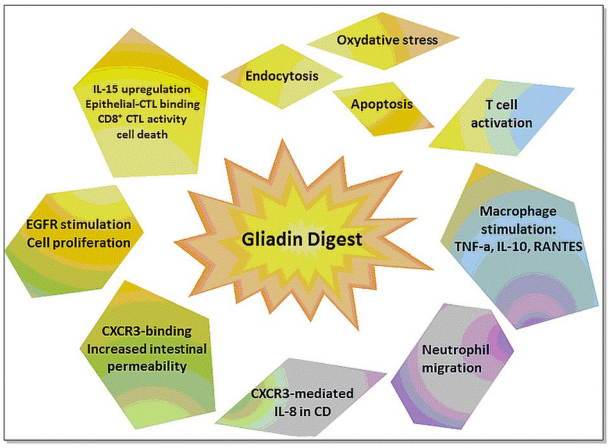

Figure 6. Summary of reported gliadin digest effects in vivo. The figure depicts the effects of gliadin digest that are reproduced by specific peptide fragments within the digest, as described in Section 7.

7. Identification of Gliadin Peptides and Their Pathological Role

Gliadin peptides can be classified as "toxic" or "immunogenic" depending on their ability to induce intestinal damage in vivo and/or in vitro or to activate T cells, respectively.[69] Specific synthetic gliadin fragments recapitulate many of the abovementioned effects of the full gliadin digest. A 13-mer peptide, derived from α9-gliadin (aa 31–43), has been reported to be toxic.[70] Another fragment 33 amino acids long is recognized as the most proteolytic-resistant and immunogenic peptide and is named a 33-mer gliadin peptide (aa 57–89 from α2-gliadin).[25a] More recently, Tuckova et al. have identified 12-mer and 14-mer peptides.[61] Lammers et al. report a CD-related 17-mer peptide (aa 270–286),[60] two intestinal-permeating 20-mer peptides (aa 120–140, aa 160–180), and a number of 20-mer neutrophil chemoattractant peptides.[65]

7.1. The Toxic 13-mer Gliadin Peptide (aa 31–43)

The 31–43 peptide sequence has been detected mainly in α-9 gliadin (Figure 7). It was first obtained by in vitro cyanogen bromide cleavage, and this sequence has been tested as a synthetic peptide in jejunal mucosa of untreated patients and has been shown to induce histological damage.[71] From an immunological perspective, this peptide induces several pleiotropic effects without binding to HLA-DQ2/DQ8 and without stimulating $CD4^+$ T cells. These effects include rapid production of inflammatory mediators, such as IL-15 and COX-2; upregulation of CD25 on non-T cells (CD3-negative cells); and CD83 expression on resident dendritic cells in the lamina propria.[70b,71] Furthermore, the peptide increases the intraepithelial migration of $CD8^+$ T cells and $CD94^+$ cells and increases enterocyte apoptosis. The results are dependent on IL-15, as the addition of neutralizing antibodies to IL-15 abrogates the effects (Figure 5g). To explore the in vivo effect of this peptide, Araya et al. have applied the peptide intraluminal in the intestine of normal mice during surgery and have studied the responses in these mice. The results show that the peptide causes histological changes similar to those observed on CD, such as increased intraepithelial lymphocytes and reduced villus-crypt ratio. Furthermore, the peptide induces an increase in epithelial cell death and increases titers of inflammatory mediators, in particular of type I interferon. These effects are dependent on the recruitment of MyD88, but a role for TLR4 can be ruled out. Interestingly, co-administration of poly I:C, the ligand for TLR3, enhances the immune response. As TLR3 is known for its specificity to recognize viral single-stranded RNA, the authors conclude that the gliadin peptide p31-43 can activate the innate immune pathways in vivo and suggest a potential interaction may exist between dietary gluten and viral infections.[72]

