lergies can also experience an immediate food allergy reaction when they eat foods containing HWP. In Japan, we reported 5 patients who were primarily sensitized to HWP present in facial soap and experienced wheat-dependent exercise-induced anaphylaxis (WDEIA) after the ingestion of normal wheat products [2]. After our report, more than 600 patients with such an allergy had been reported to date (June 10, 2012) from various institutes all around Japan. Importantly, the majority of the patients used the same facial soap (Cha no Shizuku®; Yuuka Co. Ltd., Fukuoka, Japan) containing acid-hydrolyzed wheat protein (acid-HWP).

Gluten is a salt-insoluble protein complex of wheat and exhibits viscoelastic properties. As for HWP, in most cases hydrolyzed gluten (HG) has been widely used in cosmetics. HG is a mixture of variously sized polypeptides. Their sizes depend on the condition of hydrolysis. The commercial acid-HWP contained in the facial soap (Glupearl 19S®; Katayama Chemical Industries Co. Ltd., Osaka, Japan; described as HWP1 in this article) was produced from gluten after partial hydrolysis with hydrogen chloride at 95°C for 40 min. Based on the SDS-PAGE pattern of HWP1, the molecular weight of a main band was 40–50 kDa.

In the present study, we focused on the allergenicity, as an ability to elicit mast cell activation, of acid-hydrolyzed gluten (acid-HG), which was generated by acid treatment under heated conditions, and intended to acquire new data about the reactivity of various time-treated acid-HGs and commercial acid-HWP (HWP1) using serum IgE from sensitized subjects as follows: sera from 10 subjects with wheat allergy accompanied by skin and rhinoconjunctival sensitization to HWP1 in the facial soap, 8 pediatric subjects with food allergy to native wheat, 9 adult subjects with WDEIA food allergy to native wheat, and 8 nonatopic healthy subjects.

Materials and Methods

HWP1 and Gluten

Glupearl 19S (HWP1) was supplied by Katayama Chemical Industries. Gluten was purchased from Sigma-Aldrich (catalog No. G5004).

Five hundred milligrams of HWP1 and gluten were suspended with 5 ml of 1 M Tris solution (pH 11.4) and left standing for more than 24 h at room temperature, and these suspensions were kept at 4°C and used as stock solutions.

Acid Hydrolysis of Gluten

One milligram of gluten was hydrolyzed in 1 ml of 0.1 N HCl at 100°C for 0.5, 1, 3, 6, 9, 12, 24, and 48 h. After a fixed time, the acid solution was neutralized with 0.1 N NaOH. At a point of 0 h, gluten was added to the neutralized solution and not heated.

Each sample was separated in 15–25% acrylamide gel (DRC Co., Ltd., Tokyo, Japan) according to the Laemmli method; the gel was then stained with CBB.

Sera

Sera was collected from 10 subjects with wheat allergy accompanied by skin and rhinoconjunctival sensitization to HWP1 in the facial soap (HW1–10 in table 1), 8 pediatric subjects with food allergy to natural wheat (CW1–8), 9 adult subjects with exercise-induced anaphylaxis after ingestion of native wheat (AW1–9), and 8 nonatopic healthy persons (NA1–8). The presence of specific IgEs in the sera was determined using ImmunoCAP® (Phadia AB, Uppsala, Sweden). All of the patients with wheat allergy who had an obvious clinical history and/or a gluten-specific ImmunoCAP class of 3 or above were enrolled into this study. Informed consent was obtained from all patients and volunteers. Our study was approved by the Ethics Review Committee of the National Institute of Health Sciences.

Dot Blot

One microgram of nondenatured gluten or acid-HG was spotted onto a BA83 nitrocellulose membrane (Whatman™; GE Healthcare UK Ltd., Little Chalfont, UK). After being blocked with 0.1% casein/PBS, the membrane was incubated with patients' sera (5- to 10-fold diluted with 0.1% casein/PBS) overnight at 4°C. The membrane was then washed and gently shaken in 500-fold diluted HRP-linked anti-human IgE (Nordic Immunology, Tilburg, The Netherlands) solution for 90 min at room temperature. Binding of serum IgE and dotted samples was detected as HRP conjugates using color-developing Konica Immunostain (Konica Minolta, Tokyo, Japan).

