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[Wang Zheng](#) , [Christine Yee Yan Wai](#) , Jason Ka Chun Sit , Nam Sze Cheng , Christy Wing Man Leung , [Ting Fan Leung](#) \*

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Review

# Novel Diagnostic and Immunotherapeutic Approaches for Wheat Allergy

Wanqi Zheng <sup>1</sup>, Christine Yee Yan Wai <sup>1,2</sup>, Jason Ka Chun Sit <sup>1</sup>, Nam Sze Cheng <sup>1</sup>, Christy Wing Man Leung <sup>3</sup> and Ting Fan Leung <sup>1,2,\*</sup>

<sup>1</sup> Department of Paediatrics, The Chinese University of Hong Kong, Shatin, Hong Kong

<sup>2</sup> Hong Kong Hub of Paediatric Excellence, The Chinese University of Hong Kong, Shatin, Hong Kong

<sup>3</sup> Faculty of Medicine, The Chinese University of Hong Kong, Shatin, Hong Kong

\* Correspondence: ttleung@cuhk.edu.hk; Tel.: (852) 3505 2981

**Abstract:** Wheat, a component of the staple diet globally, is a common food allergen in children. The symptoms of wheat allergy (WA) range from skin rash to shortness of breath, significantly impairing quality of life. Following initial clinical suspicion, individuals may undergo wheat allergen-specific skin prick test (SPT), blood test for serum immunoglobulin E (sIgE) levels or oral food challenge. Conventional management of WA lies in wheat avoidance, yet accidental consumption may be inevitable owing to the ubiquity of wheat in various food products. This article aims to provide an overview of the immunologic pathway of WA, followed by its novel diagnostic methods, namely alcohol-soluble SPT extracts, component-resolved diagnosis and basophil activation test (BAT). The mechanisms underlying wheat allergen-specific oral immunotherapy (OIT) as well as a summary of the efficacy, tolerability and safety of related clinical trials will then be discussed.

**Keywords:** Component-resolved diagnosis; desensitization; immunology; oral immunotherapy; wheat allergy

## 1. Introduction

Wheat (*Triticum aestivum*) plays an important role in the global human diet due to its nutritional value and adaptability to diverse climatic conditions. However, the prevalence of wheat allergy (WA) poses significant concern. Wheat is identified as one of the top five triggers of allergic reactions in children. In Germany and Japan, it ranks as the third most common food allergen, after milk and eggs [1]; according to Yu *et al*, WA is in fact the top cause of anaphylaxis in Japan and Korea [2]. A European meta-analysis of food allergy prevalence revealed that the overall lifetime prevalence of self-reported WA was around 1.6% (95% confidence interval [CI] 0.9-2.3) with the highest being among preschool children aged 2-5 years, while the point prevalence of food challenge-verified WA was 0.1% (95% CI 0.01-0.2) [3]. The lifetime prevalence for self-reported WA was lower than those of cow's milk (5.7%, 4.4-6.9) and egg (2.4%, 1.8-3.0) and comparable to the respective figures for peanut allergy (1.5%, 1.0-2.1) and fish (1.4%, 0.8-2.0).

Wheat allergy is an immune-mediated adverse reaction triggered by the consumption of wheat or wheat-based products [4]. It includes three main types: immunoglobulin E (IgE)-mediated, non-IgE-mediated, and mixed-IgE-mediated forms [2]. The latter two types are less common with mechanisms yet to be delineated, and thus are not detailed here. IgE-mediated WA involves two stages, namely sensitization and elicitation. Sensitization occurs upon initial exposure to wheat, leading to the production of wheat protein-specific IgE antibodies. During the elicitation phase, subsequent exposure triggers the interaction between cell-bound IgE and the same wheat proteins, resulting in the release of allergic mediators from mast cells and basophils. Gut microbiota appears to play a role in modulating allergic responses [2,4-7], as reflected by the increased prevalence of certain microorganisms in the gut of wheat-allergic children [8]. These processes lead to a range of clinical manifestations within minutes to hours after wheat consumption, including erythema,

pruritus, gastrointestinal reactions, oropharyngeal symptoms, urticaria, angioedema, atopic dermatitis, rhinitis, asthma; in severe cases, WA may escalate to life-threatening anaphylaxis [5].

Current treatment for WA primarily relies on strict avoidance of wheat and prompt management of adverse reactions resulting from accidental exposure [9]. However, challenges arise since wheat is widely used to manufacture various food products, increasing the likelihood of inadvertent exposure. Therefore, alternative desensitization treatments are needed. Immunotherapy, comprising oral immunotherapy (OIT), sublingual immunotherapy (SLIT), and epicutaneous immunotherapy (EPIT), holds promise in this regard [9]; in particular, OIT has been extensively studied for WA. Oral immunotherapy aims at inducing oral tolerance to allergenic foods by modulating both innate and adaptive immune mechanisms [2,7,10], reflected by reduced secretion of inflammatory mediators from mast cells and basophils, increased levels of wheat allergen-specific immunoglobulin G 4 (IgG4) and wheat allergen-specific IgE levels that initially elevate then decrease [10].