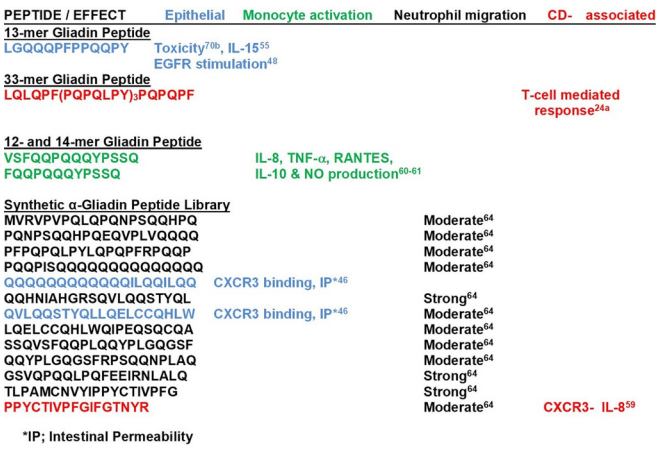

Figure 7. Summary of different gliadin fragments with their known pathological behavior.

One crucial intracellular pathway implicated in the pathogenicity of the 13-mer peptide is EGFR activation. Upon exposure to the 13-mer peptide, both the Caco-2 cell line and culture biopsy specimens from CD patients show signs of actin remodeling and cell proliferation reminiscent of the effects induced by EGF. Although not a ligand for EGFR, the 13-mer peptide prolongs activation of EGFR by interfering with EGFR endocytosis.[73] In another study, Barone et al show that in a search for sequence similarity, the 13-mer peptide resembles hepatocyte growth factor regulated tyrosine kinase substrate (Hrs), which is a crucial regulator protein of endocytic maturation. The observed accumulation of the 13-mer in the early endosomes might then occur because the peptide would interfere with Hrs and its proper functioning. As a consequence, the 13-mer delays maturation of early endosomes into late endosomes, the compartment in which degradation of peptides takes place, and additionally, it delays EGFR degradation.[73a] The perpetuation of EGFR activation may be of importance in the development of epithelial blunting, as observed in active CD (Figure 5j). Luciani et al. show the accumulation of the peptide in LAMP-containing lysosomes. The continued presence of the peptide in the cell causes cellular stress, as measured by an increase in the levels of radical oxygen species, elevated titers, and activation of tissue transglutaminase 2 and downregulation of peroxisome proliferator activator receptor (PPAR)-γ, which activates the innate immune response.[74]

Another critical topic to evaluate is how the 31–43-mer can travel through the gut mucosa. Vilasi et al. have shown with a simple micellar model that this peptide can possibly interact with membranes.[75] A recent study making use of chemical cross-linking to stabilize the peptide on proteins expressed on the cell surface and pull-down of the peptide–protein complexes with antibodies raised against peptide 31–43 did not find specific complexes between cellular proteins and the 13-mer peptide. Also, in competitive binding experiments the peptide could not displace bound peptide, which suggests nonspecific binding. On the basis of this result, the authors hypothesize that no receptor might be involved in the trafficking of the 13-mer peptide.[76]

7.2. The Immunodominant 33-mer Gliadin Peptide (aa 56–87)

The 33-mer peptide (see Figure 7) was obtained by Shan et al.[25a] after the enzymatic degradation of α-2 gliadin by gas-