In vitro Elicitation Test

An in vitro elicitation test was performed by assessing IgE crosslinking-induced luciferase expression (EXiLE) using human FcεRI genes- and nuclear factor of activated T-cell (NF-AT)-dependent luciferase reporter gene-introduced rat mast cells (RS-ATL8 cells), as previously described [9].

Briefly, RS-ATL8 cells (5×10^4 cells/50 μl/well) were sensitized with 1:100 diluted patients' sera overnight. After washing, the cells were stimulated with 100 ng/ml of acid-HGs suspended in 10% FCS-containing minimum essential medium at 37°C for 3 h in a 5% CO_2 incubator. Then, 50 μl of luciferase substrate solution containing cell lysis reagent (ONE-Glo, Promega Corp., Tokyo, Japan) was added to the cells, and chemiluminescence was measured using an EnVision multilabel plate reader (PerkinElmer, Waltham, Mass., USA). Luciferase expression levels were represented as the fold increase of light units compared with those of nonstimulated cells.

Statistical Analysis

The statistical significance of the differences between each period of the experimental groups was determined using the Steel test versus the NA group at individual time points. $p < 0.05$ was considered statistically significant.

Results

Binding of IgE from HWP- or Gluten-Sensitized Subjects to Various Time-Treated Acid-HGs

To determine the effect of the extent of the acid hydrolysis of gluten on allergenicity, gluten was treated with 0.1 N HCl at 100°C for various times up to 48 h. Figure 1a shows the CBB-stained pattern of the acid-HGs. The CBB-stained patterns changed to a smear-like pattern after acid treatment, and the molecular weight of the main protein bands gradually decreased from 40–70 kDa (0.5–1 h of treatment) to 6 kDa (9–12 h of treatment); after 24–48 h of treatment, CBB-stained bands larger than 6 kDa were no longer detected.

We then examined the binding of serum IgE to these acid-HGs. We performed immune dot blot tests using serum samples of subjects sensitized with HWP1 (HW1, 2), pediatric subjects with wheat allergy (CW1, 2), and an adult WDEIA subject (AW3). As shown in figure 1b, HW1 reacted only to acid-HGs [0.5- to 6-hour-treated acid-HGs and HWP1 (spot H)], while HW2 reacted to gluten and acid-HGs (0.5- to 3-hour-treated acid-HGs and HWP1). In contrast, CW1 and CW2 reacted to native gluten (acid-hydrolyzed for 0 h) more strongly and showed only slight reactions to 0.5- to 1-hour-treated acid-HGs. Minimal IgE binding was observed in the sample from the adult WDEIA subject (AW3).

We also examined the molecular sizes of IgE-binding proteins in acid-HGs and native gluten using Western blotting under denatured conditions (fig. 2). After 0.5–1 h of acid treatment, IgE immunoblotting with a serum sample from subject HW1 showed smears at around 40- to 70-kDa proteins, the molecular weight of which was relatively higher than that of main bands of native gluten on SDS-PAGE (around 30–50 kDa), producing the same binding results for the HWP1. However, after 3 h of acid treatment, the IgE-bindings to the 40- to 70-kDa proteins were time-dependently decreased. In contrast, a Western blot analysis using serum from a native-wheat-sensitized subject (CW1) showed clear binding to native gluten, and acid hydrolyzation decreased the binding in a time-dependent manner. On the other hand, the sample from the AW3 subject showed IgE binding to a 60-kDa protein, which corresponds to ω-5 gliadin (fig. 2c), although a weak signal was obtained using the dot blot test.

Elicitation Test of Various Time-Treated Acid-HGs Using Mast Cells Sensitized with Patients' Sera

We next performed an in vitro elicitation test to determine the ability of IgE from subjects sensitized with HWP1 or native gluten to induce mast cell activation after the addition of acid-HGs. The humanized rat mast cell line RS-ATL8, which was established by introducing the NF-AT-responsive luciferase gene into human FcεRI-expressing RBL-SX38 cells, was used to measure IgE crosslinking-induced luciferase expression (EXiLE) [9]. A two-fold increase in luciferase expression relative to those without any stimulation is considered to be a clinically relevant cut-off level according to our very recent study [10], and this level is superimposed in figure 3.

