

Update on wheat hypersensitivity

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Purpose of review

Wheat is among the six foods responsible for approximately 90% of food allergies in children, and in recent years wheat has been increasingly recognized as a cause of food-dependent, exercise-induced anaphylaxis. Wheat flour is an important cause of baker's asthma, a well-known occupational respiratory allergy to inhaled flour. This review outlines the diverse clinical manifestations of IgE-mediated wheat hypersensitivity and summarizes recent advances in characterization of clinically significant allergens.

Recent findings

Only a few of the numerous wheat proteins recognized by IgE of sensitized individuals have been characterized at the molecular level. Characterized allergens causing baker's asthma include several water/salt-soluble wheat proteins, however sensitization patterns show a great degree of individual variation. The insoluble gliadins have been implicated in IgE-mediated allergy to ingested wheat, and ω -5 gliadin has been identified as a major allergen in wheat-dependent, exercise-induced anaphylaxis. The presence of IgE to purified ω -5 gliadin in children was highly predictive of immediate clinical symptoms on oral wheat challenge.

Summary

Diagnostic skin prick and in-vitro tests measuring sensitization against water/salt-soluble wheat proteins have poor predictive values. Quantification of gliadin-specific IgE in serum or skin prick testing with gliadin could serve as an additional tool in the diagnostic work-up of allergy to ingested wheat.

Keywords

wheat allergy, gliadin, IgE, allergen

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Abbreviations

ELISA	enzyme-linked immunosorbent assay
RAST	radioallergosorbent test
SPT	skin prick test
WDEIA	wheat-dependent, exercise-induced anaphylaxis

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Introduction

Wheat is the most widely cultivated cereal and an important source of food and dietary protein worldwide. Ingestion of wheat products and inhalation of wheat flour are the two major routes of sensitization. Wheat allergy, defined as an adverse immunologic reaction to wheat [1], encompasses a broad spectrum of disorders with different pathomechanisms and clinical manifestations. Depending on the route of allergen exposure and the underlying immunologic mechanisms, wheat allergy may manifest as classic food allergy affecting the skin, gut, or respiratory tract; exercise-induced anaphylaxis; occupational asthma and rhinitis; or contact urticaria. In addition, ingestion of wheat gluten may cause celiac disease, a T-cell-mediated intestinal inflammation, or dermatitis herpetiformis, a blistering skin eruption. With the exception of the latter two, IgE antibodies play a central role in all of the above. This review focuses on the diverse manifestations of IgE-mediated wheat allergy.

Clinical manifestations of IgE-mediated wheat allergy

Sensitization to inhaled wheat flour leads to baker's asthma, whereas sensitization to ingested wheat via the gastrointestinal tract may cause food allergy or exercise-induced anaphylaxis.

Baker's asthma

Baker's asthma is an IgE-mediated occupational disease of millers and bakers caused by inhalation of cereal flour [2•]. Respiratory symptoms are usually accompanied by rhinitis, which is considered a prestage of asthma, and often by conjunctivitis and contact urticaria. Most patients with baker's asthma are, however, able to ingest wheat products without symptoms [2•,3]. An atopic constitution together with high wheat allergen concentrations in the workplace air have been shown to significantly increase the risk of sensitization and development of symptoms [4]. About 60% of bakers with work-related respiratory symptoms have IgE antibodies to wheat or rye flour [3]. Other known allergens include barley, soybean, storage mites, fungal enzymes, and other baking additives [5].

Allergy to ingested wheat in children and adults

Six major foods, milk, egg, wheat, soy, peanut, and fish, account for approximately 90% of food-allergic reactions in children [6,7•]. Wheat is introduced to the diet in early childhood, often around the age of 5 months. Sensitization to wheat may, however, occur through maternal milk during exclusive breastfeeding [8].