tric, pancreatic, and brush border peptidases. Recently, the quantification of the 33-mer by liquid chromatography tandem mass spectrometry in different wheat species and cultivars has been performed. This report shows that after proteolysis, the 33-mer remains in the range of 91 to 603 $\mu g\,g^{-1}$ in wheat flour, which justifies the importance of this fragment in the human diet.[77] This highly proteolytic-resistant peptide harbors six epitopes that are in part overlapping. By an unknown mechanism, it reaches the lamina propria, at which dendritic cells process and present it in the context of HLA-DQ2/8 to $CD4^+$ T lymphocytes.[25a] In general, proteins cross the epithelium by the transcellular pathway followed by lysosomal degradation. As for the other gliadin peptides, there is an ongoing debate on the mechanism of epithelial translocation. Schumann et al.[78] have studied 33-mer peptide transcytosis in Caco-2 cells and duodenal biopsy specimens from healthy individuals and active CD patients and have found evidence for transcellular transport of the 33-mer peptide to the lamina propria through an endocytic pathway involving Rab-5. Addition of IFN-γ, the hallmark cytokine in active celiac disease, enhances translocation of the peptide in Caco-2 cells. Compared to duodenal tissues from healthy individuals and CD patients in remission, tissues from active CD patients reveal significantly higher epithelial uptake of the peptide seemingly localized in endocytotic vesicles. The data presented by Ménard et al.[79] suggest a transcellular pathway for the 33-mer peptide. Their immunofluorescent images show co-localization of the peptide with early endosome antigen 1, which indicates that the peptide enters the early endosomes. However, no co-localization is found with lysosomal-associated protein 2, which suggests that the peptide does not enter the late endosomes or lysosomes, the cellular compartment in which peptide degradation is thought to occur. These authors hypothesize that this is in line with their other study,[80] in which they demonstrate that the 33-mer peptide is transported to the lamina propria while escaping the lysosomal compartment by binding to lumenal secretory immunoglobulin A (sIgA); thus, lysosomal degradation was impaired. The gliadin–sIgA complex is recognized by the apically expressed transferrin receptor, CD71, and is "retrotranscytosed" to the lamina propria (Figure 5h). This mechanism would only occur in active CD, for which, in contrast to the healthy condition in which CD71 is solely expressed at the basolateral site of the cells, the receptor is aberrantly expressed at the apical side of the enterocyte. Finally, Bethune et al.[81] describe two possible mechanisms for epithelial translocation of the 33-mer peptide by using a T84 cell-culture model. In response to conditioned medium from gliadin-treated T cell cultures from CD patients, epithelial cell permeability is increased and apically applied 33-mer peptides make use of the paracellular pathway to translocate. This process occurs mainly in the presence of high concentrations of IFN-γ, and the addition of neutralizing antibodies to IFN-γ or anti-IFN-γ-receptor blocking antibodies inhibits paracellular transport. In the absence of gliadin-conditioned medium or IFN-γ, transcellular transport of the 33-mer seems to take place without the involvement of a receptor. These authors conclude that under healthy conditions, gliadin peptides use the transcellular pathway in a receptor-independent way though fluid-phase endocytosis, whereas under inflammatory conditions an IFN-γ-induced increase in the intestinal permeability results in increased paracellular translocation of gliadin.

In celiac patients, a characteristic and well-known T-cell response (adaptive immune response) is observed if the peptide enters the gut mucosa. In the lamina propria, the 33-mer peptide is a high-affinity substrate for tissue transglutaminase 2, an enzyme that deamidates three glutamines to glutamic acid (Figure 5m). This deamidated peptide is much more immunogenic and induces a stronger adaptive immune response. Additionally, the 33-mer peptide contributes to β-cell hyperactivity observed before the onset of type 1 diabetes, at least in murine models.[82] Some researchers have considered that gliadin and its 33-mer fragment are handled by the host as if it were a pathogen such as a bacterium or a virus.[83] However, although there is much evidence pointing in this direction there is no sound explanation for the observed similarities.