As shown in figure 3, commercial acid-HWP (HWP1) induced a significant increase in luciferase expression in the HW and CW groups, but not in the AW group. Acid

Table 1. Information about sera from subjects with wheat-allergy in the study

Patient No.	Age, years	Gender	Total IgE IU/ml	Wheat-specific IgE, UA/ml	Gluten-specific IgE UA/ml	ω-5 gliadin-specific IgE UA/ml
HW1	42	F	4,050	23.4	31.4	2.06
HW2	22	F	412	9.23	12.4	<0.35
HW3	25	F	1,510	1.79	3.12	<0.35
HW4	55	F	123	4.38	6.15	<0.35
HW5	41	F	285	21.8	36.2	7.01
HW6	51	F	210	2.28	3.65	<0.35
HW7	45	F	3,170	14.6	24.2	<0.35
HW8	23	F	1,510	1.79	3.12	<0.35
HW9	38	F	485	1.93	1.94	<0.35
HW10	50	F	22.9	0.76	1.27	<0.35
CW1	2	M	1,050	19	50.4	1.83
CW2	3	M	512	ND	66.6	0.4
CW3	4	M	1,199	59	>100	6.38
CW4	1	F	191	17.6	ND	ND
CW5	4	M	226	15.9	20	0.44
CW6	7	M	ND	11.4	15.4	0.49
CW7	8	M	532.1	6.36	9.25	<0.35
CW8	4	F	ND	ND	ND	ND
AW1	20	M	283	2.48	3.09	22.8
AW2	16	F	2,120	4.84	7.7	24.9
AW3	62	M	1,480	1.04	3.74	11.1
AW4	56	M	ND	<0.35	0.7	4.3
AW5	49	M	182	4.3	14.2	30.4
AW6	38	M	2,160	1.16	6.63	13.5
AW7	42	M	43	<0.35	0.5	1
AW8	54	M	ND	<0.35	<0.35	10.7
AW9	69	M	1,990	9.92	11.3	26.4

ND = Not determined; HW1–10 = subjects with wheat allergy accompanied by skin and rhinoconjunctival sensitization to HWP1 in the facial soap; CW1–8 = pediatric subjects with food allergy to native wheat; AW1–9 = adult subjects with excise-induced anaphylaxis after ingestion of native wheat.

