INTRODUCTION

Among major allergic foods, wheat is the most cultivated basic staple food crop in the world, causing food allergies ranging severity. The prevalence of wheat allergy has reached significant levels in many countries. Therefore, wheat is a major global food safety and public health issue. Several researchers reveal that a family of protein in wheat may be responsible for activating inflammation in chronic health conditions. Food allergens in general are water/saline soluble proteins. However, wheat contains four different classes of protein allergens: water soluble (albumin), saline (globulin), alcohol soluble (gliadin), and acid soluble (gluten) protein allergens. The total protein content in wheat flour ranges from 8% to 12% (Yokooji et al., 2018). The term gluten includes both gliadin and glutenin. Gliadins contribute to the cohesiveness and extensibility of the gluten, whereas glutenin plays a role in the maintenance of the elasticity and strength of the gluten (Wieser, 2007). Gliadins are represented as single chain polypeptides, and are divided into four major groups: α-, β-, γ-, and ω-gliadins and connected to each other through intrachain disulfide bonds. Glutenins consist of high molecular weight glutenin subunits (HMW-GS) and low molecular weight glutenin subunits (LMW-GS). These polymeric forms contribute to the strength of gluten and improves dough quality. There is extensive evidence in the literature that food processing like fermentation and enzyme treatment can alter the allergenicity of food proteins (Verhoeckx et al., 2015; Ortiz et al., 2016; Vanga et al., 2017; Phromraksa, 2008). However, thermal processing does not reduce or eliminate allergenicity of wheat allergens. In this review we have summarized the knowledge on classification, properties, structure and role of gluten proteins in the pathogenesis of gluten intolerance manifestations.

Types of Gluten Proteins

Gluten proteins are wheat storage proteins constituting about 10% of wheat and give wheat dough its functional properties such as water absorption capacity, viscosity and elasticity which contribute to the unique baking properties. Gluten is the product of a ball of wheat flour dough that has been exhaustively washed in tap water, and the baking qualities of the wheat depends on its ability to trap carbon dioxide in dough. Gluten proteins are among the most complex proteins in nature containing hundreds of components as monomers, oligomers and polymers. No nutritional value has been attributed to gluten. Based on their physical characteristics (water/saline insoluble), gluten proteins can be divided into gliadin and glutenin. Gliadin family proteins are monomeric and can be genetically classified into α/β, γ-ω1,2- and ω5- gliadins. The glutenin family proteins are divided into low and high molecular weight glutenin (Yokooji et al., 2018). Some fragments of gluten are toxic and others are immunogenic (Fig1).

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Gluten can trigger adverse inflammatory, immunological and autoimmune reactions in some people. Gluten can produce a broad spectrum of gluten-related disorders. Two pathways have been hypothesized to be triggered by these peptides: one is the direct effect on the epithelium that involves the innate immune response (Schuppan et al., 2003), the other represents the adaptive immune response involving CD4 + T cells in the lamina propria that recognize processed gluten epitopes (Schuppan, 2000). The initial development and maintenance of tolerance to gluten when interrupted can lead to several diseases. Gluten-related diseases such as wheat allergy, celiac disease and gluten intolerance are widespread in the globe in genetically predisposed individuals. Marsh (1992) observed that gluten absorption in the intestinal revealed five interrelated lesions (preinfiltrative, infiltrative, hyperplastic, destructive, and hypoplastic) that were interpreted as cell-mediated immunologic responses. Gluten peptides can cross the basement membrane to directly interact with immune cells present in the lamina propria. In this regard, intestinal epithelial cells have been shown to release exosome-like vesicles morphologically similar to those secreted by professional antigen-presenting cells (Ciccocioppo, 2005). In 1979 Zioudrou et al. reported the opioid activity of gluten after pepsin digestion of wheat. It is well documented that opioid peptides influence the central nervous system by modulating the release of hormones and neurotransmitters via opioid receptors. Takahashi et al. (2000) observed that an opioid peptide derived from wheat gluten, gluten exorphin A5 (Gly-Tyr-Tyr-Pro-Thr) had effects on central nervous system of mammal as evidenced by memory loss and emotional distraction.

