

Wheat ω -5 gliadin is a major allergen in children with immediate allergy to ingested wheat

Kati Palosuo, MD,^{a,b} Elina Varjonen, MD,^a Outi-Maria Kekki, MD,^c Timo Klemola, MD,^a Nisse Kalkkinen, PhD,^d Harri Alenius, PhD,^b and Timo Reunala, MD^{a,c}

Helsinki and Tampere, Finland

Background: Sensitization to wheat by ingestion can lead to food allergy symptoms and wheat-dependent, exercise-induced anaphylaxis. Sensitization by inhalation causes bakers' asthma and rhinitis. Wheat allergens have been characterized at the molecular level in bakers' asthma and in wheat-dependent, exercise-induced anaphylaxis, in which ω -5 gliadin (Tri a 19) is a major allergen. However, little information is available regarding allergens responsible for hypersensitivity reactions to ingested wheat in children.

Objective: The aim of this study was to examine whether children with allergy to ingested wheat have IgE antibodies to ω -5 gliadin. **Methods:** Sera were obtained from 40 children (mean age, 2.5 years; range, 0.7–8.2 years) with suspected wheat allergy who presented with atopic dermatitis and/or gastrointestinal and/or respiratory symptoms. Wheat allergy was diagnosed with open or double-blinded, placebo-controlled oral wheat challenge.

Wheat ω -5 gliadin was purified by reversed-phase chromatography, and serum IgE antibodies to ω -5 gliadin were measured by means of ELISA. In vivo reactivity was studied by skin prick testing. Control sera were obtained from 22 children with no evidence of food allergies.

Results: In oral wheat challenge, 19 children (48%) reacted with immediate and 8 children (20%) with delayed hypersensitivity symptoms. Sixteen (84%) of the children with immediate symptoms had IgE antibodies to purified ω -5 gliadin in ELISA. In contrast, IgE antibodies to ω -5 gliadin were not detected in any of the children with delayed or negative challenge test results or in the control children. The diagnostic specificity and positive predictive value of ω -5 gliadin ELISA were each 100% for immediate challenge reactions. Skin prick testing with ω -5 gliadin was positive in 6 of 7 children with immediate challenge symptoms and negative in 2 children with delayed challenge symptoms.

Conclusion: The results of this study show that ω -5 gliadin is a

significant allergen in young children with immediate allergic reactions to ingested wheat. IgE testing with ω -5 gliadin could be used to reduce the need for oral wheat challenges in children. (*J Allergy Clin Immunol* 2001;108:634–8.)

Key words: Wheat, gliadin, food hypersensitivity, IgE antibody, allergens, enzyme-linked immunosorbent assay

Food allergy plays a pathogenetic role in up to 40% of infants and small children with moderate to severe atopic dermatitis (AD).^{1,2} In addition to cow's milk, egg, soy, peanut, and fish, wheat is one of the most common foods causing allergy in children.^{3,4} Sensitization to ingested allergens can lead to various clinical manifestations, including cutaneous, gastrointestinal, and respiratory symptoms of immediate or delayed onset.^{5,6} In adults, sensitization to wheat and related cereals usually occurs via inhalation, causing bakers' asthma and rhinitis, a well-defined IgE-mediated allergy to inhaled flour primarily seen in patients with occupational exposure.⁷ Wheat-dependent, exercise-induced anaphylaxis is a life-threatening allergy in adults, whereby ingestion of wheat in connection with physical exercise induces symptoms of anaphylaxis.^{8,9}

In our previous study, we identified and characterized ω -5 gliadin (Tri a 19) as a major allergen in wheat-dependent, exercise-induced anaphylaxis.^{9,10} In bakers' asthma, water-soluble proteins—ie, members of the α -amylase inhibitor family,¹¹ acyl-CoA oxidase, fructose-bisphosphatase aldolase,^{12,13} and peroxidase¹⁴—have been identified as important wheat allergens. In addition, binding of IgE antibodies to water-insoluble gliadins has been reported in patients with bakers' asthma.^{15,16} James et al¹⁷ identified wheat α -amylase inhibitor as a relevant allergen in 5 children with ingested wheat allergy, suggesting that it represents a sensitizing allergen both after ingestion and after inhalation. Immunoblotting studies have shown that IgE antibodies from children with hypersensitivity to ingested wheat bind to a number of water-soluble wheat proteins of different molecular weights.^{17–20} A few studies suggest that gliadins might be important allergens in wheat-allergic children with AD.^{21,22} However, these various IgE-binding proteins have not been characterized at the molecular level, and their clinical significance remains unknown.