To date, wheat allergen-specific OIT is yet to be incorporated into clinical practice due to its early stage in research. Nonetheless, this article will review the immunologic pathways of WA and mechanism of wheat allergen-specific OIT correspondingly, on a molecular level. Novel diagnostic methods and treatment options will also be introduced, with a specific focus on oral immunotherapy.

## 2. Immune Mechanism and Interaction with Microbiota

### 2.1. Sensitization

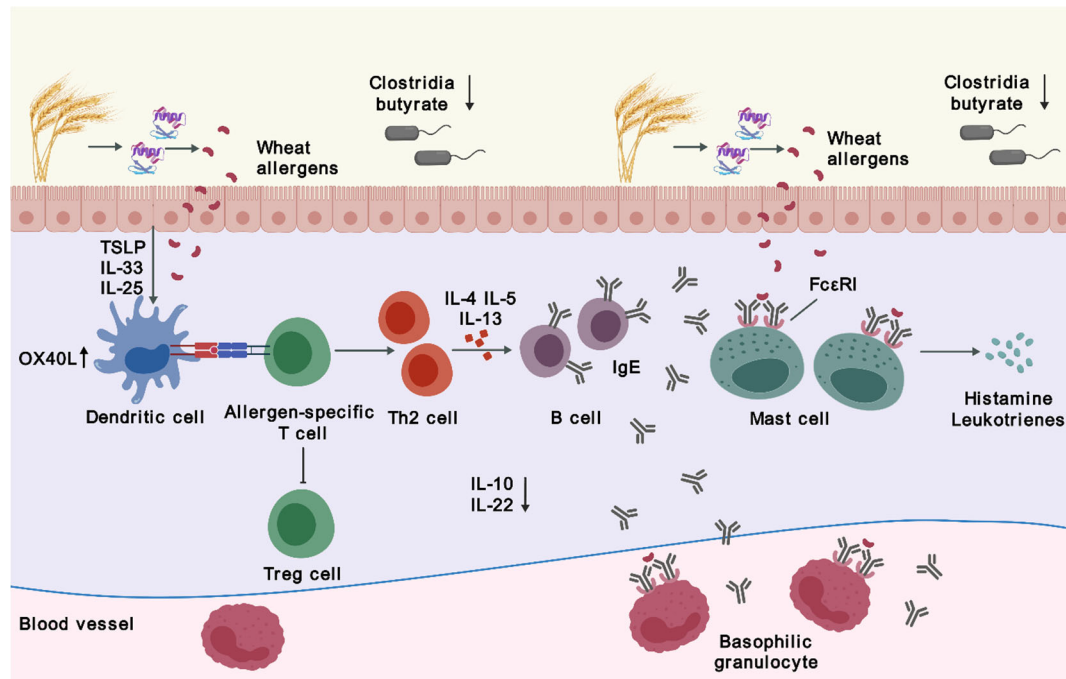
The process of IgE-mediated WA involves two stages — sensitization upon initial exposure to wheat allergens and elicitation when encountering re-exposure. Sensitization occurs when wheat allergens enter the body following digestion and absorption at the gastrointestinal tract. Epithelial-derived inflammatory cytokines, such as interleukin (IL)-25, IL-33, and thymic stromal lymphopoietin (TSLP), act on dendritic cells (DCs) and other cells to skew the immune response [7,11,12]. The tumor necrosis factor receptor superfamily member 4 (OX40) ligand (OX40L), upregulated on DCs, induces differentiation of naive T cells into T helper 2 (Th2) cells instead of regulatory T (Treg) cells, which would elicit tolerogenic responses [13]. This Th2-biased immune dysregulation results in the production of IL-4, IL-5, and IL-13, promoting class switching of B cells to produce more wheat allergen-specific IgE. These IgE antibodies bind to mast cells and basophils via their high-affinity IgE receptors (FcεRI) [14-17]. Upon re-exposure to wheat allergens, allergen-antibody binding triggers IgE crosslinking and hence the activation of tissue-residing mast cells and blood-circulating basophils, swiftly releasing inflammatory and vasoactive mediators such as histamine, platelet-activating factor, and leukotrienes to induce local or systemic allergic reactions [5,17-20] (**Figure 1**).

### 2.2. Desensitization by Immunotherapy

Immunotherapy desensitizes the immune systems of individuals via modulating the humoral immune response and activities of T cells and DCs [2,7,9]. In immunotherapy, IgE levels rise transiently then fall. The allergen-specific IgG levels, especially serum IgG4 (sIgG4), however steadily increase [21-23], likely due to steady release from the upstream IL-10 and allergen-specific regulatory B (Breg) cell pathways. The IgG isotypes also dampen IgE-mediated responses by binding to FcγRIIb and suppressing IgE-mediated activation of basophils and mast cells [2,24-26]. Allergen-specific immunoglobulin A (IgA) supports antigen exclusion and enhances the specificity of desensitization [7].

