参　考

プリックテスト

プリックテストは即時型アレルギーに適応される検査である。方法は、注射針などにより少量のアレルゲンを皮膚組織に暴露させ、15 分後に出現した膨疹径を測定し判定する。

1）適応疾患

花粉症・鼻アレルギー、喘息、アレルギー性結膜炎、食物アレルギー・OAS、ラテックスアレルギー、即時型薬剤アレルギー、アトピー性皮膚炎等が挙げられる。

2）アレルゲンの準備

市販のエキス（アレルゲンスクラッチエキス「トリイ」（鳥居薬品）など）や、新鮮な野菜・果物（そのもの）を用いる。ラテックスなどは抽出液を作製する。粗抗原抽出物に加え、近年はリコンビナント抗原（たとえば、ラテックスの Hev b 6 やシラカンバの主要抗原である Bet v 1）を用いる検査も可能である（*BIOMAY Produktions-und Handels AG Vienna Competence Center, Austria,* URL: http://www.biomay.com/）。試薬は、1、10、100 μg/ml と希釈系列を作製し検査を施行する。薬剤の場合は、既報告例の情報を確認し溶媒および至適濃度を確認し検査を実施する。

3）プリックテストの方法

テストは前腕屈側で行う。各抗原の間隔は 3 ㎝程あける。アレルゲンを 1 滴置き、注射針、バイファケイテッドニードル（東京エム・アイ商会）などでアレルゲンを静かに貫いて 1 度刺す。なお、本邦でこれまで使用されてきたプリックランセットは現在供給されていない。

4）プリックテストの判定

15 分後に膨疹の直径㎜（最長径とその中点に垂直な径の平均値）を測定する。対照液は陽性コントロールとして二塩酸ヒスタミン（和光純薬など（10 mg/ml に調製））、陰性コントロールとして生理食塩水や市販の対照液（アレルゲンスクラッチエキス対照液「トリイ」（鳥居薬品））などを用いる。ヒスタミンの 2 倍以上を 4 ＋、同等以上 2 倍未満を 3 ＋、2 分の 1 以上同等未満を 2 ＋、2 分の 1 より小さく生食より大きいものを 1 ＋、生食と同等を（－）と判定する。判定結果 2 ＋以上を陽性とする。誘発された膨疹の直径が 3 ㎜以上を陽性と判定してもよい。

付表 1
茶のしずく石鹸等に含まれた加水分解コムギ（グルパール 19S）による即時型コムギアレルギーの診断基準
（日本アレルギー学会化粧品中のタンパク加水分解物の安全性に関する特別委員会作成 2011 年 10 月 11 日 http://www.fa.kyorin.co.jp/jsa/jsa_0528_09.pdf）

【確実例】
以下の１、２、３をすべて満たす。
１．加水分解コムギ（グルパール 19S）を含有する茶のしずく石鹸等を使用したことがある。
２．以下のうち少なくとも一つの臨床症状があった。
　２－１）加水分解コムギ（グルパール 19S）を含有する茶のしずく石鹸等を使用して数分後から 30 分以内に、痒み、眼瞼浮腫、鼻汁、膨疹などが出現した。
　２－２）小麦製品摂取後４時間以内に痒み、膨疹、眼瞼浮腫、鼻汁、呼吸困難、悪心、嘔吐、腹痛、下痢、血圧低下などの全身症状がでた。
３．以下の検査で少なくとも一つ陽性を示す。
　３－１）グルパール 19S 0.1%溶液、あるいは、それより薄い溶液でプリックテストが陽性を示す。
　３－２）ドットブロット、ELISA、ウエスタンブロットなどの免疫学的方法により、血液中にグルパール 19S に対する特異的 IgE 抗体が存在することを証明できる。
　３－３）グルパール 19S を抗原とした好塩基球活性化試験が陽性である。

【否定できる基準】
４．グルパール 19S 0.1%溶液でプリックテスト陰性

【疑い例】
１、２を満たすが３を満たさない場合は疑い例となる。
＊ただし１、２を満たすが３を満たさない場合でも、血液特異的 IgE 抗体価検査やプリックテストでコムギまたはグルテンに対する感作が証明され、かつω -5 グリアジンに対する過敏性がないか、コムギおよびグルテンに対する過敏症よりも低い場合は強く疑われる例としてよい。

付表 2
小麦依存性運動誘発アナフィラキシー診断のための経口小麦負荷試験の実施例
（臨床皮膚科　62(5): 64-67, 2008 増刊号から引用）

【負荷項目】
① 小麦摂取
② 運動負荷
③ 小麦摂取＋運動負荷
④ アスピリン摂取
⑤ アスピリン摂取＋小麦摂取
⑥ アスピリン摂取＋小麦摂取＋運動負荷
注：小麦はうどんにて乾燥うどん重量 100 ～ 120g を摂取（年齢、症状により適宜増減）。
運動負荷はトレッドミルを使用し、Bruce 法で 4 ～ 5 段階、15 ～ 20 分負荷（適宜増減）。
アスピリンはアスピリン末 500 mg を内服。

【準備事項】
・文書にて試験の必要性、危険性を説明し、患者の同意、署名を得る。
・ICUスタッフに負荷試験を行う旨伝達し、必要時には対策を依頼する。
・試験当日、小麦除去食とする。試験前 6 時間は絶食するのが望ましい。
・負荷試験ではアナフィラキシーショックを起こす可能性があるため、静脈ルートを確保し、ヘパリン生食にてロックする。必要により、血液を採取、保存する。生食注、ボスミン注®、ポララミン注 5 mg®、ソル・コーテフ®を準備する。

【実施例】
［1 日目　小麦負荷］
1．乾燥うどん 120 g で作った素うどんを準備
2．ルートキープ（試験前採血）
3．うどん摂取（15 分以内）
4．食直後を 0 分とし採血開始（0、15、30、60、120 分で採血、適宜追加）、血清を分離し凍結保存
5．安静にして症状を観察、必要であれば写真撮影

［2 日目　運動負荷］
1．ルートキープ（試験前採血）
2．トレッドミル準備（心電図電極、血圧モニター装着）
3．運動負荷（Bruce 法、5 ～ 6 段階、15 ～ 20 分、患者に合わせて適宜増減）
試験中症状が確認されれば直ちに運動負荷を中止
4．運動終了直後を 0 分とし採血開始（0、15、30、60、120 分で採血、適宜追加）、血清を分離し凍結保存
5．運動負荷終了後は安静にして症状を観察、必要であれば写真撮影

［3日目　小麦＋運動負荷］
1．乾燥うどん 120g で作った素うどんを準備
2．ルートキープ（試験前採血）
3．うどん摂取（15 分以内）
4．トレッドミル準備（心電図電極、血圧モニター装着）
5．うどん食後 15 分を目標に運動負荷（Bruce 法、5 ～ 6 段階、15 ～ 20 分、患者に合わせて適宜増減）
　試験中症状が確認されれば直ちに運動負荷を中止
6．運動終了直後を 0 分とし採血開始（0、15、30、60、120 分で採血、適宜追加）、血清を分離し凍結保存
7．運動負荷終了後は安静にして症状を観察、必要であれば写真