The aim of this study was to examine whether ω -5 gliadin is a clinically relevant allergen in children with hypersensitivity reactions to ingested wheat.

From ^athe Department of Dermatology, University of Helsinki and Hospital for Skin and Allergic Diseases; ^bthe Finnish Institute of Occupational Health; ^cthe Department of Dermatology, University and University Hospital of Tampere; and ^dthe Institute of Biotechnology, University of Helsinki. Supported by grants from the Finnish Allergy Research Foundation, the Finnish Medical Society, and the Finnish Society of Allergology and Immunology.

Received for publication April 13, 2001; revised July 2, 2001; accepted for publication July 9, 2001.

Reprint requests: Kati Palosuo, MD, Laboratory of Immunotoxicology, Finnish Institute of Occupational Health, Topeliuksenkatu 41 b, 00250 Helsinki, Finland.

Copyright © 2001 by Mosby, Inc
0091-6749/2001 \$35.00 + 0 1/87/118602
doi:10.1067/mai.2001.118602

Abbreviations used

ACN: Acetonitrile
AD: Atopic dermatitis
SPT: Skin prick test
TFA: Trifluoroacetic acid

METHODS

Patients

Serum samples were collected from 40 children (mean age, 2.5 years; range, 0.7-8.2 years) who had been referred to the Helsinki University Hospital for Skin and Allergic Diseases ($n = 29$) or to the Tampere University Hospital ($n = 11$) for evaluation of suspected food allergy. The clinical histories of all 40 children were suggestive of wheat allergy, and at initial examination 23 of them had a positive skin prick test (SPT) result to wheat (1:10 w/v 0.9% NaCl, prepared as previously described⁸) and 25 of them had a positive wheat CAP RAST result (>0.35 KU/L; CAP RAST, Pharmacia, Uppsala, Sweden). Other suspected foods were cow's milk in 26 (65%), egg in 16 (40%), and cereals other than wheat in 23 (58%) children. All but 1 of the children presented with moderate to severe AD; 29% had gastrointestinal symptoms, and 12% had respiratory symptoms. Twenty-nine percent had histories of asthma, and 15% had allergic rhinitis.

Control serum samples were collected from 22 children (mean age, 10.6 years; range, 6.3-14.9 years) with no evidence of food allergy and negative SPT results to cereals. The Ethical Committees of the 2 hospitals approved the study, and informed consent was obtained from the parents.

Oral wheat challenge

Physician-supervised, open oral wheat challenges were performed in 27 children, and double-blinded, placebo-controlled wheat challenges were performed in 11 children. Two of the children were not challenged because of recent histories of severe anaphylaxis to ingested wheat. Before the challenge, each patient was instructed to strictly avoid wheat for 2 to 4 weeks.

The open challenge began with the application of a drop of wheat porridge (10% wheat, cooked in water) on intact facial skin and the upper lip. During the first day, wheat was given orally in increasing amounts (1 g, 5 g, and 10 g) at 1-hour intervals; the challenge was then continued with at least 20 g of wheat per day for 1 week. In the double-blinded, placebo-controlled wheat challenges, the placebo formula was amino acid-derived Neocate (SHS International Ltd, Liverpool, United Kingdom) and the test formula contained 5 g of wheat flour in 100 mL Neocate. On day 1, the formula was administered at 30-minute intervals (1 mL, 5 mL, 10 mL, 25 mL, and 59 mL); if no adverse reactions appeared, the challenge was continued for 1 week, the daily intake being 100 mL. On day 8, the second test formula was started; the same challenge protocol was used. In both challenge procedures, the supervising physician examined the child on appearance of any symptoms. The challenge was stopped when objective signs and symptoms indicated a positive response.