7.3. Other Identified Gliadin Peptides

It is important to note that frequently the exact peptide composition of a gliadin digest is unknown. For this reason, Tuckova et al. have identified different peptides from the peptic gliadin digest by reverse-phase (RP)-HPLC–MS. These authors report that a 14-mer fragment, VSFQQPQQQYPSSQ (named the **T** peptide), and especially its shorter 12-mer form, FQQPQQQYPSSQ (named the **B** peptide), elicit the highest TNF-α, IL-10, and RANTES secretions and an increase in IFN-γ-primed nitric oxide production in mouse macrophages.[61] Likewise, these peptides induce IL-8 and TNF-α through activation of the NFκB pathway in the human monocytic cell line, THP-1.[62] Another approach described by Lammers et al. involves the generation of a α-gliadin synthetic peptide library consisting of 25 20-mer peptides each overlapping in 10 amino acids. This library has been used to identify the fragments responsible for the observed gliadin-digest-induced effects (Figure 7).[47] A total of 16 novel gliadin peptides have been described to be involved in three different responses, for example, intestinal permeating effects,[47] immunogenic effects, and neutrophil migratory effects.[65] Using the synthetic α-gliadin peptide library, two peptides, QQQQQQQQQQQILQQILQQ (aa 120–140) and QVLQQSTYQLLQELCCQHLW (aa 160–180), have been identified as the specific gliadin fragments that bind to epithelial CXCR3 (Figure 5i). Furthermore, unlike nonbinding peptides, these two CXCR3-binding peptide fragments also induce tight junction disassembly. The second finding is related to gliadin-digest-induced immune stimulation of peripheral blood mononuclear cell cultures and shows that the cellular response to gliadin is differentially regulated in CD than in health with the involvement of the CXCR3 chemokine receptor. In this study, the cell cultures from both healthy individuals and celiac disease patients respond to gliadin digest by producing substantial titers of an array of proinflammatory mediators, including interleukin-8.[60] Solely in the wells that contain cells from celiac patients, but not those containing cells from healthy individuals, a preincubation step with an antibody that

blocks CXCR3 before stimulation with the gliadin digest results in complete inhibition of IL-8 production. With the use of the abovementioned synthetic α-gliadin peptide library, a third peptide, PPYCTIVPFGIFGTNYR (aa 270–286), shows a result similar to that obtained with the gliadin digest regarding the CXCR3-mediated production of IL-8 by cells from the phagocytic lineage from CD patients. Finally, this approach has allowed the researchers to individuate another 13 peptides that induce FPR1-mediated, moderate-to-strong neutrophil migration.

Altogether, these findings reveal exciting information on α-gliadin and illustrate the molecular complexity of the protein and its interaction with the host.

8. The Supramolecular Behavior of 33-mer Gliadin Peptide: A New Hypothesis

There is evidence that the 33-mer peptide is transported within the body because it is detected in urine and feces.[84] It seems that the 33-mer gliadin peptide can escape all the proteolytic pathways involved in digestion; it crosses the gut epithelium and reaches the lamina propria at which it induces an immune response in susceptible individuals. Up to now, the immunogenic effect of the 33-mer peptide in CD has only been explained by T-cell recognition. However, there is no explanation to put forward all the other exceptional properties of this gliadin fragment.

The immunogenicity of a protein or peptide depends not only on its primary sequence but also on the secondary and tertiary structures. Because of this, the structural features of the immunodominant 33-mer peptide are essential to understand gluten-related pathologies. Initially, it was reported that the 33-mer peptide adopts a PPII (polyproline II) structure.[25a,85] Then, a more detailed circular dichroism study, performed by Herrera et al., has shown that the 33-mer peptide suffers a conformational transition depending on the concentration under physiological conditions.[86] At low temperature (−10 °C), at a concentration below 197 μM, the peptide is in a random coil structure, whereas at a concentration exceeding 197 μM, the peptide is in an equilibrium between the random coil and a more extended structure compatible with a PPII structure. The PPII structure is, in general, in equilibrium with other conformations, such as random and β structures due to the proximity of the respective dihedral angle. In a temperature-dependence experiment from −10 to 37 °C, it is possible to determine that at a concentration of 197 μM an equilibrium between the random coil and the PPII structure exists, whereas at a concentration of 613 μM a second conformational equilibrium exists between the PPII and β structures. Under these experimental conditions, the 33-mer oligomerizes in a concentration-dependent manner. Electron microscopy observations show that the 33-mer can oligomerize to form nanospheres, fibrils, and fibers that coexist together at 613 μM. Molecular dynamics simulation and partial charge distribution calculations have revealed that the 33-mer peptide is a nonionic amphiphile with the capability to form, at least, a stable dimer.[86]