Repeated allergen exposures during OIT also induce apoptosis and anergy of allergen-specific Th2 cells alongside further activation of allergen-induced Treg cell functions. As abovenamed, Treg cells are tolerogenic, hindering mast cell activation directly via OX40-OX40L interaction and inhibiting DCs required for effector T cell activation [2,27]; clinically, they protect individuals from anaphylaxis. The synergistic effects of immunoglobulins and T cells facilitate allergen desensitization

in immunotherapy. Further studies, however, are required to observe for any wheat allergen-specific mechanisms of desensitization in wheat immunotherapy.



**Figure 1.** Pathophysiology of wheat allergy. Abbreviations: FcεRI, high-affinity IgE receptors; IgE, immunoglobulin E; IL, interleukin; OX40L, ligand for tumor necrosis factor receptor superfamily member 4; Th2, T helper 2; Treg, regulatory T; TSLP, thymic stromal lymphopoietin.

### 2.3. Microbiota

The role of microbiota in food allergy has been increasingly studied in recent years. Environmental factors such as delivery mode, antibiotic use, and diet may trigger microbial dysbiosis, predisposing individuals to allergic sensitization [7,28-30]. The gut microbiota plays a crucial role in modulating allergic responses to food antigens by activating DCs on the gut mucosal surface. These activated DCs produce IL-10 and IL-22 to stimulate maturation of naive T cells into Treg cells and enhance the production of anti-microbial and food allergen-specific IgA, establishing food tolerance. Conversely, an imbalance of gut microbiota in the intestinal epithelium may lead to elevated levels of IL-4, IL-33, and allergen-specific IgE, driving Th2 cell-biased responses and stimulating basophil activation in the intestine [6,30-32].

Studies have shown alterations in gut microbiota in children with WA compared to those without [33]. A study by Kanchongkittiphon *et al* revealed statistically significant enrichment of *Anaerostipes*, *Erysipelatoclostridium*, *Prevotella 2*, *Ruminiclostridium 5*, and *Clostridium innnocuum* species in children with physician-diagnosed WA [8]. The specific bacterial groups or species involved in the induction of Treg cells in the intestinal mucosa remain unknown. It appears that genera such as *Lactobacillus*, *Bifidobacterium*, and *Clostridium*, along with microbial fermentation products like butyrate stimulate Treg cells and alleviate food allergy symptoms [30,34,35]. Further large-scale studies may elucidate the role of the microbiome in WA-associated immune regulation.

## 3. Clinical Features and Related Disorders

Allergic responses to wheat can manifest in a variety of clinical symptoms, ranging from localized to severe systemic reactions occurring within minutes to hours after wheat exposure [2,5,36,37]. Typical immediate symptoms include gastrointestinal discomfort (nausea, abdominal pain, vomiting, and/or diarrhea), respiratory difficulties (rhinitis, wheezing, and/or asthma) and skin manifestations (eczema, redness, itching, urticaria, and/or angioedema). In severe cases, systemic



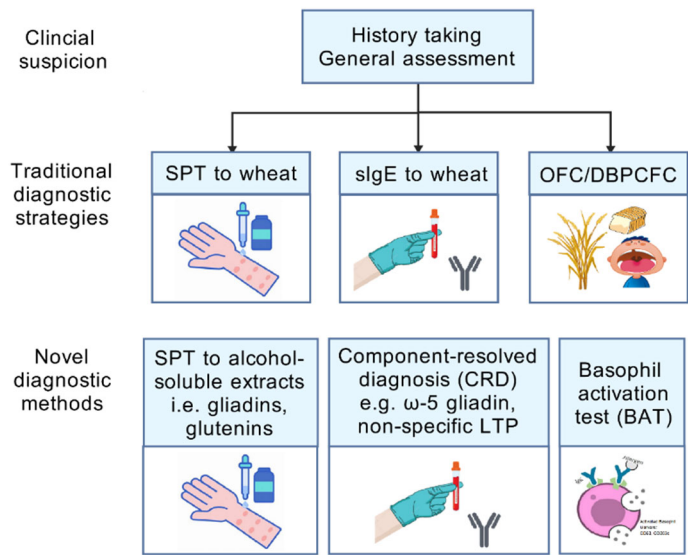
reactions such as hypotension, hypothermia or anaphylaxis may also occur; the latter affects multiple organ systems and can rapidly become life-threatening.