Purification of wheat ω -5 gliadin

Wheat ω -5 gliadin was purified as described previously in detail.⁹ Wheat grains (*Triticum aestivum*; cultivar, Tjalve) were ground and extracted with 50% acetonitrile (ACN)-0.1% trifluoroacetic acid (TFA). The sample was centrifuged, and the supernatant was collected, filtered, and concentrated to one third in a vacuum centrifuge, which resulted in precipitation of the ACN-soluble proteins. The precipitate was dissolved in 10% ACN-0.1% TFA, and ω -5 gliadin was purified from this extract by reversed-phase chromatography on a Resource RPC 3-mL column (Pharmacia) through use of a linear

gradient of ACN (10% to 60% in 60 minutes) in 0.1% TFA. Monitoring was done at 280 nm, and the fractions were collected and analyzed by SDS-PAGE and Coomassie blue staining.

IgE ELISA

ELISA was performed as described previously.²³ The concentration of ω -5 gliadin was adjusted to 2 μ g/mL for coating microtiter plates (100 μ L/well). The wells were then postcoated with 1% human serum albumin in 50 mmol/L sodium carbonate buffer, and 100 μ L patient or control serum (diluted 1:10) was added. Biotinylated goat antihuman IgE (Vector, Burlingame, Calif; diluted 1:1000) was added; this was followed by streptavidin-conjugated alkaline phosphatase (Zymed, San Francisco, Calif; diluted 1:3000) and color substrate (Bio-Rad, Hercules, Calif). The color formed was read at 405 nm with an automated ELISA reader (Titertek Multiscan, Eflab, Turku, Finland). The mean optical density value plus 3 SDs of the control sera was used as the cutoff limit for positivity.

Skin prick tests

SPT suspensions for crude wheat (1:10 w/v 0.9% NaCl) were prepared as described previously.⁸ SPT with purified ω -5 gliadin was performed at 3 different concentrations (500, 50, and 5 μ g/mL in 20% ethanol-PBS) in 7 children with immediate challenge symptoms and 2 children with delayed symptoms. Histamine dihydrochloride (10 mg/mL, ALK, Copenhagen, Denmark) was used as a positive control and 20% ethanol-PBS and physiologic saline solution were used as negative controls. A wheal diameter at least half that of the histamine reaction was considered positive.

RESULTS

Oral wheat challenge

Nineteen children (48%) reacted with immediate hypersensitivity symptoms, including urticaria, erythema, and upper and lower respiratory and abdominal symptoms (Table I). One child experienced an anaphylactic reaction requiring emergency treatment. Eight children (20%) reacted with delayed symptoms, showing flare-up AD and/or diarrhea (Table I). Thirteen children (32%) remained challenge-negative.

IgE antibodies to ω -5 gliadin in ELISA

Sixteen children (84%) with immediate challenge symptoms had IgE antibodies to ω -5 gliadin in ELISA (Fig 1 and Table I). In contrast, IgE antibodies to ω -5 gliadin were not detected in the 8 children with delayed symptoms, in the 13 children with a negative challenge, or in the 22 control children.

Skin prick tests with ω -5 gliadin

SPTs performed with purified ω -5 gliadin were positive in 6 of the 7 children with immediate symptoms at all 3 concentrations tested (Table I). The 2 children with delayed symptoms had negative SPT results to ω -5 gliadin. All 6 children with positive SPT results also had IgE antibodies to ω -5 gliadin in ELISA, and the 3 children with negative SPT results were negative in ELISA.

Predictive capacity of ELISA, CAP RAST, and SPT

The sensitivity, specificity, and positive and negative predictive values of the various tests in predicting immedi-

TABLE I. Wheat challenge, ELISA, CAP RAST, and SPT results in 40 children with suspected wheat allergy