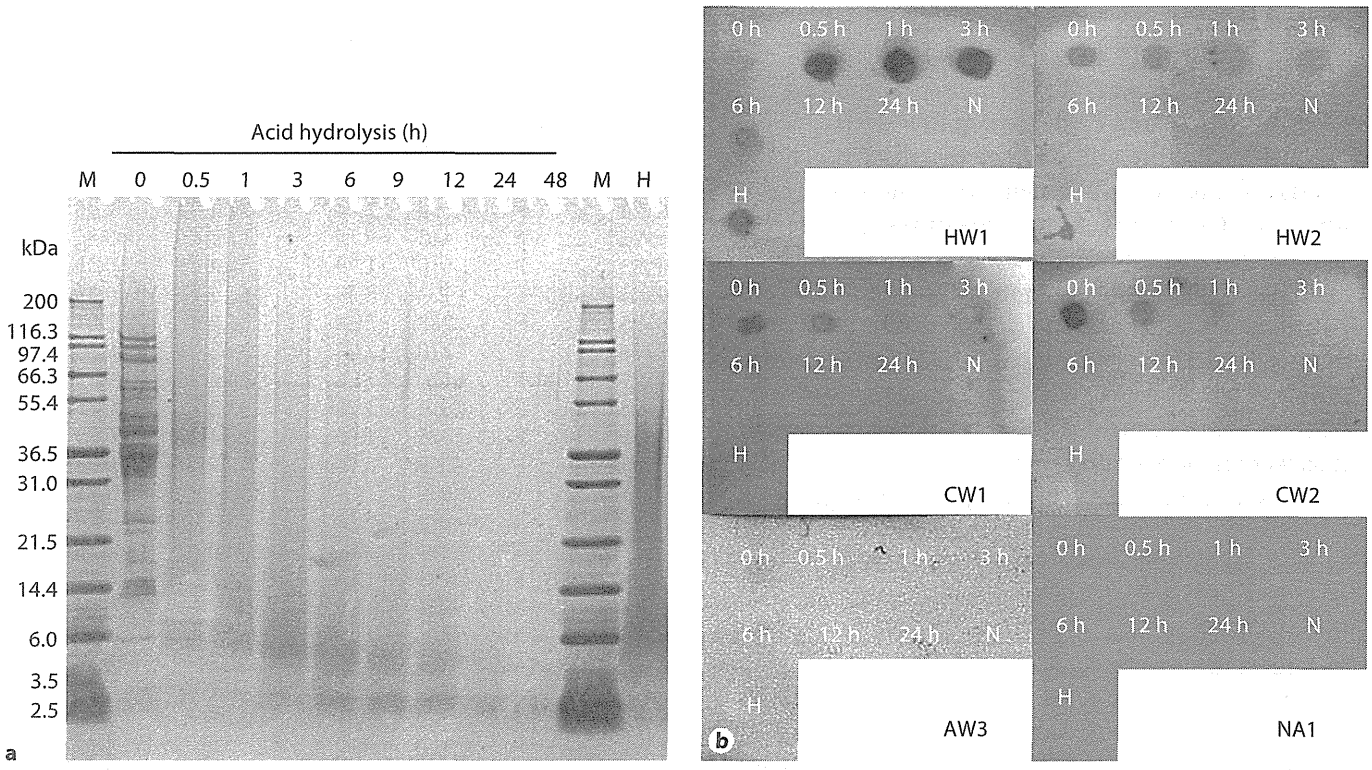

Fig. 1. Binding of IgE from subjects with wheat allergies to acid-HGs. **a** Coomassie brilliant blue-stained pattern of heat- and various time-treated acid-HGs on a 15–25% acrylamide gel. **b** Dot immunoblots of the gluten hydrolysates using sera from subjects sensitive to acid-HGs (HW1, 2), child wheat allergy (CW1, 2), adult WDEIA (AW3), and a nonatopic healthy person (NA1). M = Molecular weight marker; H = HWP1; N = PBS.

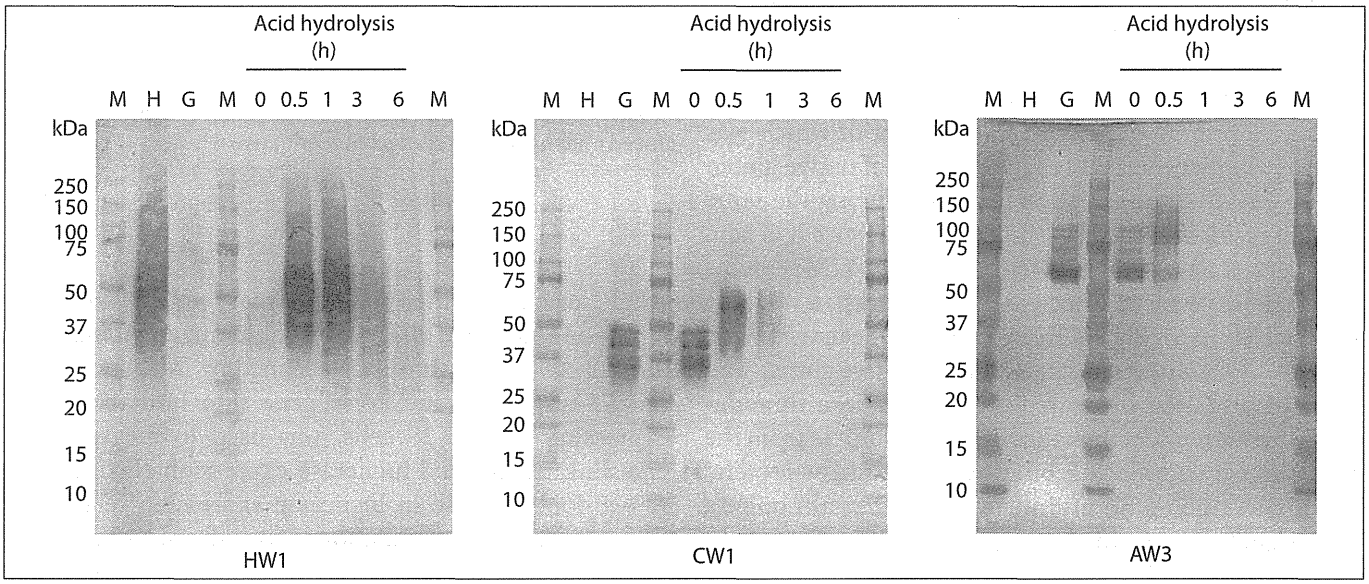

Fig. 2. Immunoblots of gluten hydrolysates using sera from subjects with wheat allergies. HWPs and gluten were separated on 10%–20% SDS-polyacrylamide gels and electronically transferred to nitrocellulose membranes, which were then incubated with serum diluted 5- (CW1, AW3) or 10-fold (HW-1). IgE-binding proteins were detected as HRP conjugates using color development. M = Molecular weight marker; H = HWP1; G = native gluten.