Wheat-dependent exercise-induced anaphylaxis (WDEIA), a distinct form of WA presenting as asthma, dyspnea, urticaria, angioedema, syncope, and more rarely shock, is typically diagnosed in adults but occasionally also in older children [38-40]. WDEIA may occur 10 to 60 minutes after physical activity when preceded by wheat ingestion 10 minutes to four hours ago, or upon wheat consumption immediately after exercise. Matsuo et al. found that exercise might accentuate allergic reactions in WDEIA by increasing gliadin absorption from the gastrointestinal tract [41], while another Japanese study suggested a role for filaggrin in WDEIA [42]. The severity of clinical manifestations may be dependent on the amount of wheat ingested and exercise intensity. Alcohol and non-steroidal anti-inflammatory drugs such as aspirin have been identified as significant risk factors for WDEIA and can trigger episodes even without exercise in a subset of patients [43,44].

4. Diagnosis

4.1. Conventional Diagnostic Strategies

The diagnostic algorithm of WA follows the sequence of history taking, general assessment, then further investigations (**Figure 2**). The two screening tests are skin prick test (SPT) and blood test for measuring serum IgE (sIgE) levels, while oral food challenge (OFC) is performed for confirmation. While the double-blind, placebo-controlled food challenge test (DBPCFC) is considered the gold standard to achieve a definitive diagnosis of WA, an objectively measured open-label OFC may suffice in certain clinical scenarios [45-47]. In OFCs, individuals receive incremental doses of whole wheat extracts in a stepwise manner, from one to 50 milligrams (mg) of wheat-specific protein to larger hourly doses, with a cumulative dose of up to 0.5-1 gram (g) of wheat protein [39]. Wheat allergy is diagnosed when (1) the OFC result is positive and (2) symptoms manifest within two hours after ingestion. Although clinical trials have demonstrated the tolerability of wheat allergen-specific OFC, on-site standby of trained healthcare providers may provide subjects with more reassurance, in the event of rare near-fatal adverse events.



**Figure 2.** Diagnostic algorithm of wheat allergy. Abbreviations: DBPCFC, double-blind, placebo-controlled food challenge; LTP, lipid transfer protein; OFC, oral food challenge; sIgE, serum immunoglobulin E; SPT, skin prick test.

The most commonly adopted diagnostic methods of WA are SPT and sIgE measurement. Although the latter enjoys higher sensitivity, these tests are both of low specificity [48]. A postulation for such drawback may be that commercially prepared wheat extracts fail to contain the salt-insoluble

major wheat allergens, i.e. gliadins and glutenins [48-50]. This concern has been addressed by novel wheat extract formulations with gliadin and glutenin-solubilizing properties, which yielded better accuracy for diagnosing WA in children than their preceding commercial wheat extracts [51-53]. Another postulation is that extensive allergen cross-reactivity between wheat flour and grass pollen may lower the specificity of SPT and sIgE to wheat. More than 60% of subjects with grass pollen allergy showed false positive results in sIgE to wheat, although they were all clinically asymptomatic [54,55]. Another observation unique to WA is that the wheat allergen-specific IgE levels may be falsely elevated in wheat-tolerant individuals — in children who previously had WA but developed tolerance, about 27% to 40% still display elevated levels of sIgE to whole wheat extract (>50 kU/L) in one to two years [56,57]. These altogether account for the low specificity of conventional SPT and sIgE measurements.

#### 4.2. Wheat Allergens and Component-Resolved Diagnosis

Wheat belongs to the grass family *Poaceae* and contains numerous allergenic proteins, which can be classified into two main fractions based on solubility in salt [4,37,58]. The salt-soluble fraction, constituting 15-20% of total proteins, includes albumins and globulins containing proteins such as  $\alpha$ -Amylase/Trypsin inhibitors (ATIs) and lipid transfer proteins (LTPs). In contrast, the salt-insoluble fraction, comprising approximately 80% of wheat protein content, consists of gliadins and glutenins. Gliadins can be categorized into  $\alpha/\beta$ ,  $\gamma$ , and  $\omega$ -gliadins based on their electrophoretic mobility under acidic conditions. Additionally,  $\omega$ -gliadins can be further subdivided into  $\omega 1$ ,  $\omega 2$ , and  $\omega 5$  components. Glutenins can be separated via electrophoresis into high molecular weight (HMW) and low molecular weight (LMW) glutenin subunits.

In recent years, component-resolved diagnosis (CRD) has been utilized to identify specific components that can predict clinical reactions in cases of IgE-mediated WA and WDEIA. Commercial tests are now available to measure IgE sensitization to Tri a 19 ( $\omega$ -5 gliadin) and Tri a 14 (non-specific LTP). Wheat  $\omega$ -5 gliadin (Tri a 19) is well understood as the major allergen in WDEIA-affected individuals [38,59-61]. Studies have also revealed that  $\omega$ -5 gliadin-specific sIgE is a strong predictor of immediate WA in children, with elevated levels correlating with positive results in OFC;  $\omega$ -5 gliadin-specific IgE was detected in more than 80% of children with WA [46,49,62]. Meanwhile, wheat flour non-specific LTP is an important allergen for IgE-mediated WA, WDEIA, and Baker's asthma; this protein exhibits resistance to heat and enzymatic degradation, retaining their allergenic potential even after food processing and digestion. Non-specific LTP also does not cross-react with grass pollen; thus, measurement of non-specific LTP may help differentiate wheat sensitization from pollen allergies, which is crucial in patients with high levels of grass pollen-specific IgE [4,36,39]. Furthermore, positive sIgE responses to glutenins,  $\alpha$ -,  $\beta$ -, and  $\gamma$ -gliadins, as well as  $\alpha$ -Amylase/Trypsin inhibitors have been found in wheat allergic children, yet none has reached a high specificity and sensitivity in WA diagnosis [48,63,64]. So far, CRD is mainly used in scientific research, and further studies are required to better understand these allergens.