［4日目　アスピリン負荷］
1．アスピリン末 500 mg を準備
2．ルートキープ（試験前採血）
3．アスピリン服用
4．安静にして症状を観察、必要であれば写真撮影
5．採血（30、60、90、120 分、適宜追加）、血清を分離し凍結保存

［5日目　アスピリン＋小麦負荷］
1．乾燥うどん 120g から作った素うどんおよびアスピリン 500 mg を準備
2．ルートキープ（試験前採血）
3．アスピリン服用
4．アスピリン服用後 30 分後にうどん摂取（15 分以内）
5．うどん食直後を 0 分とし採血開始（0、15、30、60、120 分で採血、適宜追加）、血清を分離し凍結保存
6．安静にして症状を観察、必要であれば写真撮影

［6日目　アスピリン＋小麦＋運動負荷］
1．乾燥うどん 120g から作った素うどんおよびアスピリン 500 mg を準備
2．ルートキープ（試験前採血）
3．アスピリン服用
4．アスピリン服用後 30 分後にうどん摂取（15 分以内）
5．トレッドミル準備（心電図電極、血圧モニター装着）
6．うどん食後 15 分を目標に運動負荷（Bruce 法、5 ～ 6 段階、15 ～ 20 分、患者に合わせて適宜増減）
　試験中症状が確認されれば直ちに運動負荷を中止
7．運動終了直後を 0 分とし採血開始（0、15、30、60、120 分で採血、適宜追加）、血清を分離し凍結保存
8．運動負荷終了後は安静にして症状を観察、必要であれば写真撮影

注：本実施例における採血は、経口負荷試験後に血中グリアジン濃度、あるいは血中のケミカルメディエーターを測定するために用いる。

付表 3

血清抗原特異的 IgE 検査

	単一アレルゲン測定			マルチパネルスクリーニング	
商品名	アラスタット 3gAllergy	イムノキャップ	オリトン IgE「ケミファ」	View アレルギー	マストイムノシステムズⅢ
製造元	シーメンスヘルスケア・ダイアグノスティックス(株)	ファディア(株)	日本ケミファ(株)	ファディア(株)	日立化成(株)
アレルゲン固相	*ポリスチレンビーズ	多孔質セルローススポンジ	*多孔性ガラスフィルター	多孔質セルローススポンジ	ポリスチレンウェル
アレルゲン数	207	188	57	36	33
抗体価単位	IU_A/ml	U_A/ml	IU/ml	Index 値	ルミカウント
特徴	対応抗原数が最も多い。測定範囲が＜0.1-＞500 IU_A/ml であり定量性に優れる。	最も頻用されている。プロバビリティーカーブの報告がある。	測定に要する時間が短い	必要血清量 0.7ml スクリーニングに有用	必要血清量 0.5ml

* 第 1 相では液相を使用しているが、最終的に固相化するものを記載した。

注：その他迅速検査キットとして、イムノキャップラピッド（ファディア(株)）、イムファストチェック J1/2（LSI メディエンス(株)）、スポットケム i-Line IgE シリーズ（アークレイ(株)）がある。

参考資料

■日本アレルギー学会化粧品中のタンパク加水分解物の安全性に関する特別委員会報告
(http://jsall-web.sharepoint.com/Pages/8gatsu2014.aspx)
■厚生労働科学研究班による食物アレルギーの診療の手引き 2011
■アナフィラキシーガイドライン（一般社団法人　日本アレルギー学会）

特殊型食物アレルギーの診療の手引き 2015

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http://www.med.shimane-u.ac.jp/dermatology/index.htm

ORIGINAL ARTICLE Food allergy

Wheat allergy in children evaluated with challenge and IgE antibodies to wheat components

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Keywords
allergy; children; food challenge; gliadin; glutenin; IgE; wheat; ω-5 gliadin

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Abstract

Introduction: Wheat sensitization is common but IgE antibodies (IgE-abs) to wheat are not predictive of clinical symptoms in children with suspected wheat allergy. Wheat allergen components other than ω-5 gliadin have not been well studied. Our aim was to characterize the clinical profile and investigate the value of adding measurements of IgE-abs to wheat components in a group of children with a doctor's diagnosed wheat allergy.

Method: Sixty-three children with a doctor's diagnosis of wheat allergy confirmed sensitization to wheat and, on a wheat elimination diet, went through oral wheat challenges or had a convincing recent history of wheat allergy. IgE-ab to ω-5 gliadin, low molecular weight glutenin (LMW-glutenin), high molecular weight glutenin (HMW-glutenin) and a native gliadin preparation containing α-, β-, γ-, and ω-gliadin (gliadin) were analyzed.

Results: Twenty-six children were positive in challenge, while six children were regarded as wheat allergic due to recent anaphylactic reactions. The IgE-ab levels to all four wheat components were significantly higher in the group with wheat allergy compared to the group with no wheat allergy ($p < 0.0001$). Also, the severity of symptoms at challenge correlated with the IgE-ab levels to all four components ($p < 0.05$). IgE-ab levels to ω-5 gliadin correlated best with challenge outcome, and by additional analysis of gliadin, HMW- and LMW-glutenin IgE-abs all challenge positive children could be identified.

Conclusion: Many children diagnosed as wheat allergic have outgrown their allergy and are unnecessarily on a wheat-free diet. The levels of IgE-ab to wheat gluten-derived components correlated well with wheat challenge outcome and severity.

Wheat sensitization and allergy are common in childhood, and wheat avoidance imposes major dietary restrictions (1–3). The clinical diagnosis of wheat allergy in clinical practice includes patient history and oral wheat challenge tests and is supported by skin prick test and measurement of IgE antibodies (IgE-abs) to wheat extract. The benefit of using wheat components in the diagnostic workup has not been much studied so far.

Abbreviations
HMW-glutenin, High molecular weight glutenin; IgE-abs, Immunoglobulin E antibodies; LMW-glutenin, Low molecular weight glutenin; OAS, Oral allergy syndrome; WA, Wheat allergic; WDEIA, Wheat-dependent exercise-induced anaphylaxis.

The diagnosis of food allergy to wheat is more complicated than for other food allergies, as for instance, delayed reactions that are difficult to associate with the actual intake of wheat may occur (2). Wheat allergy is also known to be outgrown during childhood, but IgE levels may remain although the allergy has resolved. Also, wheat contains many allergenic proteins, of which several cross-react with grass pollen allergens as wheat is a grass from the *Poaceae* family (4). Diagnostically, this may cause false-positive test results if the patient has an underlying grass sensitization (4, 5). Moreover, the allergen components that lately have been described as important for wheat food allergic reactions are underrepresented in whole wheat extract-based tests due to their relative insolubility. As a consequence, wheat-specific sensitizations may go undetected. Altogether, these problems may explain why decision levels for wheat IgE antibody (IgE-ab) predicting clinical reactivity have not been established (6).