Clinically wheat allergy manifests as classic food allergy affecting the skin, gastrointestinal tract, and respiratory system [9]. The majority of wheat-allergic children suffer from moderate to severe atopic dermatitis, and sensitization to other foods, such as milk and egg, often coexists. Wheat ingestion elicits typical IgE-mediated reactions of immediate onset, including urticaria, angioedema, bronchial obstruction, nausea, and abdominal pain, or in severe cases systemic anaphylaxis. Delayed hypersensitivity symptoms appear about 24 h after wheat ingestion and include gastrointestinal symptoms and deterioration of atopic dermatitis [10–12]. In a study by Majamaa *et al.* [10] oral wheat challenges frequently caused delayed-onset symptoms in children with negative skin prick tests (SPTs) and specific IgE results for wheat. Similarly, Niggemann *et al.* [13•] showed that wheat elicited positive oral challenge reactions, more often than egg or milk, in children with no evidence of sensitization.

Ingestion of wheat rarely causes classic IgE-mediated food allergy symptoms in adults, however in some cases it may lead to exacerbation of atopic dermatitis [14,15]. A study by Simonato *et al.* [16] suggested that IgE-mediated wheat allergy may play a role in adults suffering from gastrointestinal symptoms after wheat ingestion, even when tests for wheat-specific IgE remain negative.

Wheat-dependent, exercise-induced anaphylaxis

Wheat has been increasingly recognized as a cause of food-dependent, exercise-induced anaphylaxis [17,18,19•]. This is a unique form of allergy, when ingestion of a specific food together with physical exercise triggers anaphylactic reactions [20]. The severity of the reactions ranges from local or generalized urticaria and angioedema to dyspnea, hypotension, collapse, and shock. The reactions are unpredictable and difficult to diagnose, because the amount of wheat ingested as well as the degree of exercise needed to provoke symptoms varies greatly [18,21,22]. In a series of 18 Finnish patients with wheat-dependent, exercise-induced anaphylaxis (WDEIA) the time between wheat ingestion and exercise ranged from 10 min to 4 h, and the duration of exercise before the onset of symptoms from 10 to 60 min [22]. All patients were adults (mean age 31, range 18–59 years) at the onset of symptoms. None had a history of wheat allergy in childhood and less than 30% had a history of other atopic diseases. Introduction of a gluten-free diet led to symptom resolution [21,22]. In 12 Japanese patients with WDEIA, the mean age at onset was 24 (range 10–63 years) and 67% had an atopic constitution. In 70% of these patients aspirin ingestion enhanced symptoms of WDEIA [18].

Classification of wheat proteins

Cereal grains are members of the grass family (*Graminae*) that produce edible dry fruits, known as seeds or kernels.

The kernel contains on the average 10–15% protein. Early studies by Osborne [23] categorized cereal proteins into four fractions on the basis of their solubility in a series of solvents: water (albumins), dilute salt solutions (globulins), aqueous alcohol (gliadins), and dilute alkali or acid (glutelins). The water/salt-soluble albumins and globulins are mainly structural proteins and metabolically active enzymes, such as α - and β -amylases, and their inhibitors. The water/salt-insoluble gliadins and glutelins, together known as prolamins or gluten, are the major storage proteins of the wheat grain. They are responsible for the ability of wheat flour to be baked into bread by creating a visco-elastic dough capable of holding gas [24]. The ethanol-soluble gliadin group comprises over 40 structurally similar proteins, which can be further categorized into α -, γ -, and ω -types on the basis of their electrophoretic mobility. The name prolamins reflects the fact that these proteins are unusually rich in proline and glutamine, present in highly repetitive sequence motifs. Wheat prolamins share a great degree of sequence and structural homology with each other and with the corresponding proteins in rye and barley [24–26].