#### 4.3. Cell-Based Diagnosis

A new in-vitro test to complement WA diagnosis is the basophil activation test (BAT) by fluorescence-based flow cytometry [65-68]. In BAT, basophils are activated with whole blood and stimulated with an allergen-containing solution, leading to upregulated expression of cell surface proteins, namely CD63 and CD203c upon basophil degranulation, the latter also expresses in resting basophils [65,69]. Basophil activation test is established as a sensitive biomarker which corresponds to the clinical severity of anaphylactic reactions. Measurement of basophil CD203c expression induced by various preparations of wheat proteins, particularly  $\omega$ -5 gliadin, is useful in predicting causative allergens in patients with WA and WDEIA [69,70]. Particular caution is required for subjects to completely avoid wheat intake before BAT as otherwise subjects' basophils can be substantially activated at baseline. This problem can influence the accuracy of BAT results that assess the fold changes in CD63 or CD203c expression on basophils from baseline to after in-vitro wheat exposure. Meanwhile, the mast cell activation test (MAT) is similar to BAT, but MAT uses plasma or

serum to sensitize mast cells instead of whole blood [45,68]. The application of MAT in clinical research is sparse [71], but may be a novel field of study in the coming years.

## 5. Management

### 5.1. Natural History of Wheat Allergy

Despite the high prevalence of WA, a considerable proportion of wheat-allergic children develop tolerance later in childhood, reflecting WA has a better prognosis than other allergies (e.g. peanuts, shellfish or fish) which often persist into adulthood. Several studies have shown that the median age of tolerance to wheat allergens is approximately six years old [56,57]. Resolution rates were found to be 20-29% by four years, 52-56% by eight years, 65-66% by 12 years, and 76% by 18 years. The progress of tolerance can be assessed by measuring wheat allergen-specific sIgE titers repeatedly while on elimination diet. As the wheat allergen-specific sIgE concentration rises, tolerance development becomes less likely, whereas declining sIgE titers suggest a higher likelihood of tolerance [36]. Studies have shown that for children with peak wheat sIgE levels below 20 kU/L, the median age of tolerance development was 2.6 years; for those with peak sIgE levels between 20 and 49 kU/L, it was 4.5 years; and for those with peak sIgE levels  $\geq 50$  kU/L, it was 12.1 years old [56]. The abovementioned suggests that peak wheat sIgE level is a useful predictor of tolerance development.

### 5.2. Therapeutic Strategies

The current management approach for IgE-mediated WA primarily involves strict avoidance of wheat-containing foods. Patients need to be educated to accurately identify wheat allergens in food labels and written instructions should be provided to effectively eliminate wheat from their diet [4,9]. Individuals with WDEIA individuals should especially refrain from exercising for up to six hours after consuming wheat or wheat-containing products; sometimes, complete wheat avoidance may even be recommended [5].

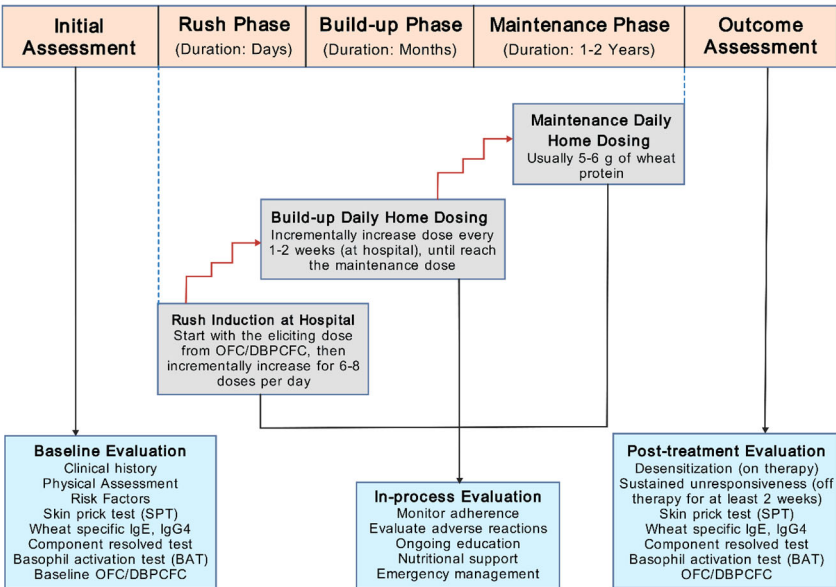
However, adhering to a wheat-free diet poses challenges due to the ubiquitous presence of wheat in various food items, including cakes, noodles, pasta, bread and condiments like soy sauce. Severe accidental exposure to wheat allergens may occur, necessitating the use of epinephrine autoinjectors to manage allergic reactions and subsequent emergency department admission for close monitoring [4]. Antihistamines, glucocorticoids and  $\beta$ -agonists are considered adjunctive treatments for anaphylaxis which may be used alone or in combination depending on the severity of the reaction [9].