**Types of Gluten Allergies**

Wheat allergies are of two types: IgE-dependent reactions and IgE-independent but eosinophil dependent reactions, however most wheat allergies are IgE-dependent reactions (Fig 1). The IgE-mediated wheat allergies include three groups of disorders: (i) Occupational allergies, such as allergic rhinitis (AR), allergic conjunctivitis (AC), bakers’ asthma (BA), and contact urticaria (CU); (ii) wheat food allergy (WFA), such as atopic dermatitis (AD), gastrointestinal allergic disease, and systemic anaphylaxis; and (iii) wheat-dependent exercise-induced anaphylaxis (WDEIA). The IgE-independent but eosinophil-mediated wheat allergies include eosinophilic esophagitis (EOE) and eosinophilic gastritis (EOG) (Jin et al., 2019).

**Wheat Allergies**

Wheat allergies are often confused with celiac disease and nonceliac gluten sensitivity. In contrast to wheat allergies that are mediated by IgE antibodies, celiac disease is an autoimmune disease in which gluten in the diet triggers WBC to attack the villi that line the small intestine leading to erosions and prevention of absorption of some nutrients (malabsorption). The non-celiac gluten sensitivity is mediated by the over-active innate immune system.

**Insights to Various Types of Inflammatory Responses to Gluten**

Several mouse models of wheat allergy have been developed and studied upon for greater insights on its allergenicity. Kozai et al. (2006) developed a mouse model to explain the molecular mechanisms of wheat-dependent exercise-induced anaphylaxis (WDEIA) (Table 1).

**Table 1 Major lessons learnt from animal models on wheat protein allergenicity**

<table>
<thead>
<tr>
<th>Species</th>
<th>Wheat Allergen Exposure</th>
<th>Route</th>
<th>Sensitization</th>
<th>Elicitation of Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog</td>
<td>Gliadins</td>
<td>IP</td>
<td>IgE</td>
<td>Vomiting, Skin reaction, Diarrhoea</td>
</tr>
<tr>
<td></td>
<td>Glutenin</td>
<td>Oral</td>
<td>IgE</td>
<td>Anaphylaxis, EIA</td>
</tr>
<tr>
<td></td>
<td>Albums</td>
<td>Skin</td>
<td>IgE</td>
<td>Anaphylaxis, EIA</td>
</tr>
<tr>
<td></td>
<td>Globulins</td>
<td></td>
<td></td>
<td>Increased</td>
</tr>
<tr>
<td>Mouse</td>
<td>Gliadins</td>
<td>IP</td>
<td>IgE</td>
<td>Anaphylaxis</td>
</tr>
<tr>
<td></td>
<td>Gluten</td>
<td>Skin</td>
<td>No IgE</td>
<td>Increased</td>
</tr>
<tr>
<td></td>
<td>Detergent</td>
<td>Skin</td>
<td>IgE</td>
<td>Anaphylaxis</td>
</tr>
<tr>
<td></td>
<td>Acid</td>
<td>Skin</td>
<td>IgE</td>
<td>Anaphylaxis</td>
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<td></td>
<td>Hydrolyzed gluten (AHG)</td>
<td>Skin</td>
<td>Increased</td>
<td>Dermatitis (Th1, Th2, Th17)</td>
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<tr>
<td></td>
<td>+ Detergent</td>
<td></td>
<td></td>
<td>Cytokines + Allergic</td>
</tr>
<tr>
<td></td>
<td>Albums</td>
<td>IP</td>
<td>IgE</td>
<td>Chemokines</td>
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<tr>
<td></td>
<td>Globulins</td>
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<td>Anaphylaxis</td>
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<tr>
<td></td>
<td>Gluten</td>
<td>IP</td>
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<td>EIA</td>
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<tr>
<td></td>
<td>AHG</td>
<td>Skin</td>
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<tr>
<td>Rat</td>
<td>Enzyme</td>
<td>Oral</td>
<td>IgE</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hydrolyzed gluten</td>
<td>Oral</td>
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<td>EIA</td>
</tr>
</tbody>
</table>

Abbreviations: IP = intraperitoneal injection; EIA = exercise-induced anaphylaxis

They sensitized mice with albumin/globulin, gliadin and glutenin fractions. Then, they tested the effect of exercise (treadmill) after oral feeding with each protein fraction. This
model showed that: (i) gliadins and glutenins not only elicited sensitization, but also caused WDEIA; (ii) salt-soluble proteins neither caused sensitization nor WDEIA; (iii) exercise caused mucosal lesions after oral challenge with wheat proteins and the leakage of gliadin and glutenin proteins into the liver. Thus, gluten proteins (gliadin and glutenin) were linked to WDEIA. Although α-5-gliadin was linked to WDEIA, whether it can cause anaphylaxis independent of exercise was unknown.