Wheat allergens in baker's asthma

Immunoblotting studies have identified numerous IgE-binding proteins in baker's asthma, both in the water/salt-soluble and insoluble fractions of wheat [27–30]. Only a small proportion of these allergens, however, have been isolated and characterized at the molecular level. The characterized allergens belong to the water/salt-soluble fraction, and include several 14–17 kDa proteins from the α -amylase/trypsin inhibitor family [31,32]. In addition a 36 kDa wheat glycoprotein with peroxidase activity [33,34], a 27 kDa protein similar to acyl-coenzyme A oxidase [32], and a 35 kDa protein similar to fructose-bisphosphate-aldolase have been identified [35]. A recent study by Sander *et al.* [36] revealed a large degree of variation in sensitization to different water/salt-soluble wheat proteins in 10 individuals with baker's asthma. In two-dimensional immunoblotting, each patient had an individual IgE-binding pattern with 4–50 different IgE-binding spots. Two of the most frequently recognized proteins were similar to isoforms of glyceraldehyde-3-phosphate dehydrogenases from barley, one was similar to triosephosphate isomerase from barley, and one was identified as serpin, a serine protease inhibitor from wheat. Interestingly, α -amylase inhibitors or other previously characterized allergens were not among the most frequently detected allergens. The authors suggested that this could result from cultivar-specific differences as well as the great degree of individual variation in sensitization patterns.

Allergens involved in childhood wheat allergy

Compared with baker's asthma, the number of studies on allergy to ingested wheat is surprisingly small. Very

few clinically significant allergens in wheat have been characterized. In wheat-allergic children the IgE response appears heterogeneous, and immunoblotting studies have detected IgE binding to a number of water/salt-soluble wheat proteins with molecular masses ranging from 15 to 69 kDa [37–40]. James *et al.* [40] showed IgE binding to a 15 kDa α -amylase inhibitor in the sera of five children with ingested wheat allergy, suggesting that it represents a sensitizing allergen both after ingestion and inhalation. Recent studies, however, provide evidence that the insoluble gliadin fraction contains clinically significant allergens responsible for symptoms after wheat ingestion in children. Varjonen *et al.* [11] showed extensive IgE immunoblot reactivity and positive SPTs to the gliadin fraction in 13 children with atopic dermatitis and positive oral wheat challenges, but not in children with negative challenges. Takizawa *et al.* [41] detected IgE binding to 35–67 and 95 kDa water/salt-insoluble proteins in the sera of seven children with wheat-induced anaphylaxis. Six wheat-allergic children with atopic dermatitis showed similar, but less intense, IgE staining patterns.

We investigated IgE reactivity to purified ω -5 gliadin, a major allergen in WDEIA, in a group of 40 children with atopic dermatitis who underwent oral wheat challenges because of suspected wheat allergy [12]. The presence of IgE to ω -5 gliadin was highly predictive of immediate oral challenge reactions; wheat ingestion elicited clinical symptoms in all 16 children with IgE to ω -5 gliadin. Eight children with delayed symptoms and 13 challenge-negative children did not have IgE to ω -5 gliadin. In contrast, wheat CAP radioallergosorbent test (RAST) (Pharmacia, Uppsala, Sweden) and SPTs (1:10 w/v in physiological saline) showed false positive results in 38% of the challenge-negative children. IgE enzyme-linked immunosorbent assay (ELISA) with ω -5 gliadin gave a diagnostic specificity as well as a positive predictive value of 100%, and a sensitivity of 84% for immediate challenge reactions.

In a recent study by Armentia *et al.* [42], immunoblotting with pooled sera from adults and children with inhalant or ingested cereal allergy gave similar IgE-staining patterns to the water/salt-soluble extracts of wheat, rye and barley. Unfortunately, the insoluble gliadin fraction was not examined. The authors suggested that similar allergens are responsible for symptoms after ingestion and inhalation of cereals. Further investigation of in-vivo and in-vitro responses to purified allergens in individual patients would clarify the clinical significance of these observations.