Patient sex/age (y)	Oral wheat challenge			Skin prick test (diameter in mm)			
	Symptoms	Time	IgE* ELISA ω-5 gliadin (2 μg/mL)	Wheat CAP RAST (KU/L)	ω-5 gliadin 500/50/5 (μg/mL)	Wheat 1:10	Histamine 10 mg/mL
	Immediate						
M/0.9	Urticaria	5 min	3.64	200.0	—	6	5
M/7.0	ND (history of anaphylaxis)		1.35	95.8	—	13	5
F/4.8	ND (history of anaphylaxis)		1.16	87.9	—	6	5
M/3.1	Urticaria, erythema	10 min	0.84	96.8	—	7	7
M/1.1	Urticaria, facial swelling	30 min	0.83	37.8	5/4/3	14	5
M/0.9	Erythema, pruritus	45 min	0.77	94.4	5/3/2	3	4
M/6.1	Anaphylaxis	10 min	0.71	387.0	—	8	5
M/3.3	Urticaria, dyspnea	25 min	0.48	280.0	8/5/3	6	4
M/1.3	Urticaria, erythema, pruritus	5 min	0.46	89.1	6/5/4	7	5
M/5.8	Pharyngeal pruritus, nasal congestion	5 min	0.31	> 400	5/5/3	5	5
M/4.5	Conjunctival pruritus, rhinorrhea, sneezing	15 min	0.31	286.0	5/4/3	12	5
M/2.2	Erythema, pruritus	5 min	0.19	49.6	—	8	5
F/0.7	Urticaria	10 min	0.14	8.5	—	5	5
F/2.5	Urticaria, vomiting†	5 min	0.14	84.2	—	5	6
M/1.5	Urticaria†	5 min	0.08	32.9	—	0	6
M/2.9	Erythema, pruritus	30 min	0.05	7.5	—	5	5
F/2.4	Erythema, abdominal pain	20 min	<0.04	<0.35	—	0	4
F/2.2	Erythema, pruritus, rhinorrhea, sneezing	10 min	<0.04	9.4	0	5	5
M/1.1	Urticaria	40 min	<0.04	3.7	—	6	5
	Delayed						
F/1.1	Atopic dermatitis†	5 days	<0.04	<0.35	—	0	4
F/1.5	Atopic dermatitis†	2 days	<0.04	8.8	—	0	5
M/1.8	Atopic dermatitis, diarrhea†	3 days	<0.04	<0.35	—	0	3
M/3.8	Atopic dermatitis†	3 days	<0.04	<0.35	—	0	5
F/8.2	Atopic dermatitis, diarrhea	5 days	<0.04	0.7	0	5	5
M/2.2	Atopic dermatitis	3 days	<0.04	<0.35	—	0	5
M/6.5	Atopic dermatitis	3 days	<0.04	<0.35	0	0	5
M/2.3	Diarrhea	2 days	<0.04	<0.35	—	0	5
	None						
M/1.9	Negative†		<0.04	<0.35	—	3	4
M/0.9	Negative†		<0.04	<0.35	—	0	5
F/1.0	Negative†		<0.04	<0.35	—	0	5
M/1.9	Negative†		<0.04	0.7	—	0	5
F/2.3	Negative†		<0.04	0.5	—	5	5
F/0.8	Negative		<0.04	33.7	—	3	4
M/2.0	Negative		<0.04	68.9	—	7	5
M/0.7	Negative		<0.04	3.0	—	5	5
F/1.3	Negative		<0.04	<0.35	—	0	5
F/1.8	Negative		<0.04	<0.35	—	0	5
M/1.3	Negative		<0.04	<0.35	—	0	5
F/2.4	Negative		<0.04	<0.35	—	0	5
F/1.0	Negative		<0.04	<0.35	—	0	6

ND, Not done.

*In ELISA absorbancy units at 405 nm; cutoff limit for positivity, ≥ 0.04 .

†Double-blinded, placebo-controlled challenge.

ate challenge reactions are presented in Table II. The specificity and positive predictive value of ω-5 gliadin ELISA were each 100%; this compared with 67% and 72% for wheat CAP RAST and 71% and 74% for wheat SPT (Table II). None of the 13 wheat challenge-negative children had IgE antibodies to ω-5 gliadin in ELISA, but 5 (38%) of these children had positive wheat CAP RAST results and 5 (38%) showed positive SPT results to wheat (Table I).

DISCUSSION

The results of this study show that the presence of IgE antibodies to ω-5 gliadin, a major allergen in wheat-dependent, exercise-induced anaphylaxis, associates strongly with immediate symptoms on oral wheat challenge in young children with food allergy symptoms. On the contrary, IgE antibodies to ω-5 gliadin were not

detected in children with delayed or no symptoms in oral wheat challenge. This suggests that ω -5 gliadin is a potent sensitizer not only in adults with exercise-induced anaphylaxis⁹ but also in children with IgE-mediated immediate hypersensitivity to ingested wheat.