Insight into the oligomerization process depending on concentration has been obtained by using dynamic light scattering (DLS) and atomic force microscopy (AFM). DLS experiments reveal that at concentrations ranging from 125 to 610 μM, the 33-mer gliadin peptide forms oligomers of different sizes. Three oligomeric populations have been identified: a population of oligomers with a size smaller than 100 nm, a second population of oligomers with sizes between 100 and 1000 nm, and a third population of oligomers with sizes greater than 1000 nm.[87] These results strongly suggest that in aqueous solution the 33-mer peptide behaves as a dynamic polydisperse system with nanometer-to-micrometer-sized particles, and at low concentrations the smaller particles associate to generate bigger ones. However, at high concentrations, the bigger particles seemingly act as new sites for nucleation, which limits the interaction between the small ones. AFM observations visualize the different oligomers in a hydrated state without chemical manipulation. Deposition of an aliquot of a 6 μM solution onto a mica surface revels that the 33-mer peptide forms isolated spherical nanostructures and clusters. In the range of 60 and 250 μM, spherical oligomers are associated with linear patterns forming annular structures (Figure 8).

Some planar structures show a sheet-like morphology. At the highest concentration of 610 μM, there are mainly filaments and plaques surrounded by spherical nanostructures. At all the concentrations tested, the 33-mer oligomers follow a diffusion-limited assembly (DLA) mechanism. The structural changes that take place during self-organization on a surface have been followed by using attenuated total reflectance Fourier-transform infrared (ATR-FTIR) spectroscopy. Here, taking advantage of the fact that the absorption coefficient of the β structure is higher in IR spectroscopy than in circular dichroism, the nature of the β structure has been elucidated. Initially, the same concentration-dependent conformational equilibrium that was previously detected in solution was confirmed. The ATR-FTIR spectrum of the 33-mer aggregates in the amide I (representative of protein secondary structure) region reveals two main bands. The intensity of the band at around $\tilde{\nu}=1630\ cm^{-1}$ increases as a function of concentration relative to intensity of the band at around $\tilde{\nu}=1660\ cm^{-1}$, which suggests that the 33-mer peptide goes from a somewhat unordered state to a more folded state, enriched in β structures. Interestingly, if the peptide concentration increases, larger structures is detected by microscopy techniques. Moreover, the absence of a band at around $\tilde{\nu}=1695\ cm^{-1}$, characteristic of an antiparallel β-sheet structure, strongly suggests a parallel β-sheet structure.[88] The PPII secondary structure is involved in protein–protein interactions, such as in motifs related to SH2 domains,[89] and in proteins that form aggregates, such as the dental enamel protein amelogenin.[90] On the other hand, the conformational transition towards a parallel β structure is the hallmark signature of amyloid or conformational diseases. These diseases involve not only misfolding of endogenous proteins, as seen in Alzheimer's disease,[91] but also exogenous proteins, as in the case of prion diseases.[92] In conformational diseases, oligomers and fibril structures are detected and associated with innate and adaptive immune activation. The observed