Allergens in wheat-dependent, exercise-induced anaphylaxis

The insoluble gliadin fraction seems to hold the majority of allergens responsible for WDEIA [18,21,22,43]. We

identified and purified a 65 kDa allergen similar to wheat γ -gliadin, later identified as ω -5 gliadin (Tri a 19) [22]. We showed that ω -5 gliadin bound IgE antibodies in ELISA in the sera of 18 patients with WDEIA and elicited positive SPTs in all 15 patients tested. Sera from 16 patients with baker's asthma did not recognize this allergen. In the same study, purified α -gliadin bound IgE in 72% of the WDEIA patient sera, but failed to elicit significant SPT responses. In ELISA inhibition studies, ω -5 gliadin (100 μ g/ml) inhibited up to 80% of serum IgE binding to a whole wheat extract containing mainly water-soluble proteins [22], and nearly 100% of that to a commercial crude gliadin preparation on solid-phase (K. Palosuo, personal communication). Thus far, we have examined altogether 32 Finnish patients with WDEIA and all of them have exhibited IgE to ω -5 gliadin in ELISA or SPTs [19*,44]. Supporting its significance as a major allergen, ω -5 gliadin induced high histamine release from the basophils of patients with WDEIA, but not from the basophils of nonallergic controls [19*]. Morita *et al.* [43] demonstrated IgE binding to γ -gliadin in the sera of four Japanese patients with WDEIA. RAST inhibition with purified γ -gliadin (200 μ g/ml) inhibited 60–90% of the IgE binding to solid-phase gluten proteins.

Cell-mediated immune responses to gliadin and a hypothesis for the mechanism of wheat-dependent, exercise-induced anaphylaxis

The pathophysiologic mechanisms underlying WDEIA are unknown, and the role of exercise remains obscure. Suggesting aberrant regulation of cell-mediated immune responses to gliadin, we showed that gliadin stimulation of peripheral blood mononuclear cells from 10 patients with WDEIA elicited enhanced proliferative responses, but significantly decreased mRNA expression of the antiinflammatory cytokine IL-10 [19*]. Recent findings by our group propose a hypothesis that tissue transglutaminase, a stress-responsive enzyme that catalyses the formation of irreversible protein cross-links, could be activated in the intestinal mucosa of patients with WDEIA during physical exercise [45]. We showed that transglutaminase treatment of ω -5 gliadin peptides created high molecular weight complexes that elicited significantly enhanced SPT and in-vitro IgE responses in patients with WDEIA. We hypothesized that these large allergen complexes could trigger anaphylactic reactions in patients with WDEIA.

Cross-reactivity of wheat allergens with cereal grains and grasses

Patients with grass allergy often exhibit clinically insignificant SPT and in-vitro IgE responses to wheat. Jones *et al.* [39] demonstrated IgE cross-reactivity between the water/salt-soluble proteins of cereal grains and grasses, but clinical reactivity to more than one

cereal was observed in only 20% of the cases. RAST inhibition studies by Sandiford *et al.* [46] detected IgE cross-reactivity between water/salt-soluble allergens in wheat, rye, barley, and soy in patients with baker's asthma. We demonstrated that rye and barley, but not oats, contained allergens cross-reacting with ω -5 gliadin [44]. The purified rye γ -secalins and barley γ -hordein bound IgE in over 80% of the WDEIA patient sera and elicited positive SPTs in 20–67% of the patients. While some patients with WDEIA have experienced symptoms when exercising after rye ingestion, others seem to tolerate rye and barley.

Diagnostic tests for wheat allergy

The diagnosis of wheat allergy is based on the patient's clinical history, detection of wheat-specific IgE and results of elimination diets and oral challenges, or bronchial challenges in cases of baker's asthma. The diagnostic accuracy of wheat SPTs and in-vitro IgE assays is unsatisfactory [47,48]. Sampson *et al.* [48] used the CAP system to determine food-specific IgE concentrations more than 95% predictive of clinical reactivity, but in the case of wheat the positive predictive value remained less than 75%. This poor capacity could be explained by the fact that commercial test reagents are mixtures of water/salt-soluble wheat proteins that lack allergens from the insoluble gliadin fraction. Moreover, cross-reactivity of water/salt-soluble wheat proteins with grasses increases the frequency of clinically insignificant positive test results. As long as purified and well characterized allergens are not available for diagnostic purposes, detection of gliadin-specific IgE in serum or skin prick testing with a gliadin preparation could serve as an additional tool in the diagnostic work-up of IgE-mediated allergy to ingested wheat. The atopy patch test, an epicutaneous test with foods, has given promising results in predicting delayed wheat-allergic reactions in children [10,13•].