The finding that the same allergen—ie, ω -5 gliadin—triggers production of IgE antibodies both in children with wheat allergy and in adults with exercise-induced anaphylaxis is of interest. Although both conditions represent allergies to ingested wheat, the clinical manifestations differ clearly and the underlying pathophysiologic mechanisms appear to be different.⁵ The wheat-allergic children have cutaneous, gastrointestinal, or respiratory food allergy symptoms and present mainly with AD,⁶ whereas the adults experience recurrent episodes of anaphylaxis only during exercise.⁹ Most children outgrow their sensitivity to wheat,² and it remains to be seen whether IgE reactivity to ω -5 gliadin subsides together with clinical reactivity. In contrast, symptoms of wheat-dependent, exercise-induced anaphylaxis do not appear until the onset of adulthood and tend to persist thereafter.^{8,9}

Gliadins are the major storage proteins of the wheat grain.²⁴ They are a polymorphic group of ethanol-soluble proteins that contain regions of repeated sequences consisting of proline- and glutamine-rich peptide motifs.^{25,26} Gliadins can be classified into α , γ , and ω groups on the basis of their electrophoretic mobility. In contrast to the sulfur-rich α and γ gliadins, ω -gliadins consist almost entirely of repeats and are characterized by a low content of sulfur amino acids and a lack of cysteine residues.²⁶ ω -5 gliadin seems to differ from other ω gliadins in its higher proportion of glutamine and its lower proportion of proline.²⁷

Physician-supervised oral food challenge is considered the only reliable means of diagnosing food allergy,² though attempts have been made to develop diagnostic tests predictive of clinical reactivity. Several studies have correlated SPT wheal sizes^{3,28,29} or specific IgE levels^{3,21,29,30} with results of oral food challenges. Sampson and Ho³⁰ measured concentrations of food-specific IgE with the CAP System FEIA for egg, milk, peanut, and fish; they could thereby predict clinical reactivity with greater than 95% certainty. However, for wheat this test was too nonspecific and a predictive value of 90% could not be reached.³⁰ This could be explained by the use of antigen extracts containing wheat proteins mainly from the water/salt-soluble albumin and globulin fractions rather than from the ethanol-soluble gliadin fraction, which seems to hold the clinically relevant allergens in ingested wheat allergy.²² In contrast, patients with allergy to inhaled wheat—ie, bakers' asthma and rhinitis—react mainly to the water-soluble proteins and are usually able to consume wheat without symptoms.⁷

Jones et al¹⁹ demonstrated clinically insignificant cross-reactivity between the water/salt-soluble proteins of cereal grains and grasses, accounting for the frequently observed false positive IgE reactions to wheat and other cereals in grass pollen-allergic patients. In the same study, approximately 20% of patients with cereal allergy reacted in double-blinded, placebo-controlled

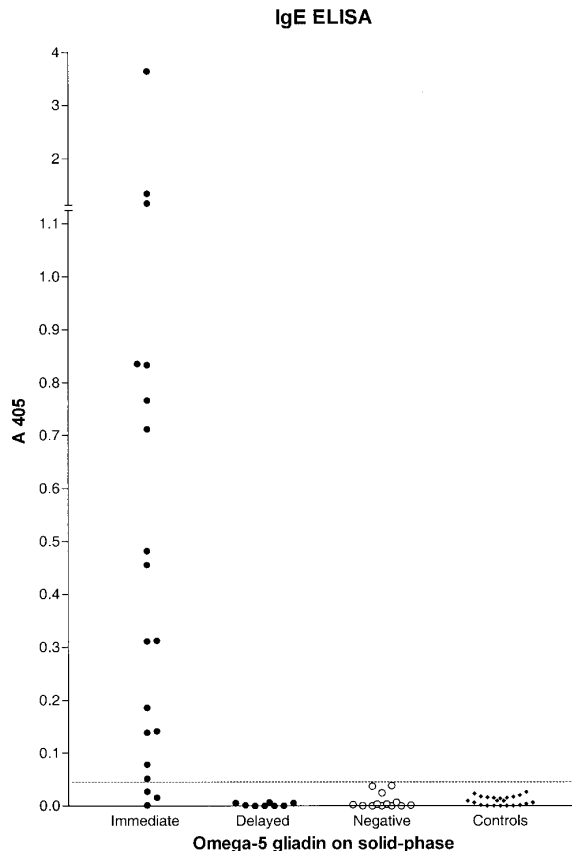


FIG 1. IgE antibody levels to purified ω -5 gliadin in ELISA (absorbance units) in 19 children with immediate, 8 children with delayed, and 13 children with no symptoms in oral wheat challenge and in 22 controls.