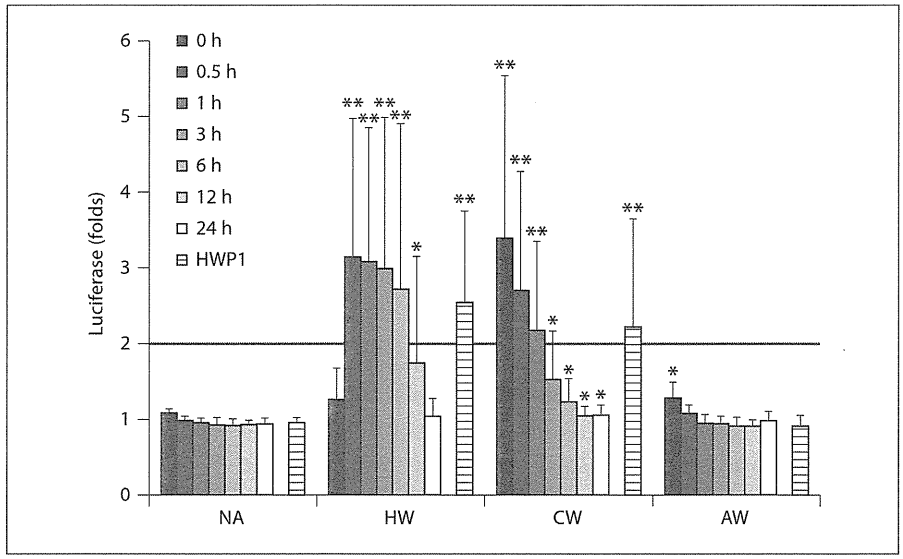

Fig. 3. In vitro elicitation (EXiLE) test using IgE in sera from subjects with wheat allergies. RS-ATL8 cells were sensitized with 1:100-diluted sera overnight and stimulated for 3 h with 100 ng/ml of acid-HGs or HWP1 suspended/dissolved in culture medium. Data are means ± SD (NA, n = 8; HW, n = 10; CW, n = 8, and AW, n = 9), shown as fold increases. The cut-off level (2-fold increase) is indicated by the gray line. * $p < 0.05$, ** $p < 0.01$ according to Steel's test vs. the NA group at a specific hydrolyzation period.

hydrolyzation of the gluten attenuated antigen-induced luciferase expression in a time-dependent manner for sera from native-wheat-sensitized pediatric subjects (CW4). On the other hand, in the sera samples from HW subjects, acid hydrolyzation of the gluten for 0.5 h dramatically increased luciferase expression, whereas native gluten had little effect on mast cell activation, suggesting the contribution of neo-epitopes. Even after prolonged hydrolyzation for 6 h, acid-HG still retained the ability to activate mast cells, with an activity level that was only slightly decreased. Contrary to the pediatric subjects, in the case of the adult WDEIA subject (AW), none of the HG, including HWP1, was capable of inducing mast cell activation.

Discussion

Although it is generally believed that hydrolysis reduces the allergenicity of allergenic protein, the number of studies that have shown that HWP induces immediate hypersensitivity via skin contact and/or food ingestion has increased [1–8]. The immunoreactivity of HWP has been reported by us and other groups [2, 6, 7]. Lauriere et al. [7] reported that the immunoblotting analysis of European patients' sera showed strong IgE binding to ω-1, ω-2, and γ-gliadin, but not to ω-5 gliadin. Bouchez-Mahiout et al. [6] reported that acid-HGs contained highly IgE-reacting high molecular weight entities and they have been suggested to have neo-epitopes which would be produced by rearrangements of hydrolyzed peptides. In the case of Japanese patients with HWP, their sera showed a low level of IgE-positive binding to ω-5 gliadin, and it seems that they were sensitized by gliadins other than ω-5 (or glutenins), which can be clearly distinguished from conventional WDEIA [2]. However, the detailed allergenicity of HWP has not yet been examined. In our present study, the acid hydrolyzation of gluten has generated not only prolonged IgE binding activity but also an ability to elicit mast cell activation in cases of sera from HW subjects (fig. 1–3). In contrast, IgE from native gluten-sensitized pediatric subjects showed gradually decreased reactivities to gluten acid hydrolysates time dependently, suggesting that native gluten epitopes, which were recognized by these IgE, were labile by acid- and heat-treatment. Acid lability of native gluten epitopes is consistent with previous reports that wheat proteins which were extensively hydrolyzed by acid could not inhibit the binding ability between the IgE antibodies in the patient sera and wheat proteins [11]. These results also suggested that the neo-epitopes in acid hydrolysates are relatively stable after prolonged acid- and heat-treatment.