Conclusion

The route of sensitization is important in the development of wheat allergy. Patients with baker's asthma are mostly sensitized to inhaled raw flour particles via the respiratory mucosa, whereas patients with ingested allergy are primarily sensitized to heat-treated and digested wheat proteins absorbed through the gastrointestinal epithelium. This could explain the apparent differences in sensitization to various allergens, and the finding that most patients with baker's asthma tolerate ingested wheat. A large amount of work is still required, before the clinically significant wheat allergens and their IgE-binding epitopes have been identified. Detailed knowledge on the structure and the immunologic and biochemical properties of major wheat allergens is a prerequisite for the development of novel strategies for diagnosis and treatment. The immunologic mechanisms

underlying delayed wheat-allergic reactions as well as the pathophysiologic events leading to WDEIA require further investigation.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

- 1 Johansson SG, Hourihane JO, Bousquet J, *et al.* A revised nomenclature for allergy: an EAACI position statement from the EAACI nomenclature task force. *Allergy* 2001; 56:813–824.
- 2 Brisman J. Baker's asthma. *Occup Environ Med* 2002; 59:498–502.
- This is a comprehensive review on the epidemiology, allergens, exposure, and prevention of baker's asthma.
- 3 Baur X, Degens PO, Sander I. Baker's asthma: still among the most frequent occupational respiratory disorders. *J Allergy Clin Immunol* 1998; 102:984–997.
- 4 Houba R, Heederik D, Doekes G. Wheat sensitization and work-related symptoms in the baking industry are preventable: an epidemiologic study. *Am J Respir Crit Care Med* 1998; 158:1499–1503.
- 5 Baur X, Posch A. Characterized allergens causing bakers' asthma. *Allergy* 1998; 53:562–566.
- 6 Burks AW, James JM, Hiegel A *et al.* Atopic dermatitis and food hypersensitivity reactions. *J Pediatr* 1998; 132:132–136.
- 7 Ellman LK, Chatchatee P, Sicherer SH, Sampson HA. Food hypersensitivity in two groups of children and young adults with atopic dermatitis evaluated a decade apart. *Pediatr Allergy Immunol* 2002; 13:295–298.
- This paper shows that a relatively small number of foods continue to account for the vast majority of food-allergic reactions in children and young adults.
- 8 Linna O. Specific IgE antibodies to uningested cereals. *Allergy* 1996; 51:849–850.
- 9 Sicherer SH. Determinants of systemic manifestations of food allergy. *J Allergy Clin Immunol* 2000; 106:251–257.
- 10 Majamaa H, Moisio P, Holm K, Turjanmaa K. Wheat allergy: diagnostic accuracy of skin prick and patch tests and specific IgE. *Allergy* 1999; 54:851–856.
- 11 Varjonen E, Vainio E, Kalimo K. Antigliadin IgE-indicator of wheat allergy in atopic dermatitis. *Allergy* 2000; 55:386–391.
- 12 Palosuo K, Varjonen E, Kekki OM, *et al.* Wheat ω -5 gliadin is a major allergen in children with immediate allergy to ingested wheat. *J Allergy Clin Immunol* 2001; 108:634–638.
- 13 Niggemann B, Reibel S, Roehr CC, *et al.* Predictors of positive food challenge outcome in non-IgE-mediated reactions to food in children with atopic dermatitis. *J Allergy Clin Immunol* 2001; 108:1053–1058.
- This paper provides evidence that non-IgE-mediated mechanisms are responsible for about 10% of positive oral food challenges in children, and for over 20% of positive challenge reactions to wheat.
- 14 Yokota S, Tsubaki K, Shimizu H, *et al.* Study of immune-responsiveness to wheat antigen by IgG, IgA, and IgE immunoblotting with sera from patients with atopic dermatitis. *Acta Derm Venereol Suppl* 1992; 176:45–48.
- 15 Varjonen E, Savolainen J, Mattila L, Kalimo K. IgE-binding components of wheat, rye, barley and oats recognized by immunoblotting analysis with sera from adult atopic dermatitis patients. *Clin Exp Allergy* 1994; 24:481–489.
- 16 Simonato B, De Lazzari F, Pasini G, *et al.* IgE binding to soluble and insoluble wheat flour proteins in atopic and non-atopic patients suffering from gastrointestinal symptoms after wheat ingestion. *Clin Exp Allergy* 2001; 31:1771–1778.
- 17 Romano A, Di Fonso M, Giuffreda F, *et al.* Food-dependent exercise-induced anaphylaxis: clinical and laboratory findings in 54 subjects. *Int Arch Allergy Immunol* 2001; 125:264–272.
- 18 Aihara M, Miyazawa M, Osuna H, *et al.* Food-dependent exercise-induced anaphylaxis: influence of concurrent aspirin administration on skin testing and provocation. *Br J Dermatol* 2002; 146:466–472.