TABLE II. Predictive capacity of ω -5 gliadin ELISA, wheat CAP RAST and wheat SPT for immediate positive challenge reactions in 40 children with suspected wheat allergy

Test	Sensitivity* (%)	Specificity† (%)	Positive predictive value‡ (%)	Negative predictive value§ (%)
ω -5 gliadin ELISA (>0.04 AU)	84	100	100	88
Wheat CAP RAST (>0.35 KU/L)	95	67	72	93
Wheat SPT wheal diameter (3 mm)	89	71	74	88

*Proportion of true positives detected.

†Proportion of true negatives detected.

‡Proportion of symptomatic individuals among test positives.

§Proportion of nonsymptomatic individuals among test negatives.

food challenges to more than 1 cereal.¹⁹ Previously, we have shown that rye γ -70 and γ -35 secalins and barley γ -3 hordein cross-react with wheat ω -5 gliadin in patients with wheat-dependent, exercise-induced anaphylaxis.¹⁰ Further studies will determine whether the binding of

IgE antibodies to these cross-reacting rye and barley allergens correlates with clinical reactivity in children.

In the present study, the sensitivity of wheat CAP RAST in predicting immediate positive challenge reactions was 95%; its specificity was 67%, and its positive predictive value was 72%. Similarly, the sensitivity of wheat SPT was 89%, its specificity 71%, and its positive predictive value 74%. However, the presence of IgE antibodies to purified ω -5 gliadin in ELISA or a positive SPT was invariably associated with immediate symptoms on oral wheat challenge. No false positive reactions were seen, and a specificity of 100% as well as a positive predictive value of 100% was reached. Sixteen of the 19 children with immediate challenge symptoms showed IgE to ω -5 gliadin in ELISA, giving this assay a sensitivity of 84%. The children with delayed hypersensitivity reactions were negative for ω -5 gliadin, a finding that could be due to the presence of non-IgE-mediated immune mechanisms or to the binding of IgE antibodies to different wheat proteins in this subset of patients.^{21,31}

Oral food challenges carry the risk of provoking serious, even life-threatening reactions in children with immediate hypersensitivity.³² In this study, high levels of IgE antibodies to ω -5 gliadin were detected in 2 children with recent histories of anaphylaxis to ingested wheat and in 1 child who experienced an anaphylactic reaction during the wheat challenge. Detection of IgE antibodies to ω -5 gliadin might offer a specific diagnostic test that would reduce the need for potentially dangerous oral wheat challenges, especially in children at high risk of serious reactions.

In conclusion, this study showed that ω -5 gliadin is a major allergen in children with immediate hypersensitivity to ingested wheat. Patients from other populations must be examined to confirm the results of this study. Further studies are also needed to clarify the significance of other gliadins and cereal prolamins as allergens in hypersensitivity to ingested wheat.

We thank Mrs Leena Petman for expert skin prick testing and Mrs Sari Tillander for technical assistance. We are grateful to Laura Linkosalo, MD, for the control sera.