Recent studies show that the main wheat allergens are found among the relatively insoluble gluten proteins, consisting of gliadins and glutenins, and both are wheat-specific proteins that do not cross-react with grass pollen allergens (7). Many wheat-allergic individuals have been shown to be sensitized to α-, β-, γ-, and/or ω-gliadins as well as high and low molecular weight glutenins (HMW-glutenin and LMW-glutenin, respectively) (8–11). Several studies have demonstrated that IgE-ab to ω-5 gliadin is associated with wheat allergy, both wheat-dependent exercise-induced anaphylaxis (WDEIA) in adolescents and adults (10, 12) and immediate allergic reactions to wheat in children (13–17). However, the latter has not been confirmed in all studies (18).

The aim of this study was to characterize the clinical profile and investigate the value of adding IgE-ab measurements to wheat components in a large group of children with a doctor's diagnosed wheat allergy.

Material and methods

Study population

One hundred and two children aged 1–17 yr with wheat allergy were identified; the families were contacted by telephone, and 76 children were willing to participate in the study. Inclusion criteria were a doctor's diagnosed wheat allergy, IgE-ab (≥0.35 kU_A/l), and/or positive skin prick test (≥3 mm) to wheat and a wheat-free diet. Exclusion criteria were celiac or other autoimmune diseases. Thirteen children were excluded; four children did not fully participate; three were not wheat-sensitized; in three, blood samples could not be obtained, two developed autoimmune diseases, and one child did not complete the wheat challenge. Thus, 63 patients were included in the study. The study was approved by the ethics committee in Stockholm, Sweden Dnr 2008-562-31/3, and the parents provided written consent.

Study design

At the first study visit, medical histories and previous symptoms to wheat ingestion were recorded and subjects were physically examined and assessed for lung function by spirometry and FeNO measurements. Blood samples were taken for analyses of IgE-ab to common food and inhalant allergens. Children revisited the clinic for open oral wheat challenge within thirteen months of their first visit.

Challenge test

The child's current health was recorded, a peripheral venous catheter was inserted, and a clinical examination was performed. Open oral challenge tests were performed using wheat bread (Pågens, Sweden) with well-defined wheat content of 0.1/1 g wheat protein/g bread in increasing doses every 30 min in 5 or 7 steps from 0.005 g to 1.7 g of wheat protein. The maximum cumulative dose was 3.38 g of wheat protein. Symptoms developing within 2 h were scored according to the criteria of Astier (19) (Table 1). A negative test was defined as no objective symptoms within 2 h after the challenge. Challenges were stopped upon appearance of objective symptoms. A final diagnosis of wheat allergy (WA) or not (non-WA) was made by one physician (N.N.) after discussion with a senior pediatric allergist (C.N.). The six children not challenged for ethical reasons (Table 3) were considered wheat allergic but were omitted in the analysis of severity of reactions.

Serological analyses

Venous blood samples were drawn; serum was separated and stored at −20°C pending IgE-ab quantification. Sensitization to common food and inhalant allergens was analyzed using ImmunoCAP® (Phadia AB, Uppsala, Sweden). IgE-ab to whole wheat, recombinant ω-5 gliadin (Tri a 19), and a native gliadin preparation containing α-, β-, γ-, and ω-gliadins (gliadin) was analyzed in sera collected on the day of challenge. Experimental ImmunoCAP tests were prepared with recombi-

Table 1 Symptom score according to Astier (19) for evaluation of clinical reactions in challenges

Symptom score	Classification of symptoms
0	No symptoms
1	Abdominal pain that resolved without medical treatment, rhino-conjunctivitis, or urticaria <10 papules, rash
2	One organ involved: abdominal pain requiring treatment, generalized urticarial, non-laryngeal angioedema, or mild asthma (cough, fall of peak expiratory flow <20%)
3	Two organs involved (of symptoms mentioned under 2)
4	Three organs involved (of symptoms mentioned under 2) or asthma requiring treatment or laryngeal edema, or hypotension
5	Cardiac and respiratory symptoms requiring hospitalization in the intensive care unit

Table 2 Demographic data and clinical characteristics of all study subjects and in subjects confirmed as wheat allergic (WA) or non-wheat allergic (non-WA) based on challenge outcome (n = 57) convincing history (n = 6)

		Final wheat allergy diagnosis		
Patient characteristics	All	Non-WA	WA	p-value*
Total number	63	31	32	ns
Sex, male/female	41/22	19/12	22/10	ns
Age, yr; median (range)	5.0 (1–17)	4.0 (1–17)	6.5 (1–17)	ns
Reported allergies; number (%)				
Asthma	41 (65)	17 (55)	24 (75)	ns
Rhino-conjunctivitis	35 (56)	17 (55)	18 (56)	ns
Eczema	40 (63)	18 (58)	22 (69)	ns
Any pollen allergy	43 (68)	22 (71)	21 (66)	ns
Grass pollen allergy	25 (40)	12 (39)	13 (41)	ns
Furry animal allergy	29 (46)	13 (42)	16 (50)	ns
Other food allergy	55 (87)	27 (87)	28 (88)	ns
Egg	49 (78)	24 (77)	25 (78)	ns
Milk	45 (71)	22 (71)	23 (72)	ns
Fish	10 (16)	6 (19)	4 (12)	ns
Peanut/tree nuts	33 (52)	13 (42)	20 (62)	ns

*Comparison between the study groups was performed using Fisher's exact test. A p-value <0.05 was considered significant, ns, not significant.

nant LMW-glutenin (Tri a 36) (20) and recombinant HMW-glutenin (Tri a 26) (10, 21) as described previously (22). The cutoff for positive IgE levels was set to ≥0.35 kU_A/l, except for LMW-glutenin which had a high non-specific background binding, and therefore, the cutoff was set to ≥1.5 kU_A/l based on the mean signal from 20 healthy blood donors +3 s.d.

Statistical analysis

Fisher's exact test was used for pairwise comparison and Mann–Whitney *U*-test for comparison of IgE-ab levels. A p-value of <0.05 was considered significant. IgE-ab levels <0.35 kUA/l were set to 0.175 and for LMW-glutenin levels <1.5 kUA/l were set to 0.75 for statistical analyses. Receiver operating characteristic (ROC) curves were calculated for IgE-ab levels with the food challenge results as reference and reported as area under the curve (AUC). Diagnostic performance in terms of sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) was calculated. IgE-ab levels producing specificities and sensitivities of at least 95% (here called positive and negative decision points, respectively) were calculated for all tests. All analyses were performed using Prism 5, GraphPad Software, La Jolla, CA, USA.

Results

Patient characteristics

Of the 63 children included in the study, 41 (65%) were boys and the median age was 5 (1–17) yr (Table 2). Upon inclusion, 41 (65%) children had doctor's diagnosis of asthma, 35 (56%) suffered from allergic rhino-conjunctivitis and 40 (63%) had eczema (Table 2). Food allergies beside wheat allergy were reported by 87% of the patients (Table 2). Allergy to pollen was claimed by 68% of the children and of these 58% specifically stated grass pollen as the trigger. Children in the study were on average sensitized to more than four of the six food allergen tested and to more than four of eight inhalant allergens tested. Sensitization to grass pollen was demonstrated in 73% (46/63) of the study subjects.