There have been some previous reports regarding assessments of the allergenicity of processed foods. Díaz-Perales et al. [12] reported that some pepsin digestion-resistant peptides (MW, 1,400–5,100 Da) could bind to a patient's IgE, inducing allergic symptoms. Takagi et al. [13] also kinetically analyzed the digestion of ovomucoid in simulated gastric fluid and showed that in 21% of the examined patients, the capacity of their IgE to bind to the small 4.5-kDa fragment was retained. Furthermore, another reference reported an increase in allergenicity after the transglutaminase-mediated crosslinking of ω-5 gliadin [14]. In the case of HWP1, acid hydrolyzation seems

to have induced the physicochemical changes in gluten proteins after acid and heat treatment and some modifications to the amino acid molecules and protein aggregates. These changes may lead to an increase in the allergenicity of gluten. The remaining allergenicity of the 6-hour-treated acid-HG, as shown in figure 3, can be explained by the trace amount of persistent protein aggregates with a high molecular weight or by acid treatment-resistant peptide fragments with a lower molecular weight.

The results of the in vitro elicitation test (fig. 3) were consistent with those of the dot blot analysis (fig. 1b), suggesting that the antigen-specific IgE that bound to the hydrolyzed proteins with a lower molecular weight in the dot blot analysis might have the ability to crosslink to FcεRI on mast cells in the HW subjects. On the other hand, the AW3 subject had negative results for both the dot blot analysis (fig. 1b) and the EXiLE test (fig. 3), although strong IgE binding to ω-5 gliadin was observed in the Western blotting analysis (fig. 2c). This discrepancy can be explained by the difference in IgE accessibility to the antigen under denatured (Western blot) and nondenatured (dot blot and EXiLE) conditions. In addition, in previous studies describing allergenicity assessments of processed foods [12–14], ELISA and skin prick tests were mainly used. However, ELISA only provides binding information for IgE and the allergen, and skin prick tests are followed by a slight risk of anaphylaxis and sensitization. In this context, the EXiLE test may be a useful method for assessing the allergenicity of processed foods depending on IgE crosslinking-induced mast cell activation without any risk of anaphylaxis. Furthermore, we could confirm that the EXiLE system is a sensitive and convenient method to detect functional epitopes. The basophil activation test seems to be useful for similar purposes; however, this ex vivo test requires whole blood specimens which cannot be preserved for long periods.

In conclusion, this study showed production of neo-epitopes (and/or new arrangements of hidden epitopes) that are stable after prolonged acid and heat treatment. How such epitopes were generated by acid hydrolysis remains to be determined.