### 5.3. Immunotherapy

Immunotherapy is a promising method for treating food allergies, with three main routes of administration — oral immunotherapy (OIT), sublingual immunotherapy (SLIT), and epicutaneous immunotherapy (EPIT) [9,72], among which OIT is the most studied. Oral immunotherapy works by inducing oral tolerance to allergenic foods through modification of both innate and adaptive immune response and adopts the abovementioned desensitization mechanism. While Tomsitz *et al* reported that SLIT increased reaction thresholds in three WDEIA-affected patients [73], there has not been any reported studies on EPIT for WA. However, the feasibility of SLIT in inducing unresponsiveness to wheat can be limited by the daily dosing of wheat that subjects can receive. Oral administration of OIT is the natural way through which subjects are exposed to wheat in their diet, and hence the plethora of research data that evaluated the potential usefulness of OIT in treating WA. As discussed above, several probiotics were a feasible treatment option for food allergy via the stimulation of Treg cells [30,34,35]. An Australian multi-center clinical trial reported safety benefit of a probiotic adjuvant during OIT for peanut allergy, while it did not improve the occurrence of sustained unresponsiveness [74]. There has not been any clinical trial that investigated the therapeutic benefits from a combination of probiotics and wheat OIT.

#### 5.3.1. OIT Protocol

Standard OIT protocols typically comprise the rush, long-term build-up and maintenance phases (**Figure 3**) [10]. During the rush phase, patients first consume a very small dose of the test food (equivalent to the eliciting dose from DBPCFC), followed by a gradual increase in the dosage with six to eight doses per day. This phase is usually conducted in a hospital due to the high risk of systemic reactions. Once completed, patients are advised on a safe starting dose for self-administration at home. The subsequent build-up phase involves incremental increase in immunotherapy dosage every one to two weeks, typically by 20-30 percent increment per visit, until the maintenance dose (usually one serving dose) is reached. Patients maintain this dose for at least one to two years, followed by evaluation for desensitization and tolerance [also known as sustained unresponsiveness (SU)]. Desensitization indicates subjects’ ability to tolerate the maintenance dose when regularly consuming the food, whereas SU reflects their ability to tolerate doses even after discontinuing regular dosing. Throughout the treatment process, oral food challenge tests are performed to assess desensitization. Subjects with established SU should discontinue the allergenic food doses for at least 2 weeks before undergoing another oral food challenge [10]. The occurrence of SU is the closest surrogate for immunological tolerance as subjects are only intermittently exposed to the previously allergic food item. Nonetheless, long-term follow up of subjects for at least 1-2 years is required to ascertain if they have achieved immunological tolerance.



**Figure 3.** Schematic view on study design of oral immunotherapy for wheat allergy. Abbreviations: DBPCFC, double-blind, placebo-controlled food challenge; g, gram; IgE, immunoglobulin E; IgG4, immunoglobulin G 4; OFC, oral food challenge.

5.3.2. Clinical Trials

**Table 1** summarizes the published clinical trials for wheat OIT protocols demonstrating wide variability, with efficacy being defined differently between trials [21-23,50,75-83]. For example, the types of wheat products utilized for the immunotherapy varied among studies — some used pasta, others used udon and bread, to name a few. Additionally, the target maintenance doses in different OIT trials ranged from 53 mg to 13 g of wheat protein (WP). Most studies used a maintenance dose of 5-6 g of WP [22,76,77,82], which is approximately equivalent to two to three slices of bread. The varying dosages may have been affected by the degree of wheat sensitization and reports on subjects’ tolerance level. For instance, in a wheat DBPCFC trial by Rodriguez del Rio *et al.* with higher threshold doses (ranging from 0.8 g to 12.5 g of WP), subjects were reported to be tolerant even to high maintenance doses of wheat (13 g of WP) without experiencing systemic reactions [75]; this may



be attributed to a lower degree of wheat sensitization among this group of patients. Furthermore, maintenance durations varied widely, ranging from two months to three years. Some trials defined their primary endpoint as desensitization to a maintenance dose of OIT, with effectiveness ranging from 30% to 100%. Others defined their primary endpoint as SU to an oral wheat challenge after abstaining from wheat consumption for at least two weeks, with efficiency ranging from 13% to 100%. These altogether accounted for the varying degrees of measured efficacy and effectiveness, posing challenges for head-to-head comparison across trials.