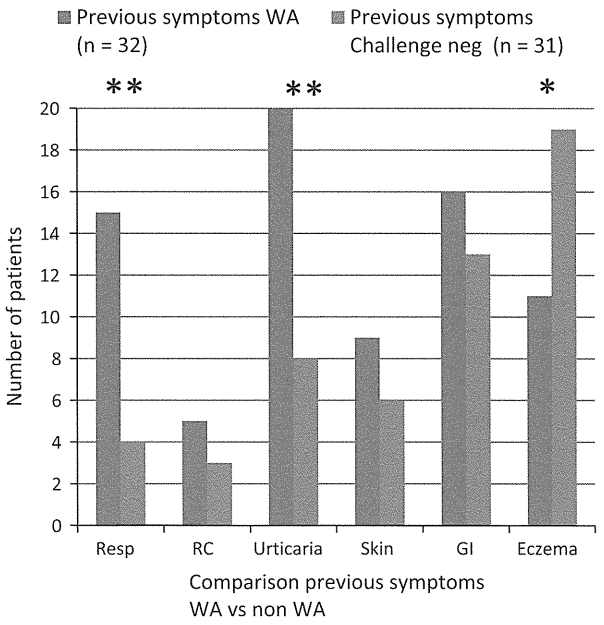

Figure 1 Comparison of previous wheat symptoms reported between wheat-allergic (WA) and non-wheat-allergic (non-WA) children (* refers to a level of significance <0.05, ** <0.01).

Table 3 Age, symptoms*, and IgE-ab levels (in kU_A/l) for children not challenged due to *recent history of severe reactions within 6 months from study inclusion*

		IgE antibody level (kU_A/l)				
Age		Wheat (f4)	ω-5 gliadin	Gliadin	HMW-glutenin	LMW-glutenin
13	A, RC, U	281	6.2	46	7.8	12
14	A, U, Other	818	13	262	44	46
5	A	846	19	240	27	32
4	A, GI, U	3247	52	1161	30	53
14	A, GI	877	28	291	23.3	24
6	A, GI, U	926	31	316	49	25

*A, asthma, GI, gastrointestinal, RC, rhino-conjunctivitis, U, urticaria, Other, angioedema/rash/OAS.

Challenge test results

Twenty-six children reacted with objective symptoms, while 31 children passed the oral wheat challenge test without objective symptoms. Six children were not challenged due to a recent history of severe reactions. Thus, in total, 32 children in the study were classified as wheat allergic (Table 2).

The first dose at challenge (0.005 g wheat protein) elicited subjective symptoms (OAS) in one child which, however, resolved, and thus, the challenge was continued. No child reacted at the next dose step (0.025 g), while three reacted objectively at a dose of 0.05 g wheat protein. The highest dose at which any child reacted corresponded to a total intake of 3.38 g wheat protein.

The most common symptoms at challenge or in the 2 h observation time were respiratory symptoms (n = 10) and rhino-conjunctivitis (n = 8) and 'other' symptoms (angioedema, rash, and OAS) (n = 8). Urticaria and gastrointestinal symptoms were both elicited in seven subjects (data not shown).

Comparisons of wheat-allergic and non-wheat-allergic subjects

There was no difference in the median age between wheat-allergic (6.5 yr, range 1–17) and wheat-tolerant children (4 yr, range 1–17). Neither was there any significant difference in prevalence of other allergic diseases, and symptoms of grass pollen allergy were found to be equally common in both groups (Table 2). However, sensitization to grass pollen was more common in the wheat-allergic group than in the non-wheat-allergic group (87.5% vs. 58%, $p < 0.05$) (results not shown).

Previous symptoms to ingested wheat reported by the study subjects differed between the two diagnosis groups; children who were confirmed as wheat-allergic after the challenge had significantly more often experienced respiratory symptoms and urticaria upon wheat intake ($p < 0.001$), while children who passed the challenge more often reported eczema ($p < 0.05$) (Fig. 1). Rhino-conjunctivitis, gastrointestinal symptoms, and other symptoms (rash, OAS, angioedema) did not differ between the two groups.

Sensitization to each of the four studied components was significantly more common in WA children compared to non-WA ($p < 0.001$) (data not shown), and the six children were considered wheat allergic but not challenged; all had detectable IgE-ab to all four wheat components (Table 3). Also, the levels of IgE-ab to wheat and the four wheat components were significantly higher in the WA than in the non-WA children ($p < 0.001$, Fig. 2).

Receiver operating characteristic curves calculated show that the HMW-glutenin test had the largest AUC of 0.88, while AUCs varied between 0.78 and 0.82 for the other wheat components tested (Table 4). At the respective assay cutoff points for each test, ω-5 gliadin had a specificity of 84% and a sensitivity of 62%, while gliadin, HMW-glutenin, and LMW-glutenin had sensitivities of 81–94% and specificities between 29 and 52% (Table 4). The positive decision points based on at least 95% clinical specificity in the diagnosis of wheat allergy were 70 kU_A/l for wheat, 1.3 kU_A/l for ω-5 gliadin, 6.0 kU_A/l for gliadin, 1.4 kU_A/l for HMW-glutenin, and 4.0 kU_A/l for LMW-glutenin (Table 4). Negative decision points, defined as a sensitivity of at least 95%, were only possible to calculate for wheat (8 kU_A/l) and HMW-glutenin (0.35 kU_A/l) (Table 4).

Relation between symptom score and IgE levels to wheat components

The severity of reactions at challenge was graded resulting in a median score of 2.5 on a five-point graded scale. Thirteen

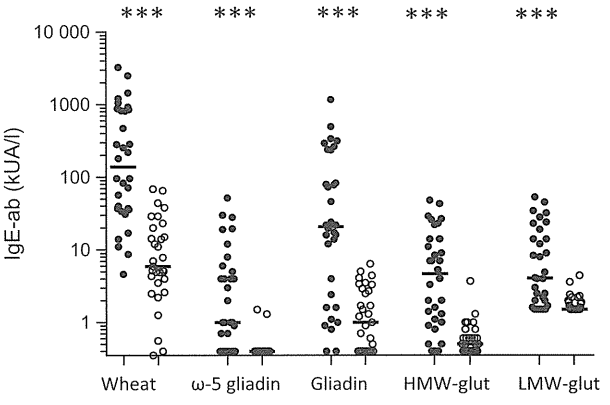

Figure 2 IgE-ab levels to wheat and wheat components in 32 children with WA (O) and 31 non-WA (●) children. Statistical significance was calculated based on the presence or absence of IgE-ab above the set thresholds for each test (***refers to a level of significance <0.001).