Acknowledgments

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References

1 Lauriére M, Pecquet C, Bouchez-Mahiout I, Snégaroff J, Bayrou O, Raison-Peyron N, Vigan M, Branlard G: Hydrolysed wheat proteins present in cosmetics can induce immediate hypersensitivities. Contact Dermatitis 2006;54:283–289.
2 Fukutomi Y, Itagaki Y, Taniguchi M, Saito A, Yasueda H, Nakazawa T, Hasegawa M, Nakamura H, Akiyama K: Rhinoconjunctival sensitization to hydrolyzed wheat induced luciferase expression protein in facial soap can induce wheat-dependent exercise-induced anaphylaxis. J Allergy Clin Immunol 2010;127:531–533.
3 Snégaroff J, Bouchez-Mahiout I, Pecquet C, Branlard G, Lauriére M: Study of IgE antigenic relationships in hypersensitivity to hydrolyzed wheat proteins and wheat-dependent exercise-induced anaphylaxis. Int Arch Allergy Immunol 2006;139:201–208.
4 Pelkonen AS, Mäkinen-kiljunen S, Hilvo S, Siltanen M, Mäkelä MJ: Severe allergic reaction to gluten hydrolysate without reaction to wheat. Ann Allergy Asthma Immunol 2011;106:343–344.
5 Chinuki Y, Kaneko S, Sakieda K, Murata S, Yoshida Y, Morita E: A case of wheat-dependent exercise-induced anaphylaxis sensitized with hydrolysed wheat protein in a soap. Contact Dermatitis 2011;65:43–58.
6 Bouchez-Mahiout I, Pecquet C, Kerre S, Snégaroff J, Raison-Peyron N, Lauriére M: High molecular weight entities in industrial wheat protein hydrolysates are immunoreactive with IgE from allergic patients. J Agric Food Chem 2010;58:4207–4215.
7 Lauriére M, Pecquet C, Boulenc É, Bouchez-Mahiout I, Snégaroff J, Choudat D, Raison-Peyron N, Vigan M, Branlard G: Genetic differences in omega-gliadins involved in two different immediate food hypersensitivities to wheat. Allergy 2007;62:890–896.
8 Ledue V, Moneret-Vautrin DA, Guerin L, Morrisset M, Kanny G: Anaphylaxis to wheat isolates: immunochemical study of a case proved by means of double-blind, placebo-controlled food challenge. J Allergy Clin Immunol 2003;111:897–899.
9 Nakamura R, Uchida Y, Higuchi M, Nakamura R, Tsuge I, Urisu A, Teshima R: A convenient and sensitive allergy test: IgE cross-linking-induced luciferase expression in cultured mast cells. Allergy 2010;65:1266–1273.
10 Nakamura R, Ishiwatari A, Higuchi M, Uchida U, Nakamura R, Kawakami H, Urisu A, Teshima R: Evaluation of the luciferase assay-based in vitro elicitation test for serum IgE. Allergol Int 2012;61:431–437.
11 Akiyama H, Sakata K, Yoshioka Y, Murata Y, Ishihara Y, Teshima R, Sawada J, Maitani T: Profile analysis and immunoglobulin E reactivity of wheat protein hydrolysates. Int Arch Allergy Immunol 2006;140:36–42.
12 Díaz-Perales A, Blanco C, Sánchez-Monge R, Varela J, Carrillo T, Salcedo G: Analysis of avocado allergen (Prs a 1) IgE-binding peptides generated by simulated gastric fluid digestion. J Allergy Clin Immunol 2003;112:1002–1007.
13 Takagi K, Teshima R, Okunuki H, Itoh S, Kawasaki N, Kawanishi T, Hayakawa T, Kohno Y, Urisu A, Sawada J: Kinetic analysis of pepsin digestion of chicken egg white ovomucoid and allergenic potential of pepsin fragments. Int Arch Allergy Immunol 2005;136:23–32.
14 Palosuo K, Varjonen E, Nurkkala J, Kalkkinen N, Harvima R, Reunala T, Alenius H: Transglutaminase-mediated cross-linking of a peptic fraction of omega-5 gliadin enhances IgE reactivity in wheat-dependent, exercise-induced anaphylaxis. J Allergy Clin Immunol 2003;111:1386–1392.

