

EDITORIAL

Clinical & Experimental Allergy

Wheat-dependent exercise-induced anaphylaxis: Pros and cons of recombinant ω -5gliadin and glutenins, or their epitope peptides, in diagnosis

This editorial discusses the findings of the paper in this issue by H. Takahashi et al. [10], pp. 1293–1298.

L. Tuckova¹, D. Sanchez¹, H. Tlaskalova-Hogenova¹ and P. Panzner²

¹Department of Immunology and Gnotobiology, Institute of Microbiology, v.v.i., Academy of Sciences of the Czech Republic and ²Department of Immunology and Allergology, Medical Faculty in Pilsen, Charles University, Czech Republic

Wheat (*Triticum aestivum*) one of the major cereals, is essential in daily nutrition for a majority of people worldwide, but its ingestion can trigger adverse reactions, most often presenting as IgE-mediated food hypersensitivity or T-cell mediated gluten enteropathy – coeliac disease. IgE-mediated hypersensitivity reactions to wheat proteins are clinically distinct dependent on the route of allergen exposure, target organs, and involve immunological mechanisms. They may manifest as a traditional food allergy to ingested wheat proteins (including cutaneous, gastrointestinal, and respiratory symptoms), as wheat-dependent exercise-induced anaphylaxis (WDEIA) – when gluten intake is followed by exercise or occupational (baker's) asthma and rhinitis reactions to inhaled flour. In contrast, coeliac disease (gluten-sensitive enteropathy) is a genetically predisposed (HLA-DQ2/DQ8 restricted) disease, mediated by autoimmune mechanisms.

The ingestion of foods (such as celery, tomato, nuts, shell-fish, and wheat) before physical exercise can trigger food-dependent exercise-induced anaphylaxis (FDEIA) [1, 2]. The clinical manifestation is highly variable, from urticaria or angioedema to dyspnoea, hypotension, collapse, and shock. The threshold amount of ingested allergen and the threshold intensity and duration of exercise that induces symptoms varies considerably among patients. The FDEIA diagnosis is based on the detection of food-specific IgE antibodies (Abs), a

history compatible with IgE-mediated symptoms, skin prick test (SPT), and/or basophil activation test (BAT), often having a high sensitivity, but low specificity. In most sensitive RIAs or enzymatic immunoassays, including a CAP-fluorescent enzymatic-immunoassay (CAP-FEIA), a certain extent of cross-reactivity cannot be excluded (depending on the purity of the allergens and/or their structural homologies). The diagnosis is more reliable when confirmed by exercise challenge testing, which is time consuming and also risky, as it can induce severe clinical symptoms. However, the specificity of all diagnostic approaches depends on the purity of allergens applied, thus considerable attention has been focused on the identification, isolation, and employment of defined allergens instead of commonly used allergen extracts, i.e. the use of purified native or recombinant allergens or their defined epitope peptides in diagnosis and potentially also in immunotherapy.

The wheat-exercise association is not obvious in many patients, highlighting the need to consider WDEIA in the differential diagnosis of all patients presenting with idiopathic systemic reactions. In a portion of patients with WDEIA, more severe reactions were observed in combination with COX-1 inhibitors administration and other factors like psychical stress, fatigue, analgesic or alcohol intake, menstruation, and/or infections could be responsible for disease triggering [3].

Wheat proteins can be classified, based on differential solubility, into: water/salt soluble albumins and globulins, and water/salt insoluble gliadins and glutenins. IgE Abs specific to wheat albumins and globulins were found in the sera of patients having wheat allergy or baker's asthma. In patients with WDEIA, the most severe form of wheat allergy, appearing predominantly in young adults and adolescents, IgE Abs against water insoluble gliadins and glutenins were detected. Interest-

Correspondence:

Ludmila Tuckova, Department of Immunology and Gnotobiology, Institute of Microbiology, v. v. i., Videnska 1083, 142 20 Praha, Czech Republic. E-mail: tuckova@biomed.cas.cz

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ingly these gliadin family members and their antigenic epitopes differ substantially from those recognized by gliadin-specific T cell receptors and specific IgA and IgG Abs, in patients with coeliac disease. The major wheat allergens in WDEIA were identified as ω -5 gliadin (Tri a19) and HMW-glutenin subunits of wheat gluten in Japan and Finland; interestingly, in two countries with a different history of wheat consumption in each [4, 5]. The ω -5 gliadin was also found as a major allergen in relatively rare wheat-induced anaphylaxis, developing without any medication or exercise after the ingestion of a wheat-containing diet (WIA).