REFERENCES

- Eigenmann PA, Sicherer SH, Borkowski TA, Cohen BA, Sampson HA. Prevalence of IgE-mediated food allergy among children with atopic dermatitis. *Pediatrics* 1998;101:E8.
- Sicherer SH, Sampson HA. Food hypersensitivity and atopic dermatitis: pathophysiology, epidemiology, diagnosis, and management. *J Allergy Clin Immunol* 1999;104:114-22.
- Sampson HA, Albergro R. Comparison of results of skin tests, RAST, and double-blind, placebo-controlled food challenges in children with atopic dermatitis. *J Allergy Clin Immunol* 1984;74:26-33.
- Sicherer SH, Morrow EH, Sampson HA. Dose-response in double-blind, placebo-controlled oral food challenges in children with atopic dermatitis. *J Allergy Clin Immunol* 2000;105:582-6.
- Sampson HA. Food allergy. Part 1: immunopathogenesis and clinical disorders. *J Allergy Clin Immunol* 1999;103:717-28.
- Sicherer SH. Determinants of systemic manifestations of food allergy. *J Allergy Clin Immunol* 2000;106:251-7.
- Baur X, Posch A. Characterized allergens causing bakers' asthma. *Allergy* 1998;53:562-6.
- Varjonen E, Vainio E, Kalimo K. Life-threatening, recurrent anaphylaxis caused by allergy to gliadin and exercise. *Clin Exp Allergy* 1997;27:162-6.
- Palosuo K, Alenius H, Varjonen E, Koivuluhta M, Mikkola J, Keskinen H, et al. A novel wheat gliadin as a cause of exercise-induced anaphylaxis. *J Allergy Clin Immunol* 1999;103:912-7.
- Palosuo K, Alenius H, Varjonen E, Kalkkinen N, Reunala T. 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;31:466-73.
- 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;104:171-4.
- Posch A, Weiss W, Wheeler C, Dunn MJ, Gorg A. Sequence analysis of wheat grain allergens separated by two-dimensional electrophoresis with immobilized pH gradients. *Electrophoresis* 1995;16:1115-9.
- Weiss W, Huber G, Engel KH, Pethran A, Dunn MJ, Gooley AA, et al. Identification and characterization of wheat grain albumin/globulin allergens. *Electrophoresis* 1997;18:826-33.
- Sanchez-Monge R, Garcia-Casado G, Lopez-Otin C, Armentia A, Salcedo G. Wheat flour peroxidase is a prominent allergen associated with bakers' asthma. *Clin Exp Allergy* 1997;27:1130-7.
- 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;76:23-8.
- Sandiford CP, Tatham AS, Fido R, Welch JA, Jones MG, Tee RD, et al. Identification of the major water/salt insoluble wheat proteins involved in cereal hypersensitivity. *Clin Exp Allergy* 1997;27:1120-9.
- James JM, Sixbey JP, Helm RM, Bannon GA, Burks AW. Wheat alpha-amylase inhibitor: a second route of allergic sensitization. *J Allergy Clin Immunol* 1997;99:239-44.
- Sutton R, Hill DJ, Baldo BA, Wrigley CW. Immunoglobulin E antibodies to ingested cereal flour components: studies with sera from subjects with asthma and eczema. *Clin Allergy* 1982;12:63-74.
- 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;96:341-51.
- Varjonen E, Vainio E, Kalimo K, Juntunen-Backman K, Savolainen J. 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;25:1100-7.
- Räsänen L, Lehto M, Turjanmaa K, Savolainen J, Reunala T. Allergy to ingested cereals in atopic children. *Allergy* 1994;49:871-6.
- Varjonen E, Vainio E, Kalimo K. Antigliadin IgE-indicator of wheat allergy in atopic dermatitis. *Allergy* 2000;55:386-91.
- Alenius H, Kalkkinen N, Yip E, Hasmin H, Turjanmaa K, Mäkinen-Kiljunen S, et al. Significance of rubber elongation factor as a latex allergen. *Int Arch Allergy Immunol* 1996;109:362-8.
- Mifflin BJ, Field JM, Shewry PR. Cereal storage proteins and their effect on technological properties. In: Daussant J, Mossé J, Vaughan J, editors. Seed proteins. London: Academic Press; 1983. p. 255-319.
- Bietz JA. Cereal prolamin evolution and homology revealed by sequence analysis. *Biochem Genet* 1982;20:1039-53.
- Shewry PR, Napier JN, Tatham AS. Seed storage proteins: Structure and biosynthesis. *Plant Cell* 1995;7:945-56.
- Kasarda D, Autran J, Lew E, Nimmo C, Shewry P. N-terminal amino acid sequences of ω -gliadins and ω -secalins: implications for the evolution of prolamin genes. *Biochim Biophys Acta* 1983;747:138-50.
- Sporik R, Hill DJ, Hosking CS. Specificity of allergen skin testing in predicting positive open food challenges to milk, egg and peanut in children. *Clin Exp Allergy* 2000;30:1541-6.
- Majamaa H, Moisio P, Holm K, Turjanmaa K. Wheat allergy: diagnostic accuracy of skin prick and patch tests and specific IgE. *Allergy* 1999;54:851-6.
- 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;100:444-51.
- Bengtsson U, Nilsson-Balknas U, Hanson LA, Ahlstedt S. Double blind, placebo controlled food reactions do not correlate to IgE allergy in the diagnosis of staple food related gastrointestinal symptoms. *Gut* 1996;39:130-5.
- Reibel S, Rohr C, Ziegert M, Sommerfeld C, Wahn U, Niggemann B. What safety measures need to be taken in oral food challenges in children? *Allergy* 2000;55:940-4.