Table 4 Diagnostic performance of wheat and four wheat allergen component tests based on specific IgE antibody quantifications and outcomes of oral wheat challenges

	Wheat allergens				
Diagnostic performance	Wheat (f4)	ω-5 gliadin	Gliadin	HMW-glutenin	LMW-glutenin
ROC/AUC*	0.91	0.78	0.83	0.88	0.82
Assay cutoff point**					
Specific IgE level (kU_A/l)	0.35	0.35	0.35	0.35	1.5
Sensitivity, Specificity (%)	100, 6	62, 84	94, 29	97, 42	81, 52
PPV, NPV (%)	52, 100	80, 68	58, 82	63, 93	63, 73
Positive decision point (95% spec)					
Specific IgE level (kU_A/l)	70	1.3	6.0	1.4	4.0
Sensitivity, Specificity (%)	62, 97	44, 97	69, 97	66, 97	56, 97
PPV, NPV (%)	95, 71	93, 62	96, 75	95, 73	95. 68
Negative decision point (95% sens)					
Specific IgE level (kU_A/l)	8.0	None	None	0.35	None
Sensitivity, Specificity (%)	97, 58			97, 42	
PPV, NPV (%)	70, 95			63, 93	

*Receiver operating characteristic (ROC) curves were constructed for wheat and the four components with the food challenge result as reference. The area under the curve (AUC) was calculated for each component.
**0.35 kU/l for all except LMW-glutenin for which an assay cutoff of 1.5 kU/l was set.

children were graded as score 1, four score 2, and nine score 4. No child was classified as having scores 3 or 5.

The challenge positive children (n = 26) were divided into two severity groups (mild = severity score 1 and 2; severe = score 4), and the IgE-ab levels to wheat components were compared to those of non-WA children (Fig. 3). Both severity groups had significantly higher levels of IgE-ab to all four components as compared to non-WA (p between >0.05 and >0.001), apart from gliadin where there was no difference between mild reacting children and non-WA (Fig. 3). Children with severe symptoms compared to those with mild symptoms had significantly higher IgE-ab levels to gliadin, HMW-glutenin, and LMW-glutenin ($p > 0.05$) but not to ω-5 gliadin. IgE-ab levels for ω-5 gliadin, HMW-glutenin, and LMW-glutenin ($p > 0.05$) but not for gliadin were significantly different when comparing children with mild symptoms to non-WA.

Discussion

In this study, we have characterized the clinical profile of wheat-allergic children and investigated the diagnostic properties of wheat component IgE-ab tests in a large group of children with a previous diagnosis of wheat allergy. Children with confirmed wheat allergy (WA) had a history of more severe symptoms, significantly higher IgE-ab levels to gluten-derived wheat components than the challenge negative children. In addition, the IgE-ab levels to these wheat components correlated with the severity of reactions at challenge.

Only half of the children with a previous diagnosis of wheat allergy reacted at the oral wheat challenge test, which may be explained by that most wheat-allergic children develop tolerance over time but still have detectable wheat IgE-ab (2). A third of the challenge positive children reacted severely, the majority with respiratory symptoms. We found a significant difference between the severity of historically reported symptoms to wheat in WA and non-WA. Respiratory symptoms

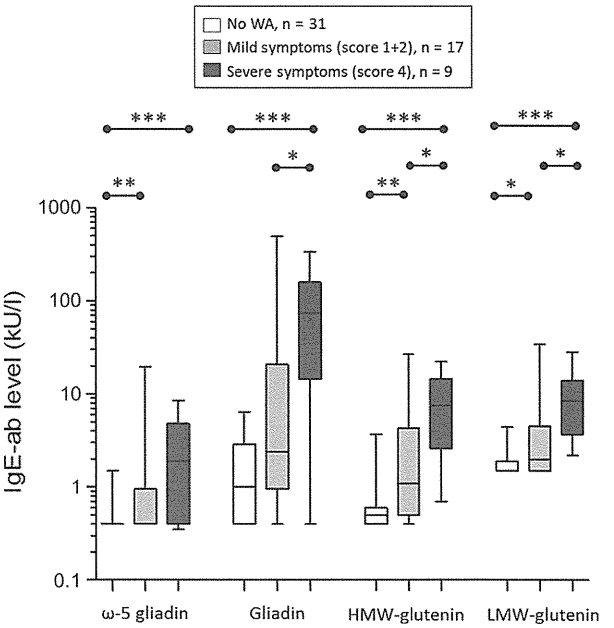

Figure 3 IgE-ab levels to four gluten-derived wheat components in 31 non-WA and 26 children positive in wheat challenge divided into groups with mild and severe symptoms, respectively. Results are presented as the 25th to 75th percentile of the values with the median and min to max indicated. Statistical significant differences between the groups are indicated (*refers to a level of significance <0.05, **<0.01 and ***<0.001).

and urticaria were more prevalent in WA children while eczema was more frequently reported by non-WA children.

Wheat allergy diagnosis is many times confounded by underlying grass pollen sensitizations that through cross-reactivity give rise to false-positive wheat extract test results, and thus, there is a need for more specific wheat allergy biomarkers. We show here that four wheat-specific components from the gluten fraction are useful in differentiating clinically relevant sensitizations to wheat. The IgE-ab levels to gliadin, ω-5 gliadin, HMW-glutenin, and LMW-glutenin were all associated with the challenge outcome and were also very high in children with a convincing recent history of wheat allergy.

While the wheat extract test had high sensitivity in this study (at least partly due to the inclusion criteria of positive wheat IgE tests), the specificity was very low, showing the need for more specific diagnostic tools. The ω-5 gliadin was the test showing the highest specificity and best in discriminating between children with WA from non-WA children. Only five of 31 non-WA children had detectable albeit low levels of IgE-ab (data not shown). However, the sensitivity of the ω-5 gliadin test was rather low (62%), and one-third of the WA children were not sensitized to ω-5 gliadin. However, the eleven children negative to ω-5 gliadin IgE-ab were all sensitized to one or more of the other three wheat-specific components. In addition, the levels of IgE-ab to the four gluten components all correlated with the severity of the symptoms at challenge in this study.

Our findings are supported by previous reports in the literature; gliadins were previously shown using skin prick testing to be useful in the diagnosis of wheat-allergic children (8). In other studies, wheat-allergic patient has been shown to more frequently have IgE-ab to LMW-glutenin than to ω-5 gliadin (20). However, the combination of IgE-ab against LMW-glutenin and ω-5 gliadin did not allow identification of all wheat-allergic patients in that study. An association between the presence of IgE-ab to LMW-glutenin and gastrointestinal symptoms after wheat ingestion could be demonstrated by Simonato et al. (23).

Many studies have been focusing on WDEIA patients and the usefulness of IgE-ab to HMW-glutenin (10, 21, 24) in their diagnosis. In a German study on WDEIA patients (24), sensitization to ω-5 gliadin, gliadin, and HMW-glutenin concluded that analyzing IgE-ab to gliadin and HMW-glutenin gives additional information in patients negative to ω-5 gliadin IgE-abs. The results from our study indicate that also in immediate wheat allergy, a combination of gluten-derived component tests improves the prediction of clinical reactions.