According to Galipeau et al. (2015), intestinal microbiota modulates gluten induced immunopathology in mice. They investigated whether specific microbiota compositions influence immune responses to gluten in mice expressing the human DQ8 gene, which confers moderate celiac disease (CD) genetic susceptibility. Germ-free mice, clean specific-pathogen-free (SPF) mice colonized with a microbiota devoid of opportunistic pathogens and proteobacteria, and conventional SPF mice that harbor a complex microbiota that includes opportunistic pathogens were used. Clean SPF mice had attenuated responses to gluten compared to germ-free and conventional SPF mice. Germ-free mice developed increased intraepithelial lymphocytes, markers of intraepithelial lymphocyte cytotoxicity, gliadin-specific antibodires, and a proinflammatory gliadin-specific T-cell response. Antibiotic treatment, leading to Proteobacteria expansion, further enhanced gluten-induced immunopathology in conventional SPF mice. Protection against gluten-induced immunopathology in clean SPF mice was reversed after supplementation with a member of the proteobacteria phylum, an enteroadherent Escherichia coli isolated from a CD patient.

Deamidation of gluten is a common practice used by the food industry because this modification of gluten increases its solubility, thus making it a preferred form of gluten to use as a food ingredient and in cosmetics. However, there are concerns on the potentially enhanced allergenicity of such modified gluten. Studies show that deamidated gliadin (DG) sensitizes mice more effectively than the native gliadin and that the DG elicited IgE profile in mice was very similar to that seen in wheat allergic human (Fig 3).

After gluten enters (Fig 3) into the digestive system, glutamine and proline-rich gluten composing proteins are partially hydrolyzed by proteases presented in the gastrointestinal tract. The upregulation of intestinal peptide zonulin, involved in tight junction regulation, appears to be partly responsible for the increased permeability characteristic of the gut. As a result, generated gluten-derived peptides reach the lamina propria (mucosa) by transcellular or paracellular transport where they are modified by tissue transglutaminase (tTG) enhancing their affinity to MHC II molecules, and thereby making them toxic and immunogenic in HLA-DQ2 or DQ8 (human leukocyte antigen Class II with DQ2 and/or DQ8 molecules on antigen-presenting cells) containing patients. The repetitive presence of glutamine and proline residues determines the gluten-derived peptides as a preferred substrate for tTG. tTG-mediated modifications occur in two ways: deamidation or more frequently transamidation. Further peptides presentation by HLA-DQ2/DQ8 protein subunits in the surface of dendritic cells to gluten-specific T cells induces two levels of immune response: the innate response and the adaptive (T-helper cell mediated) response with the production of interferon-γ and IL-15. As a result, it causes immune-mediated enteropathy, intestinal inflammation, followed by the atrophy of villi, crypt hyperplasia and increased infiltration by intraepithelial lymphocytes. It also produces weight loss and chronic diarrhea.

Gourbeyre et al. (2012) used the Balb/c mouse model to test this hypothesis. They found that: i) Native gliadin elicited a higher T helper 1 type of immune response and the deamidated gliadin (DG) elicited a higher T helper 2 or allergic immune response and histamine response. However, both types of proteins elicited anaphylaxis to the same extent; ii) native gliadin elicited IgE against all five gliadins (α/β, γ, α1.2, and α5). However, these antibodies did not bind to DG. In contrast, DG elicited IgG against all five deamidated gliadins, which also bound the native gliadin. These data support that DG is more allergenic than the native gliadin.

Yokooji et al. (2018) elucidated the role of Aspirin in facilitating intestinal absorption of the wheat allergen gliadin. They performed concentration checks on the plasma of gliadin after oral administrations by gavage or administration into a closed intestinal loop. They found that aspirin increased plasma concentrations of gliadin after oral administration but had no effect in the closed intestinal loop study. Aspirin increased the absorption of intact and pepsin-digested gliadin via the paracellular pathway maintaining their allergenicity.