- 19 Lehto M, Palosuo K, Varjonen E, *et al.* Humoral and cellular responses to gliadin in wheat-dependent, exercise-induced anaphylaxis. *Clin Exp Allergy* 2003; 33:90–95.
- This study suggests that aberrant regulation of allergen-specific, cell-mediated immune responses may play a role in the pathogenesis of wheat-dependent, exercise-induced anaphylaxis.
- 20 Kidd JM, Cohen SH, Sosman AJ, Fink JN. Food-dependent exercise-induced anaphylaxis. *J Allergy Clin Immunol* 1983; 71:407–411.
- 21 Varjonen E, Vainio E, Kalimo K. Life-threatening anaphylaxis caused by allergy to gliadin and exercise. *Clin Exp Allergy* 1997; 27:162–166.
- 22 Palosuo K, Alenius H, Varjonen E, *et al.* A novel wheat gliadin as a cause of exercise-induced anaphylaxis. *J Allergy Clin Immunol* 1999; 103:912–917.
- 23 Osborne TB. The vegetable proteins. London: Longmans Green; 1924. pp. 1–151.
- 24 Shewry PR, Halford NG. Cereal seed storage proteins: structures, properties and role in grain utilization. *J Exp Bot* 2002; 53:947–958.
- 25 Shewry PR, Tatham AS. The prolamin storage proteins of cereal seeds: structure and evolution. *Biochem J* 1990; 267:1–12.
- 26 Shewry PR, Napier JN, Tatham AS. Seed storage proteins: structure and biosynthesis. *The Plant Cell* 1995; 7:945–956.
- 27 Walsh BJ, Wrigley CW, Musk AW, Baldo BA. A comparison of the binding of IgE in the sera of patients with bakers' asthma to soluble and insoluble wheat-grain proteins. *J Allergy Clin Immunol* 1985; 76:23–28.
- 28 Weiss W, Vogelmeier C, Gorg A. Electrophoretic characterization of wheat grain allergens from different cultivars involved in bakers' asthma. *Electrophoresis* 1993; 14:805–816.
- 29 Sandiford CP, Tatham AS, Fido R, *et al.* Identification of the major water/salt insoluble wheat proteins involved in cereal hypersensitivity. *Clin Exp Allergy* 1997; 27:1120–1129.
- 30 Maruyama N, Ichise K, Katsube T, *et al.* Identification of major wheat allergens by means of the *Escherichia coli* expression system. *Eur J Biochem* 1998; 255:739–745.
- 31 Fränken J, Stephan U, Meyer HE, König W. Identification of α -amylase inhibitor as a major allergen of wheat flour. *Int Arch Allergy Immunol* 1994; 104:171–174.
- 32 Posch A, Weiss W, Wheeler C, *et al.* Sequence analysis of wheat grain allergens separated by two-dimensional electrophoresis with immobilized pH gradients. *Electrophoresis* 1995; 16:1115–1119.
- 33 Sanchez-Monge R, Garcia-Casado G, Lopez-Otin C, *et al.* Wheat flour peroxidase is a prominent allergen associated with Baker's asthma. *Clin Exp Allergy* 1997; 27:1130–1137.
- 34 Yamashita H, Nanba Y, Onishi M, *et al.* Identification of a wheat allergen, Tri a Bd 36K, as a peroxidase. *Biosci Biotechnol Biochem* 2002; 66:2487–2490.