Although the number of children with symptoms of grass pollen allergy was equal in WA and non-WA children, we found significantly higher levels of IgE-ab to timothy grass pollen in the allergic children. These children also had higher IgE-ab levels to wheat extract, indicating that sensitization to grass pollen influences the apparent IgE-ab level to wheat. A previous study has shown that 65% of the patients with grass pollen allergy had false-positive IgE-ab test results to wheat extracts (25). There is thus a need for markers that truly can differentiate between primary wheat sensitization and cross-reactive sensitization.

Quantification of IgE-ab to ω-5 gliadin, gliadin, HMW-glutenin, and LMW-glutenin all seems clinically useful in our study population. The results indicate that a negative ω-5 gliadin test result is a good predictor of a negative wheat challenge. By combining results from the ω-5 gliadin test, having the highest specificity, with the results for gliadin and HMW-glutenin tests, having the highest sensitivities, it was possible to identify all children in the positive challenge group.

A limitation of this study is that all the wheat challenges were performed as open food challenges but as the Astier scoring method only takes objective symptoms into consideration, this challenge procedure might be a minor drawback to the study design. However, the ability of the gluten-derived components to separate wheat allergy from tolerance is high taking into consideration our study group was highly selected for wheat allergy. Had the same analyses been made in a group of children with weaker suspicion of wheat allergy, we believe that the differentiating ability of the components may have been even better.

In summary, it seems that many children are unnecessarily on a wheat-free diet. In our population, the levels of IgE-ab to ω-5 gliadin, gliadin, HMW-glutenin, and/or LMW-glutenin, all wheat specific without any homologous proteins in grass pollen, all are associated with clinical reactions to wheat and also predict the severity of the wheat allergy.

Acknowledgments

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Conflict of interest statement

Sigrid Sjölander, Malin Berthold, and Magnus P. Borres are employed by ThermoFisher Scientific, Uppsala, Sweden. All other authors have declared they have no conflict of interest.

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Acta Derm Venereol 2014; 94: 78–79

SHORT COMMUNICATION

Olopatadine Hydrochloride Decreases Tissue Interleukin-31 Levels in an Atopic Dermatitis Mouse Model

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Atopic dermatitis (AD) is an inflammatory skin disease characterized by an intensely pruritic skin rash (1). A variety of mediators, including histamine and neuropeptides, are involved in pruritus. We previously reported that olopatadine hydrochloride (olopatadine), a histamine H1 receptor antagonist, significantly suppresses the number of scratching events associated with a decreasing number of intraepidermal nerve fibres via increased semaphorin 3A expression and decreased nerve growth factor (NGF) levels in NC/Nga mice (2). Oral olopatadine (Kyowa Hakko Kirin, Tokyo, Japan) has been prescribed in Japan and Korea for treatment of allergic rhinitis, urticarial, pruritus, eczema, prurigo, psoriasis vulgaris, and erythema multiforme, which was covered by insurance.

Recently, interleukin (IL)-31 was found to play a role in pruritus and skin barrier function in AD (3–5). It was reported that transgenic mice overexpressing IL-31 exhibit spontaneous pruritus and develop severe dermatitis (6). Moreover, serum and tissue IL-31 levels in patients with AD were increased compared with levels in control subjects, and IL-31 levels correlated with both disease activity and severity of AD (3, 7–9). Thus, we evaluated the effect of olopatadine on tissue IL-31 levels in an AD model using NC/Nga mice.

MATERIALS AND METHODS

AD-like dermatitis was induced by the topical application of *Dermatophagoides farinae* body (Dfb, 100 mg/mouse/application) ointment 3 h after barrier disruption by sodium dodecyl sulphate on shaved dorsal skin of NC/Nga mice. These procedures were repeated twice per week for 4 weeks. After 2 weeks of Dfb application, mice were treated orally with either distilled deionized water (control) or olopatadine (3 or 10 mg/kg/day) daily, whereas another group of mice received 0.1% (w/w) topical tacrolimus (100 mg/mice/application) applied to the back skin twice per week for 2 weeks. Mice treated with only sodium dodecyl sulphate served as the sham group. Skin samples from the lesional skin were homogenized as previously reported (2). Subsequently, the tissue concentrations of IL-31, NGF, E-selectin, and amphiregulin were determined by enzyme-linked immunoassay (ELISA) according to the manufacturer's protocol (CSB-E13660m: Cusabio Biotech, Wuhan, Hubei Province, China, NGF Emax Immunoassay system: Promega, Madison, WI, USA, CD62E Quantikine ELISA kit and mouse amphiregulin Duoset: R&D systems, Minneapolis, MN, USA, respectively). The number of scratching episodes was determined by taking video images for 90 min. Both the F-test and Aspin-Welch test were used for analysis of differences between sham and control groups. Multiple comparisons among treatment groups were made using the Kruskal–Wallis test, followed by the Steel test.

RESULTS

As shown in Fig. 1A, IL-31 levels were significantly increased in mice that received Dfb application ($n=10$) compared with sham-treated mice ($n=6$). Olopatadine at 3 and 10 mg/kg/day ($n=10$ each) significantly suppressed this increase in IL-31 levels by 88.1% and 94.5%, respectively. Tacrolimus ointment also significantly suppressed the increase in IL-31 production by 94.3%.

In the sham, control, and olopatadine-treated groups, IL-31 correlated positively with the tissue concentra-

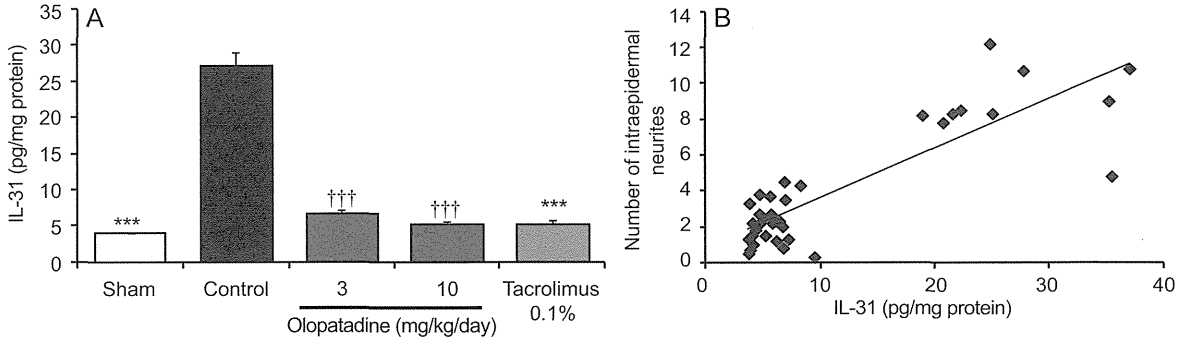

Fig. 1. (A) Effects of olopatadine and tacrolimus ointment on interleukin (IL)-31 levels in lesioned skin of NC/Nga mice. Each column represents the mean ± standard error of 6–10 mice. ***$p<0.001$, significant difference from the control group (Aspin-Welch test). †††$p<0.001$, significant difference from the control group (Steel test). (B) Relationship between IL-31 levels and the number of intraepidermal neurites. A correlation was assessed by Pearson's correlation analysis. Each square represents individual data-points from individual mice.

tions of several inflammatory and pruritus mediators, including NGF, IL-1b, E-selectin, and amphiregulin ($r=0.7574$, $r=0.7324$, $r=0.8368$, and $r=0.6970$, respectively). In particular, the correlation between IL-31 levels and the number of intraepidermal nerve fibres was strong (Pearson's correlation analysis: $r=0.8523$, $p<0.0001$; Fig. 1B), whereas olopatadine decreased IL-31 levels, as well as the number of scratching events, with a weak correlation ($r=0.5426$).