Verdu et al. (2007) used HLA-DQ8 mice to investigate the effects of gliadin sensitization on innate immune markers and on neuromotor and epithelial cell secretory functions in the gut to provide symptoms in humans without celiac disease. CD3+ intraepithelial lymphocyte, macrophages and FOX-P3 positive cell counts were determined. Acetylcholine release, small intestinal contractility, and epithelial ion transport were measured and compared with controls to find recruitment of intraepithelial lymphocyte, macrophages and FOX-P3 cells in G/G (gliadin sensitized and gavaged with gliadin), but not in control mice. This was paralleled by increased acetylcholine release from the myenteric plexus, muscle hypercontractility, and increased active ion transport in G/G mice. Gluten sensitivity in HLA-DQ8 mice induces immune activation in the absence of intestinal atrophy. This is associated with cholinergic dysfunction and a prosecretory state that may lead to altered water movements and dysmotility.

Dietary gluten reduces the number of intestinal regulatory T cells in mice. Ejsing-Duun et al. (2008) hypothesized that
gluten is responsible for mediating its effect on T1D (type 1 diabetes) through the influence on Treg development independent of gluten-induced Lactococci. Dietary gluten significantly decreased the occurrence of Treg by 10–15% in mice compared with those fed a standard diet. The prevalence of Treg was 5- to 10-fold more abundant in the Peyer’s patches than in the spleen. They concluded that the dietary gluten has a significant negative quantitative impact on the generation of Treg in mice, independent of gluten-induced Lactococcus garvieae, and Treg are far more abundant in Peyer’s patches than in the spleen.

Vijakrishnaraj et al. (2017) developed a wheat gluten induced BALB/c model for addressing wheat gluten related disorders by sensitizing the wheat gluten through route of intraperitoneal and oral challenge in prolonged days. They found that prolonging sensitization of gluten can moderate the antigen-specific inflammatory markers such as IL-1β, IL-4, IL-15, IL-6, IFN-γ, and TNF-α level in mice sera. Histopathology staining of jejunal sections indicated that enterocyte degeneration in the apical part of villi and damage of tight junction in G+ (gliadin and gluten) sensitized murine model.

CONCLUSION

Wheat allergies are a significant public health problem and food safety issue at the global level. Untreated gluten-sensitive enteropathy manifests diarrhea, abdominal distention, developmental delay, severe malnutrition, dental enamel defects and ultimately celiac diseases. Fundamental mechanisms underlying this problem are incompletely understood at present. A number of valuable animal models have been developed for wheat food allergy and anaphylaxis, but not for other types of wheat-induced allergies. Currently, animal models are markedly underutilized to advance mechanistic knowledge on wheat allergies. There are ample opportunities for further improvement of current models as well as to develop new models.

This review work provides insight into the IgEepitope structure of wheat allergens, effects of detergents and other chemicals on wheat allergenicity, and the role of genetics, microbiome, and food processing in wheat allergenicity. This study can also serve as source of experimental models useful for pre-clinical testing tools to develop safer genetically modified wheat, hypoallergenic wheat products, pharmaceuticals and vaccines. The great deal of research work done on gluten allergies and the findings have shed light on newer aspects and approaches towards this greater health issue. Though the detailed mechanism of IgE independent reactions of wheat allergies and uses of less used physiological routes (skin, eyes, airways, oral) of exposure to wheat proteins to elicitation sensitivity seem to be less explored till now. Treatment of gluten-sensitive enteropathy remained empiric until the middle of the 20th century when patients were noted to improve dramatically after wheat was removed from their diet.

The currently prevalent type 1 diabetes (T1D) and celiac disease (CD) and with their increasing incidence, the potential effects of gliadin intolerance of T1D are alarming. This very common dietary intolerance may increase T1D risks (Galipeau et al., 2011) at many folds. A person with gluten intolerance should eliminate gluten from their diet. Specific microbiota-based therapies may aid in prevention in gluten induced enteropathy and the pathobionts are capable of modulating gluten sensitization by increased T cell proliferation and proinflammatory cytokines production.

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