In attempts to increase the diagnostic value of specific IgE assays in allergic diseases, two strategies are applied: a) the use of a recombinant form of allergens or b) their specific IgE-binding peptides in tests (CAP-RAST or in CAP-FEIA or in rapid microarrays or multi-allergen assays). Despite the limited knowledge of wheat DNA, the recombinant peptides and ω -5 gliadin protein were cloned, purified, and applied in a CAP-System fluorescent enzyme immunoassay for specific IgE detection in WDEIA patient diagnosis [6, 7].

The fine specificity of IgE Abs in WDEIA patients was characterized using an array with overlapping synthetic peptides covering the whole primary sequence of ω -5 gliadin [8]. Four of these IgE-binding epitope peptides, QQIPQQQ, QQFPQQQ, QQSPEQQ, QQSPQQQ, were shown to be dominant, recognized by a vast majority of WDEIA patients. These findings were supported by Battais et al. [9], who characterized IgE reactivity with other members of the gliadin family, and identified linear sequential epitopes in repetitive domains of α/β , γ , ω 2, or ω 5 gliadins. Comparative epitope analysis led to ω -5 gliadin-derived consensus motif QXX₁PX₂QQ (X₁ being L, F, S, or I and X₂Q, E, or G) for IgE-binding epitopes recognized by WDEIA patients.

In a minority group of WDEIA patients, the reactivity of IgE with ω -5 gliadin was low if any, but significantly increased binding of purified wheat glutenins, high-molecular weight glutenin subunits (HMW-GS), and/or low-molecular weight glutenin subunits (LMW-GS) was detected in these patients. The mapping of IgE-binding epitopes of HMW-glutenins led to the identification of three dominant linear epitopes in its primary sequence as: QQPGQ, QQPGQGQQ, and QQSGQGQ. Based on this knowledge these consensus sequences, QQPGX and QXSGQXQ were derived that differ from the sequence reported for ω -5 gliadin IgE-binding epitopes. Interestingly, two combined epitopes, A- KPQQQ SPQQQFPQQIPQQQ and B- PTSPQQSGQGQQPGQGQQ, derived from ω -5 gliadin and HMW-glutenin were designed; and their capacity to bind patient IgE was found to be higher and more specific than the binding capacity of wheat extract or isolated gluten fractions. Moreover, simultaneous detection of specific IgE to

both antigenic peptides was suggested as a useful *in vitro* diagnostic approach/tool for WDEIA patients [6–9].

In this article, Takahashi et al. [10] who significantly contributed to the knowledge of allergens in WDEIA, have described the preparation of the recombinant form of the second main allergen - rHMW-GS. Employment of two main recombinant allergens (r ω -5 gliadin and rHMW-GS) in CAP-FEIA enabled comparison of IgE testing with the combined peptide epitope (B) of HMW-glutenin, native HMW-glutenin, recombinant HMW-glutenin and recombinant ω -5 gliadin, as well as combination of the last two allergens in cohorts of WDEIA and atopic dermatitis patients and healthy controls. The positive rate of allergen-specific IgE was highest for the simultaneous estimation/detection of specific IgE against the recombinant form of both allergens (r ω -5 gliadin and rHMW-glutenin). This combined testing helps to diagnose WDEIA in a majority of adults, as well as in children. They documented the maintenance of a high specificity and an increase in sensitivity (reaching 92.9% and 93.8% respectively), when compared to the sensitivity of the ω -5 gliadin test being 81.3%. This confirms preliminary data obtained from a smaller number of patients, published earlier in the review article by Morita et al. [11]. Moreover, they showed the lower specificity of ω -5 gliadin IgE detection in children in contrast to adults (being 46% in age category below 20). It must be pointed out, that six of eight patients negative for r ω -5 gliadin (including young ones) were positive for rHMW-glutenin IgE Abs assay. Thus, the authors [10] recommend employing CAP-WDEIA combined testing of specific IgE against recombinant ω -5-gliadin and recombinant HMW-glutenin in WDEIA. The preferential use of the rHMW-glutenin protein in CAP-FEIA rather than the HMW-derived epitope peptide B (both giving similar data) was explained by the lower stability of this synthetic peptide coupling on a solid phase matrix (cellulose). However, other synthetic or recombinant epitope peptides and/or their modified forms, with enhanced IgE reactivity and/or their hypoallergenic forms, are currently being tested for diagnosis and treatment [11–15]. On the other hand, using whole recombinant allergens enabled simultaneous binding of various structural and conformational epitopes and the strengthening of binding affinity to the IgE molecule.