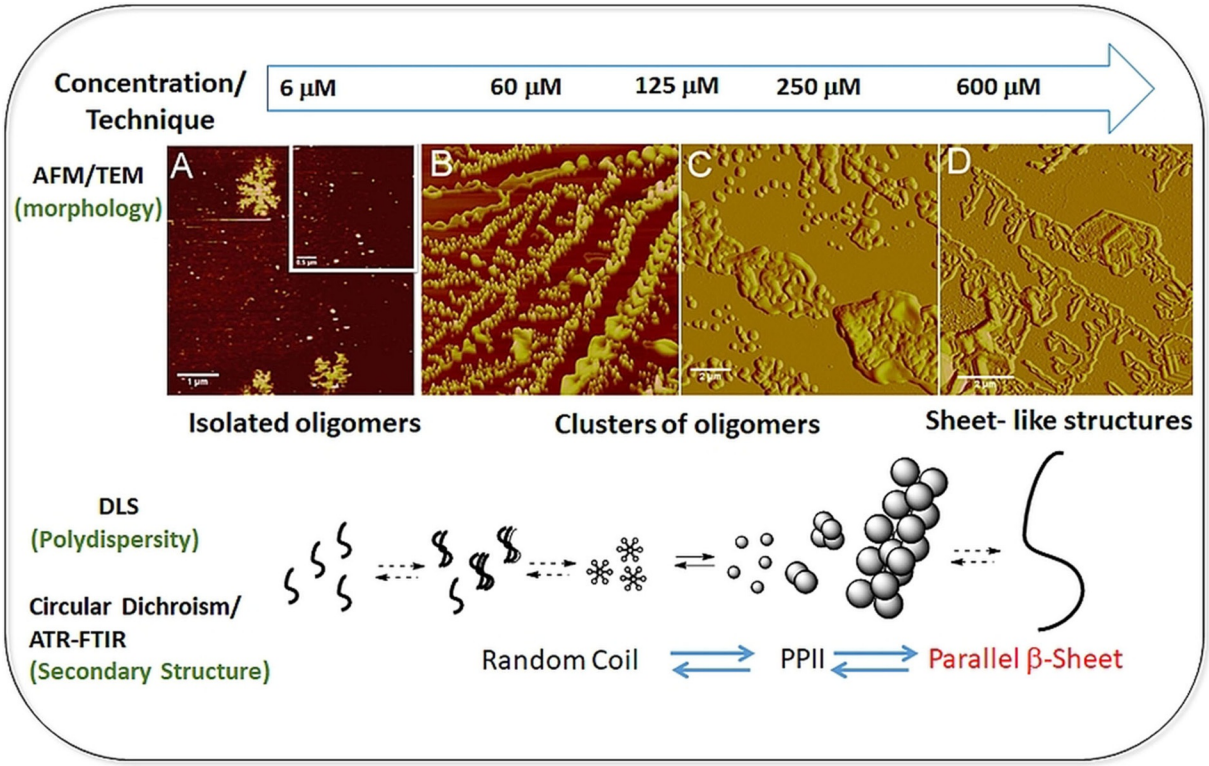

Figure 8. Spontaneous oligomerization of 33-mer depends on peptide concentration. On increasing the peptide concentration, spherical-like oligomers undergo a conformational transition towards sheet-like structures with a parallel β structure.[86,87]

conformational changes and oligomerization behavior of the 33-mer gliadin peptide could be the up-to-now unknown molecular trigger that modulates immune activation in gluten-consumption disorders. At the molecular level, the forces involved in the self-assembly process of the 33-mer are correlated with the primary structure of the 33-mer. The 33-mer peptide contains 65% proline and glutamine, which suggests that hydrophobicity could be a driving force for self-assembly. Moreover, it is known that glutamine residues can interact through complementary hydrogen bonding, in addition to the aforementioned hydrophobic effect.[93] Glutamine (Q) can link β strands together into β sheets by a network of hydrogen bonds between the amide groups of the chain and the polar side chains.[94] The importance of the Q lateral chain in the formation of protofilaments has been highlighted in different amylogenic diseases, including Huntington's disease.[95] Furthermore, supramolecular organization of the 33-mer peptide in monomers, oligomers (with different morphologies and sizes), and protofilaments might explain the different mechanisms of epithelial transport that have been reported for different groups (Figure 9). Further experiments are needed to test 33-mer oligomers in the cellular context.