DISCUSSION

It has been reported that an increased number of peripheral nerve fibres may contribute to a reduction in the itch sensation threshold (alloknesis) in human AD patients and in NC/Nga mice (8, 9). The current study suggests that IL-31, as well as NGF, may increase scratching events by reducing the itch sensation threshold of NC/Nga mice. The weak association between scratch number and IL-31 may arise from the time restriction in recording the video image. Although neither the source of IL-31 in this model nor the mechanism of olopatadine on reducing IL-31 are clear, our study suggests that olopatadine may affect local skin lesions by reducing pruritus via decreasing tissue levels of IL-31.

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INTERNAL MEDICINE

□ CASE REPORT □

Chronic Headaches and Sleepiness Caused by Facial Soap (Containing Hydrolyzed Wheat Proteins)-Induced Wheat Allergy

Chifumi Iseki[1], Toru Kawanami[1], Takahiko Tsunoda[2], Yuko Chinuki[3] and Takeo Kato[1]

Abstract

A 38-year-old woman was suffering from irregular headaches and sleepiness. She had used soap containing Glupearl 19S (hydrolyzed wheat proteins) every day for approximately one year and had experienced an episode of rash eruption on her face seven months ago. Wheat-specific IgE antibodies were detected in her serum. A Western blot analysis revealed a high titer of IgE antibodies against Glupearl 19S and wheat proteins. The patient was sensitive to these compounds in a skin prick test. After avoiding eating wheat, her headaches and sleepiness disappeared. A hidden food allergy is a possible cause of these symptoms.

Key words: Glupearl 19S, food allergy, additive, mild symptoms, prostaglandin D2

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Introduction

In October 2010, the Ministry of Health, Labour and Welfare in Japan announced that the best-selling "green tea soap" contains allergens capable of sensitizing the user to a wheat allergy and may cause a food allergy to wheat, sometimes in the form of anaphylaxis. Following this announcement, the responsible cosmetics company conducted a voluntary recall of the soap in May 2011. By that time, however, at least 471 users of the soap had suffered from wheat allergies after consuming foods containing wheat products. The company changed the formulation of the soap to make it free of the hydrolyzed wheat protein additive (Glupearl 19S) in December 2010. By that time, however, more than 40 million bars of the soap had been sold, suggesting that some consumers may have suffered from a wheat allergy without being diagnosed. The mechanism underlying the development of the facial soap allergy is as follows: the hydrolyzed products of the wheat proteins in the facial soap sensitized the user through the skin or rhinoconjunctival mucous membranes, after which IgE antibodies against the proteins caused an allergic reaction upon the natural uptake of wheat proteins in foods through the alimentary tract (1). Indeed, a severe clinical form of food allergies, wheat-dependent exercise-induced anaphylaxis (WDEIA), attracted nationwide attention. WDEIA is the most common form of food-dependent exercise-induced anaphylaxis (FDEIA). In patients who experience strenuous exercise, such as running, after eating a causative food (e.g., wheat; case of WDEIA), usually within two hours, FDEIA can emerge as a serious systemic reaction, including generalized urticaria, angioedema, erythema, dyspnea, abdominal pain and/or loss of consciousness (2). FDEIA is the most severe form of allergic food reactions. Considering the countless number of facial soap users, of whom a portion were sensitized to wheat additives, it is likely that there are many patients with milder symptoms of wheat allergies who did not develop FDEIA.

Our report describes the case of a woman who was later found to have a wheat-dependent food allergy. She exhibited atypical symptoms for an allergy; however, we considered that the symptoms were caused by an allergic reaction.

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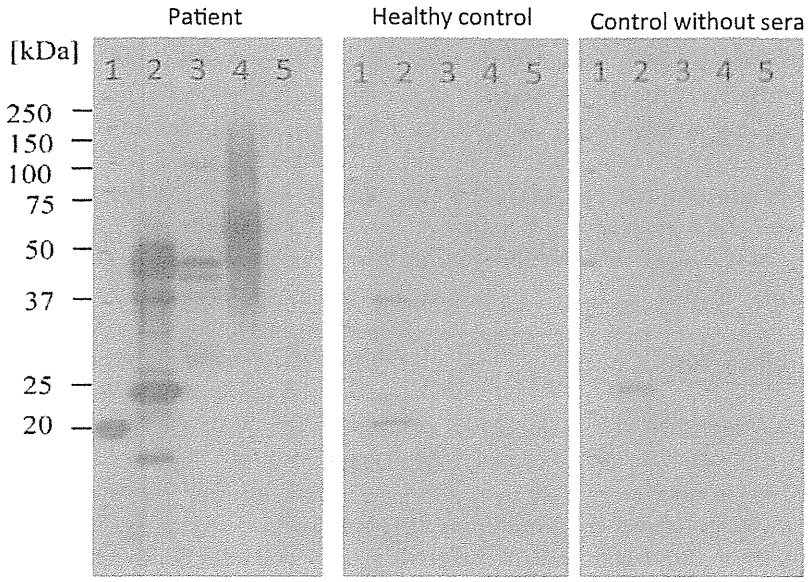

Figure. **A Western blotting analysis of wheat protein fractions and hydrolyzed wheat proteins in the sera of the patient, a healthy control subject and a control subject without sera. Lane 1, marker; Lane 2, soluble wheat proteins; Lane 3, insoluble wheat proteins; Lane 4, Glupearl 19S; Lane 5, omega-5 gliadin. The IgE in the patient's serum reacted to soluble and insoluble wheat proteins and Glupearl 19S, but not to omega-5 gliadin.**

Case Report

A 38-year-old healthy woman suddenly developed itchy edema on her face one day in December 2010. Since then, she sometimes experienced itching in her eyes and on her face, regardless of the season. Her family doctor prescribed antipruritic eye drops and antihistamine medicines, which reduced her symptoms, after which she stopped using the medicines.