Analysis from independent studies suggests that therapeutic outcomes are more dependent on the target dose of wheat protein and the duration of maintenance rather than the type of wheat products used. OIT trials employing higher maintenance doses and longer durations have demonstrated higher rates of desensitization. In a multicenter randomized controlled trial comparing lower (1445 mg) to higher dose (2748 mg) OIT, 30.4% versus 57.1% of subjects were able to tolerate a challenge of 7443 mg of WP respectively [78]. In another study which children were randomly

**Table 1.** Overview of wheat oral immunotherapy trials.

Study	Design	Patients treated with OIT, n	Age, mean (range)	Form of wheat used	Up-dosing phase	Maintenance phase	Target dose	Changes in SPT scores (mean/median)	Changes in sIgE (mean/median)	Changes in sIgG (mean/median)	Efficacy after OIT, % desensitization	Efficacy after OIT, % sustained unresponsiveness	Adverse reaction (%)
Rodríguez del Río (2014) [75]	Open-label, nonrandomized, control	6	5.5 (5-11) years	Semolina porridge and boiled semolina pasta	3-24 days	6 months	13 g of WP	No significant changes in SPT scores but showed a trend to decrease after 6 months follow-up (47.5 mm vs 28.75 mm)	No significant changes in sIgE but showed an increase after 6 months follow-up (47.5 kUA/L vs 28.75 kUA/L)	Increased sIgG4 and sIgG1 to wheat and a trend of decrease in all wheat proteins after 6 months follow-up	83%	Not assessed	6.25% of doses during up-dosing, none treated with IM epi
Sato (2015) [76]	Open-label, nonrandomized, historical control	18	9.0 (5.9-13.6) years	Boiled udon noodles	5 days	>3 months	5.2 g of WP	Not assessed	Decreased sIgE to wheat (>100 vs 43.5 kU/L) after 2 years	Not assessed	88.9%	OIT: 61.1% Historical control: 9.1%	26.4% of inpatient doses, 6.8% of outpatient doses with 1 treated with IM epi





										vs 0.09 mgA/L ) after 1 month		
Ogur a (2020 )[79]	Multic enter, open- label, a rando mized, non- placeb o control	Low dose group : 5.5 Boiled udon noodle s, High boiled pasta and bread (3.7- 5.5) years	24 months	Low dose: 650 mg of WP; High dose: 2.6 g of WP	Not assess ed	Decreas ed sIgE to wheat after 1 year in both groups and decrease d sIgE to ω-5 gliadin in low dose group	No chang es in sIgG and to High dose group: 33.3% at year 1 both groups	Low dose group: 66.7% ; High dose group: 33.3% at year 1	Low dose group: 16.7% at year 1, 58.3% at year 2; High dose group: 50.0% at year 1, 58.3% at year 2	Low dose group: 4.76% of doses with 0.02% treated with IM epi; High dose group: 8.82% of doses, none treated with IM epi		
Sugiu ra (2020 )[81]	Open- label, nonran domize d, non- placeb o control	35 5 (4- 6) years Boiled udon and somen noodle s	12 months	10 times greater than the initial dose	Not assess ed	Decreas ed sIgE to wheat (97.0 vs 51.9 UA/mL) and ω-5 gliadin (4.8 vs 1.4 UA/mL) after 12- 15 months sIgE to wheat increase d after up- dosing, followed by a decrease after 3- month mainte nance phase	Not assess ed	OIT: 37.5% Contro l (wheat avoida nce): 10.0%	Not assess ed	0.64% of doses, none treated with IM epi		
Babai e (2022 )[22]	Open- label, nonran domize d, no control	20 6 (2- 17) years Cake and bread Not menti oned hs	3-27 mont hs	5.28 g of WP		Decre ased (9.8 mm vs 4.3 mm) after 3- month mainte nance phase	Not assess ed	Not assess ed	Not mentio ned	7.2% of doses during up- dosing, with 0.4% treated with IM epi		
Naga kura (2022 )[80]	Open- label, nonran domize d, histori cal control	29 6.7 (6.3- 7.9) years Boiled udon noodle s	1 mont h	35 mont hs	53 mg of WP	Not assess ed	Decreas ed sIgE to wheat (278 vs 89.3 kUA/L), gluten (358 vs	Not assess ed	100%	OIT: 7% at year 1, 28% at year 2, 41% at year 3; Historic	7.7% of doses at year 1, 3.9% at year 2,	



Assigned to the low-dose (650 mg) versus high-dose (2.6 g) OIT groups, 17% and 50% of subjects achieved SU after one year respectively [79]. In terms of OIT safety, the incidence of total adverse reactions per total ingestion time was significantly lower in the low-dose OIT compared to the high-dose OIT (4.76% vs 8.82%), suggesting that the low-dose protocol may be safer despite lower effectiveness. Furthermore, the duration of treatment may also impact OIT effectiveness. Nagakura *et al* reported that during OIT treatment, the proportion of patients achieving short-term unresponsiveness increased by 7%, 28%, and 41% after one, two and three years respectively [80]. Moreover, the frequency of adverse reactions per OIT dosing significantly decreased annually, indicating that the longer OIT protocol may be more effective and safer. Overall, exploring the maintenance dose and the duration of OIT will crucially impact the optimization of wheat OIT protocol.