9. Conclusions and Perspectives

In the 1940s, the Dutch pediatrician Dr. Dicke observed a link between celiac disease (CD) and gluten consumption, and since then, the pathogenesis of CD has been widely studied. However, even after all these years of efforts, crucial knowledge is still lacking about the early interaction of gliadin with the gut epithelium and, hence, the first steps in the pathogenesis of this autoimmune enteropathy. Even more, the effects of gliadin in healthy individuals have not been systematically studied. The broad spectrum of responses with the gliadin digest and the more specific responses reported with the 20-mer, 13-mer, and 33-mer peptides in different assays shows that gliadin protein contains immune and toxic fragments in its sequence. As mentioned, CD is characterized as a Th1-mediated autoimmune disease with high mucosal titers of interferon-γ. As such, the adaptive immune response that is triggered by gliadin has been extensively studied. However, recent data, discussed extensively in this Review, have shed light on the involvement of innate immunity in the host's early response to gluten. Gliadin alone and the remaining large peptides (gliadin digest) are highly immunogenic if exposed to different cell types. Moreover, if it is taken into account that innate immunological responses elicited by gliadin are reported at pH 7.0, the hypothesis of colloidal assemblies of this protein as a real potential trigger, instead of randomly dispersed gliadin monomers, gains strength and may explain the reported immunogenicity of gliadin and its tissue stress effects. Likewise, the micellar aggregates formed at pH 3.0 may be responsible for the inability of pepsin to cleave gliadin in smaller fragments efficiently.

Moreover, the proteolytic resistance of the 33-mer and its self-assembly behavior on impaired cellular clearance conditions could explain the up-to-now unresolved question about the initial stages of gluten-related disorders in connection with

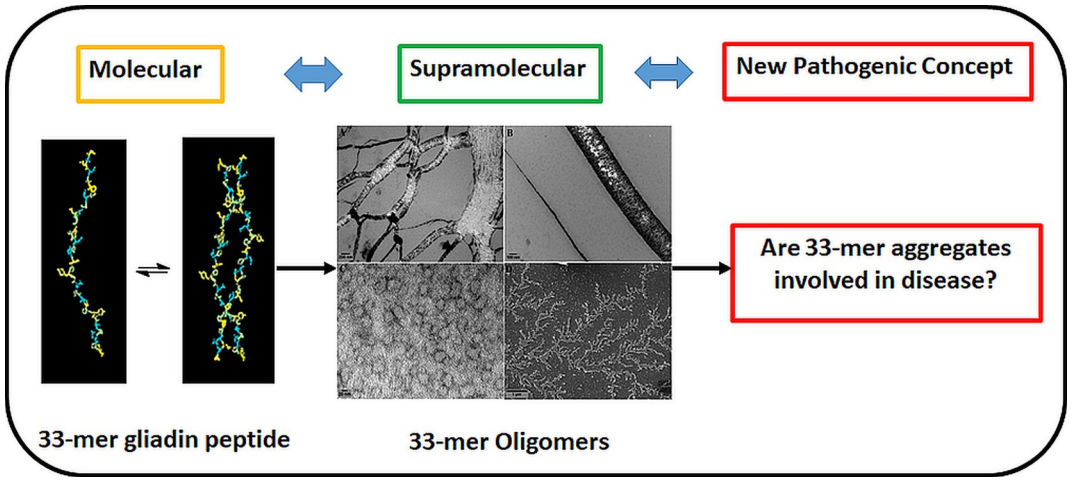

Figure 9. The new pathogenic concept might involve the occurrence of 33-mer oligomers on accumulation conditions. The molecular structure of the 33-mer gliadin peptide obtain by molecular dynamics in equilibrium with its dimer (left). The different structures of the 33-mer (right), as characterized by transmission electron microscopy and scanning electron microscopy.[86]

the immunodominant 33-mer peptide. The formation of 33-mer oligomers with different sizes and morphologies could also explain the different mechanisms of cellular transport described by the different research groups.

It seems that the 33-mer gliadin fragment might be not only the immunodominant peptide in CD, but also its accumulation and conformational transition towards the β-parallel structure might connect a common food intolerance with conformational or amyloid diseases.

Recently, oligomers of a radiolabeled mutated ^{3}H-33-mer were found in blood plasma and accumulated in different organs in murine models after oral and intravenous administration.[96] These findings support the relevance of 33-mer oligomers in vivo. Research efforts are directed towards understanding the role of 33-mer aggregates in the context of the gut immune response. Moreover, considering the relevance of protein aggregates in disease, modulation/inhibition of the oligomerization process of the 33-mer may open new perspectives for the treatment of gluten-related diseases beyond the gluten-free diet.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords: gluten-related disorders · immunology · oligomerization · peptides · protein structures

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