The recombinant forms of a LMW member of the glutenin family were employed in IgE-binding assays for diagnostic purposes in patients with backer's asthma, immediate hypersensitivity to hydrolysed wheat proteins, and also tested in patients with WDEIA. Patients developing positive skin prick tests – not only with gluten, wheat flour extracts, but also with purified natural LMW-GS – were included in tested cohorts. Recombinant forms (constructs) and two chimeras combining the halves of two genes were applied in the assays. The IgE-

binding of various constructs was detected in all patients; however, it differed in intensity also within the LMW-GS group. The mapping of IgE specificity showed some common and cross-reactive epitopes, shared with ω -5 gliadins, and also other ω -5 gliadin independent epitopes [16]. The character of LMW-specific antigenic epitopes is supposed to be conformational, and its precise analysis and evaluation of diagnostic value, alone or in combination with ω -5 gliadin and/or HMW-GS remains to be determined in future study.

We must take into account that wheat proteins are subjected to thermal and other types of processing during food preparation similarly to other food components and are exposed to pH changes and to numerous enzyme treatments in the digestive tract after consumption. All these processes could affect both the conformational and structural/sequential epitopes recognized by IgE Abs. Gliadin and glutenin peptides formed by digestive enzymes (*i.e.* pepsin, trypsin...) could cross the epithelial layer and meet enzymes present in lamina propria, such as tissue transglutaminase-TG2. Wheat gliadins (members of the group of prolamins – molecules rich in glutamine and proline) belong to well defined substrates of TG2. Under physiological conditions TG2 is an intracellular enzyme that is activated (changes its conformation) and released under stress-conditions such as infection, inflammation, or oxidative stress. TG2 can cross-link peptide-bound glutamine with lysine on an acceptor peptide by forming isopeptide [ϵ -(γ -glutamyl)lysine] binding. In the absence of lysin containing peptide, TG2 deamidates glutamine to glutamic acid. This TG2 activity is involved in the molecular mechanism of coeliac disease. By deamidation, a negative charge of gliadin peptides increases, enhancing their binding affinity to HLA-DQ2/8 molecules and antigen presentation to specific CD4⁺ T lymphocytes, playing a central role in disease development. In contrast to coeliac disease, the cross-linking activity of TG2 was suggested to participate in wheat allergies. The most frequent explanation of the pathophysiology of WDEIA is that exercise may lead to the activation of TG2, which is a stress-responsible enzyme. Indeed, TG2 irreversible protein cross-linking of ω -5 gliadin molecules and the formation of complexes of higher IgE

reactivity has been documented [17]. Based on these data we can speculate that: TG2 treatment could also affect the immune response to ω -5 gliadin and HMW-glutenin derived antigenic epitope peptides; and that aggregates of a certain size could be bound with higher stability in CAP-immunoassays for IgE detection in WDEIA patients.

When sensitized, patients have IgE Abs against food components, but their ingestion alone is insufficient to trigger allergic reaction. Exercise-induced alterations could stimulate immune and neuroendocrine system, similarly to stress conditions (trauma, tissue damage, infection), by increasing intestinal permeability, allergen uptake, and altered cytokine milieu. Proinflammatory cytokine IL-6, produced by immune cells but also by skeletal muscles during active exercise, has been shown to increase TG2 expression, which could cross-link allergenic peptides, strengthen their binding to IgE, and mast cell activation.

In general FDEIA syndrome occurs in association with allergen consumption and physical exercise. The time interval between food ingestion and exercise is individually unique (but usually between 30–120 min); as well exercise duration before the onset of symptoms lasts anywhere from 10–50 min. Symptoms may occur just as likely after mild physical effort such as walking as they would after a hard load of physical activity (*e.g.* in tennis players or other athletes). Thus, individual patients could not only significantly differ in their threshold allergen dose, but also in physical intensity regarding the clinical manifestation and anaphylactic shock occurrence after a provocation/challenge test.

We can conclude that a combination of skin tests, basophil activation, and detection of specific IgE Abs using well-defined recombinant single or combined allergens or their epitope peptides and/or their combination in ELISAs (most often in CAP-FEIA; described previously [8]) currently offers the optimal diagnostic approach. Ongoing studies using microarray-based detection (ISAC) or individually focused component-resolved molecular diagnosis presented and discussed by authors in this article, as well as by others [10, 14, 15, 18–20] represent promising diagnostic, prognostic, and eventually therapeutic tools for the future.

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