At around the same time, she sometimes experienced headaches and sleepiness/drowsiness without any other symptoms. Occasionally, her eyes were itchy for an unknown reason when she experienced headaches and sleepiness. Her symptoms prompted her to visit our outpatient clinic in August 2011. Her headaches were mild and not pulsatile, although they emerged almost every day at irregular times of the day. She described the symptom as being a type of sleepiness/drowsiness that continued for two to three hours. The headaches were unlikely tension-type headaches. Possible organic causes of headaches were excluded on cranial computed tomography; however, we were unable to identify the cause of the headaches in the first series of medical examinations. The patient sometimes used loxoprofen, a nonsteroidal anti-inflammatory drug (NSAID), for her headaches; however, the medication neither alleviated nor enhanced her symptoms. Since the cause of the allergic reaction and headaches was unknown at that time, we conducted a multiple antigen simultaneous test (MAST). Her serum contained a high titer (16.80 LC (<1.39)) of IgE antibodies specific for wheat allergens, as determined on an enzyme immunoassay that detected no IgE antibodies against 32 other allergens, such as cedar pollen, house dust, milk, eggs, soybeans, fungus, etc. The results surprised the patient because she had been eating bread every day for breakfast and lunch. At that time, however, we did not anticipate the clinical significance of the RAST results with respect to the patient's primary symptoms: headaches and sleepiness.

After seeing the issues related to the tea soap-associated wheat allergy on TV and in the newspaper in November 2011, the patient recalled that she had used the tea soap every day for approximately one year from 2009 to 2010. She wondered about the possible association between the use of the soap and the results of the RAST and consulted us again. We subsequently conducted specific examinations for the allergy induced by the soap. A skin prick test showed a positive reaction to a solution of the tea soap (100×, 1,000×), wheat extract (Torii Pharmaceutical Co. Ltd., Tokyo, Japan), a solution of the hydrolyzed products of the insoluble fraction of the wheat proteins (HWP) (provided by Shimane University) and a 0.01% solution of Glupearl 19S (a HWP contained in the tea soap) provided by the National Hospital Organization Sagamihara Hospital. A Western blot analysis also revealed the presence of IgE antibodies specific for Glupearl 19S in the patient's serum, whereas no antibodies to omega-5 gliadin were detected (Figure). The results fulfilled the diagnostic criteria for the immediate-type wheat allergy induced by Glupearl 19S proposed by the Japan Society of Allergology (1, 3). Realizing that she had a food allergy to wheat, the patient recalled that her first allergic episode occurred while she had worked hard to remove snow from her car parking lot after eating

bread for lunch. The episode did not appear to be as severe as anaphylaxis, although it was probably caused by the same mechanism as WDEIA, which is triggered by exercise after wheat intake. To treat the itching on her eyes and face, the patient avoided eating any products containing wheat, and the symptoms disappeared. Unexpectedly, her headaches and sleepiness also disappeared. After recovering, the patient reported that she experienced headaches and sleepiness one or two hours after eating bread or pasta.

Discussion

The present patient did not exhibit any of the typical severe symptoms of a food allergy to wheat products. However, we suspected that her headaches and sleepiness were associated with a wheat allergy caused by sensitization to the hydrolyzed products in the facial soap. This is the first case of headaches and sleepiness as symptoms of an allergic reaction among facial soap users.

The antigens and characteristics of soap-induced wheat allergies differ from those associated with conventional food wheat allergies, including WDEIA (1, 4). The mechanism underlying the development of the soap-induced allergy is as follows: the HWP (Glupearl 19S) contained in the facial soap sensitizes the user through the skin and/or rhinoconjunctival mucous membranes, after which he/she develops allergic symptoms to natural wheat proteins absorbed through the gastrointestinal tract (1, 5). Glupearl 19S is a product made via the acidic hydrolysis of the insoluble fraction of wheat in order to increase the solubility of gluten (1, 6). A portion of patients sensitized to the HWP exhibit a contact allergy several months after sensitization and subsequently develop WDEIA, a generalized allergic reaction induced by wheat ingestion (1). Patients with this soap-induced wheat allergy rarely show sensitivity to omega-5 gliadin (1, 4), a component protein of wheat to which up to 80% of conventional WDEIA patients are sensitive (7). The serum of the present patient contained IgE antibodies specific for Glupearl 19S but no IgE antibodies for omega-5 gliadin. The antigens and characteristics of the soap-induced wheat allergy are different from those of conventional food wheat allergies.

The headaches and sleepiness observed in the present case may have been caused by an allergic reaction to food. Symptoms of food allergies include not only urticaria, erythrogenic skin, edema and anaphylaxis, but also fatigue and headaches, which are often overlooked because it is difficult to identify the cause of the symptoms (8). Wheat and hydrolyzed products of wheat are used not only in bread and pasta, but also in food additives in many kinds of processed foods, which are consumed by patients unconsciously. The cause of the present patient's symptoms were difficult to recognize because she unconsciously kept eating many kinds of wheat products.

Although the symptoms observed in the present case did not fulfill the criteria for migraines, previous research on migraines strongly suggests the important role of mast cells in the brain in the development of headaches. As early as the 1930s, however, clinical researchers have recognized a possible association between allergies and migraines and concluded that wheat intake is one of the most frequent triggers of migraines (9, 10). Subsequently, the levels of histamine in the plasma and total IgE in some migraine patients were shown to be high (11, 12). Mast cells are located perivascularly in proximity to neurons, especially in the dura, and are sometimes activated by allergic reactions, inflammatory conditions or neurologic triggers (13). The headaches observed in the present case were not typical for migraine headaches; however, the high titer of IgE antibodies in the serum strongly suggest that the IgE-mediated release of chemical mediators/cytokines from mast cells may cause headaches.

It is well established that mast cells sensitized to IgE antibodies release not only histamine and leukotrienes, but also prostaglandin D_2 (PGD_2) upon cross-binding of IgE antibodies by antigens (14) and that PGD_2 is an endogenous sleep inducer (15). Physiologically, PGD_2 is synthesized in the dura, the membrane system surrounding the brain, and is naturally secreted into the cerebrospinal fluid where it acts as the most important endogenous sleep inducer (13). It has been reported, however, that patients with mastocytosis exhibit marked overproduction of histamine and PGD_2 in addition to headaches and sleepiness (16). We wonder whether the headaches and sleepiness observed in the present case were possibly caused by this PGD_2-mediated mechanism. It is also known that cross-binding of IgE on mast cells with antigens induces the synthesis of inflammatory cytokines, such as IL6 and TNFα (17). We wonder whether the IgE-mediated release of chemical mediators, such as PGD_2 and histamine, and the subsequent formation of inflammatory cytokines by brain mast cells may be the cause of the patient's sleepiness and headaches.

The NSAID the patient sometimes used for her headaches had no effect. We speculated that the NSAID did not block the allergic reactions, but rather adversely affected the patient's allergic reactions. Aspirin itself, with or even without exercise, induces wheat-dependent anaphylaxis (18, 19). Blocking the COX-1 pathway with NSAIDs precipitates histamine release from mast cells and basophils (20). As other presumed roles of NSAIDs in wheat allergy patients, aspirin and loxoprofen are suspected to facilitate the absorption of wheat through the gastrointestinal tract and/or promote the generation of IgE against wheat allergens (20). Although the present patient did not always use loxoprofen for her headaches, loxoprofen may have affected her allergic symptoms, including headaches and sleepiness.

We expect that the present case will call the attention of physicians and neurologists to the possibility that headaches and/or drowsiness without typical allergic symptoms may be caused by an IgE-mediated mechanism.

The authors state that they have no Conflict of Interest (COI).

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