Apart from influencing individuals' clinical response to allergens, the efficacy of wheat OIT is also reflected by changes in the SPT and sIgE test results. Several studies, though not all, have reported initial increase and subsequent decrease in sIgE levels to wheat or  $\omega$ -5 gliadin over the course of OIT [21,22]. Some studies reported reduction in sIgE levels to wheat or  $\omega$ -5 gliadin upon completion of the OIT regimen [23,76,79-83]. However, in many cases, individuals still exhibited elevated sIgE levels even when upon desensitization or sustained unresponsiveness. Alongside changes in sIgE, decrease in SPT wheal sizes were reported in some wheat OIT trials [21,22,77], although this was not observed in several other studies [74,77,82]. Levels of sIgG4 to wheat and  $\omega$ -5 gliadin generally increased with wheat OIT [23,75,78], but such finding was not consistent in other studies [79,83,84]. In summary, OIT may bring about changes in SPT, sIgE and sIgG4 levels to wheat or  $\omega$ -5 gliadin in some cases which can estimate treatment efficacy, although the correlation of these changes with desensitization or SU remains unclear. Apart from SPT and serum Ig levels, the basophil activation test is another possible biomarker of wheat OIT treatment response, yet little has been researched in this area. These diagnostic fields present interesting questions for future research to address.

### 5.3.3. Precautions for OIT

Exercise should be avoided after wheat ingestion in individuals undergoing OIT, even in those who have achieved short-term SU, since they may be at risk of WDEIA or exhibit exercise-induced allergic reactions on desensitization (EIARDs). A study evaluating the two-year follow-up prognosis for WA patients who achieved short-term SU after receiving wheat OIT revealed six out of eight symptomatic patients experienced adverse allergic reactions after exercise and three of them developed anaphylaxis [85]. Studies reported that EIARDs occurred not only after rush OIT but also after slow OIT for wheat, with 66.7% (14/21) and 48.4% (15/31) of WA patients developing EIARDs respectively [86,87]. Therefore, exercising after wheat intake may be the primary trigger for allergic symptoms during or after OIT.

### 5.4. Other Therapeutic Approaches

The combination of biologics with food OIT is a heated research topic, yet the use of omalizumab that removes circulating IgE antibodies for wheat OIT has not been tested to date. A case report demonstrated that administering omalizumab three weeks before increasing the wheat dose helped achieve higher build-up doses [88]. Chinuki *et al* suggested that short-term (12 weeks) omalizumab inhibited wheat allergen-induced basophil activation in hydrolyzed wheat protein-allergy patients, but the effect diminished after treatment cessation [66]. In a long-term (48 weeks) omalizumab study involving 20 adult patients with WDEIA, over 80% of the subjects achieved a basophil activation rate of less than 10% against all fractionated wheat preparations, with 68.8% spared from any allergic reactions [67]. Further studies with larger sample sizes and controlled trials are needed to fully evaluate the efficacy of omalizumab in conjunction with wheat OIT. Meanwhile, several strategies have been proposed to improve outcomes in wheat OIT, including wheat modification, adjunct medications such as antihistamines or leukotriene antagonists, and probiotics [4], but the safety and effectiveness profiles of these treatments are yet to be presented.

## 6. Conclusions and Future Trends

IgE-mediated WA is characterized by an immune dysregulation leading to the release of inflammatory mediators from mast cells and basophils, with microbial dysbiosis also potentially playing a role in its pathogenesis. It can manifest as a spectrum of allergic reactions which impair individuals' quality of life. For children with IgE-mediated WA, the prognosis is generally positive. The diagnosis of WA involves SPT and serum Ig measurement to wheat extracts, yet in view of their low specificity, novel approaches like alcohol-soluble extracts for SPT, component-resolved diagnosis and the basophil activation test are under research. While the principal management strategy for patients lies in dietary wheat avoidance, oral immunotherapy has recently emerged as a promising approach for treating WA, with trials using higher maintenance doses and extended durations showing increased rates of desensitization. Changes in SPT, sIgE and sIgG4 levels to wheat or  $\omega$ -5 gliadin may help assess treatment efficacy, but the inconsistency in current published trials warrant further confirmation through large-scale studies. BAT could play a potential role in evaluating treatment effectiveness, although more research is needed. Lastly, integrating omalizumab as an adjunctive therapy to wheat OIT holds potential for enhancing OIT effectiveness and represents a future direction of research. Further studies are crucial to determine the optimal OIT protocol that would safely promote tolerance in children with WA.

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