- 35 Weiss W, Huber G, Engel KH, *et al.* Identification and characterization of wheat grain albumin/globulin allergens. *Electrophoresis* 1997; 18:826–833.
- 36 Sander I, Flagge A, Merget R, *et al.* Identification of wheat flour allergens by means of 2-dimensional immunoblotting. *J Allergy Clin Immunol* 2001; 107:907–913.
- 37 Räsänen L, Lehto M, Turjanmaa K, *et al.* Allergy to ingested cereals in atopic children. *Allergy* 1994; 49:871–876.
- 38 Varjonen E, Vainio E, Kalimo K, *et al.* Skin-prick test and RAST responses to cereals in children with atopic dermatitis: characterization of IgE-binding components in wheat and oats by an immunoblotting method. *Clin Exp Allergy* 1995; 25:1100–1107.
- 39 Jones SM, Magnolfi CF, Cooke SK, Sampson HA. Immunologic cross-reactivity among cereal grains and grasses in children with food hypersensitivity. *J Allergy Clin Immunol* 1995; 96:341–351.
- 40 James JM, Sixbey JP, Helm RM, *et al.* Wheat alpha-amylase inhibitor: a second route of allergic sensitization. *J Allergy Clin Immunol* 1997; 99:239–244.
- 41 Takizawa T, Arakawa H, Tokuyama K, Morikawa A. Identification of allergen fractions of wheat flour responsible for anaphylactic reactions to wheat products in infants and young children. *Int Arch Allergy Immunol* 2001; 125:51–56.
- 42 Armentia A, Rodriguez R, Callejo A, *et al.* Allergy after ingestion or inhalation of cereals involves similar allergens in different ages. *Clin Exp Allergy* 2002; 32:1216–1222.
- 43 Morita E, Kameyoshi Y, Mihara S, *et al.* gamma-Gliadin: a presumptive allergen causing wheat-dependent exercise-induced anaphylaxis. *Br J Dermatol* 2001; 145:182–184.
- 44 Palosuo K, Alenius H, Varjonen E, *et al.* Rye γ -70 and γ -35 secalins and barley γ -3 hordein cross-react with ω -5 gliadin, a major allergen in wheat-dependent, exercise-induced anaphylaxis. *Clin Exp Allergy* 2001; 31:466–473.
- 45 Palosuo K, Varjonen E, Nurkkala J, *et al.* Transglutaminase-mediated cross-linking of a peptic fraction of ω -5 gliadin enhances IgE reactivity in wheat-dependent, exercise-induced anaphylaxis. *J Allergy Clin Immunol* (in press).
- 46 Sandiford CP, Tee RD, Newman-Taylor AJ. Identification of crossreacting wheat, rye, barley and soya flour allergens using sera from individuals with wheat-induced asthma. *Clin Exp Allergy* 1995; 25:340–349.
- 47 Sampson HA, Ho DG. Relationship between food-specific IgE concentrations and the risk of positive food challenges in children and adolescents. *J Allergy Clin Immunol* 1997; 100:444–451.
- 48 Sampson HA. Utility of food-specific IgE concentrations in predicting symptomatic food allergy. *J Allergy Clin Immunol* 2001; 107:891–896.