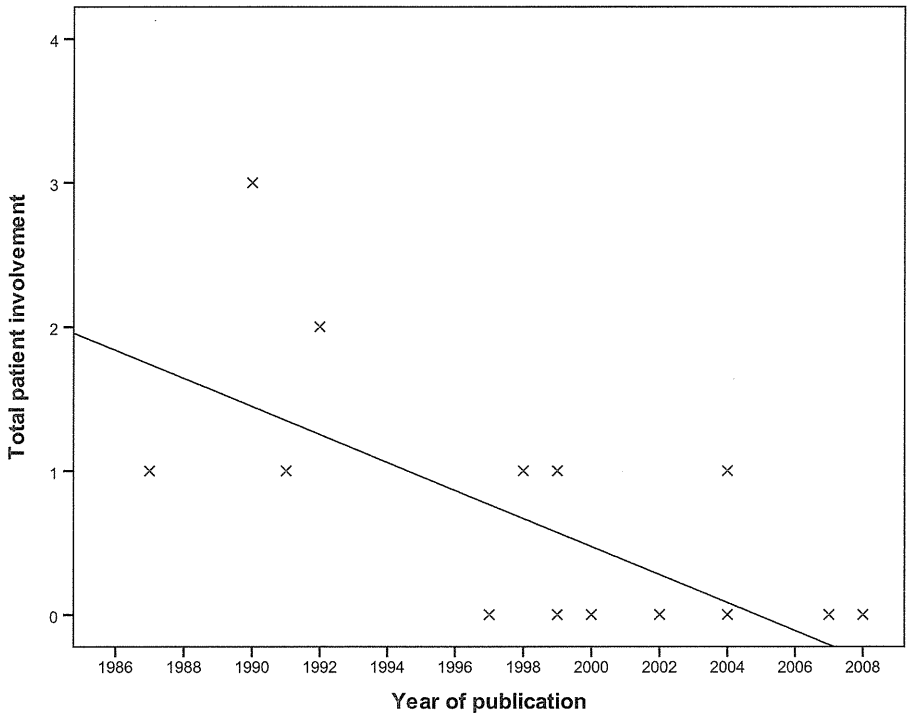

FIG 1. Patient involvement over time (1987 to 2008).

resulted in an improved PROM. However, this underscores our conclusion that future developers of asthma-specific PROMs should be attentive to appropriate patient involvement at all stages of the development cycle, and to documenting the nature of this involvement, if PROMs are to reflect health outcomes relevant to the patient.

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REFERENCES

1. Valderas JM, Alonso J. Patient-reported outcome measures: a model-based classification system for research and clinical practice. Qual Life Res 2008; 1125-35.
2. The Netherlands Cancer Institute. Assessing health status and quality-of-life instruments: attributes and review criteria. Qual Life Res 2002;11:193-205.
3. Staniszewska S, Haywood KL, Brett J, Tutton E. Patient and public involvement in PROMS: evolution not revolution. Patient 2012;5:79-87.
4. Apfelbacher CJ, Hankins M, Stenner P, Frew A, Smith H. Measuring quality of life in asthma: a systematic review. Allergy 2011;66:439-57.
5. Gibbons E, Fitzpatrick R. A structured review of patient-reported outcome measures for people with asthma: an update. Report to the Department of Public Health. Oxford, UK: Patient-reported Outcome Measurement Group; 2010.
6. Wilson SR, Rand CS, Cabana MD, Foggs MB, Halterman JS, Olson L, et al. Asthma outcomes: quality of life. J Allergy Clin Immunol 2012;29:S88-123.
7. Nilsen ES, Myrhaug HT, Johansen M, Oliver S, Oxman AD. Methods of consumer involvement in developing healthcare policy and research, clinical practice guidelines and patient information material. Cochrane Database of Systematic Reviews 2006, Art. No.: CD004563. DOI: 10.1002/14651858.CD004563.pub2.
8. Staniszewska S, Haywood KL, Brett J, Tutton L. Patient and public involvement in patient-reported outcome measures. The Patient: Patient-Centered Outcomes Research 2012;5:79-87.
9. Haywood KL, Staniszewska S, Chapman S. Quality and acceptability of patient-reported outcome measures used in chronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME): a systematic review. Qual Life Res 2011;21:35-52.
10. Van Oort L, Schröder C, French DP. What do people think about when they answer the Brief Illness Perception Questionnaire? A 'think-aloud' study. Br J Health Psychol 2012;16:231-45.
11. Branski RC, Cukier-Blaj S, Pusic A, Cano SJ, Klassen A, Mener D, et al. Measuring quality of life in dysphonic patients: a systematic review of content development in patient-reported outcomes measures. J Voice 2010;24:193-8.
12. INVOLVE (2012) Briefing notes for researchers: involving the public in NHS, public health and social care research. Eastleigh, UK: INVOLVE; 2012.

Available online August 31, 2013.
http://dx.doi.org/10.1016/j.jaci.2013.05.047

Tissue transglutaminase generates deamidated epitopes on gluten, increasing reactivity with hydrolyzed wheat protein–sensitized IgE

To the Editor:

Exercise-induced systemic allergy after wheat ingestion experienced by "Cha no Shizuku" facial soap users has become a serious problem in Japan.[1,2] The soap contained the acid-hydrolyzed wheat protein (HWP) Glupearl 19S (Glp19S), and soap sensitization can be diagnosed by using Glp19S-specific IgE measurement, a skin prick test (SPT) for Glp19S, or both. HWP-sensitized subjects are generally less responsive to wheat extract on SPTs, although they exhibit obvious clinical symptoms after wheat ingestion and some exercise.[1] However, it is unclear why HWP-sensitized subjects exhibit systemic allergic reactions after ingestion of normal wheat that does not contain HWP.

We recently developed a cell-based IgE reactivity (mast cell–activating) assay that measures IgE crosslinking–induced

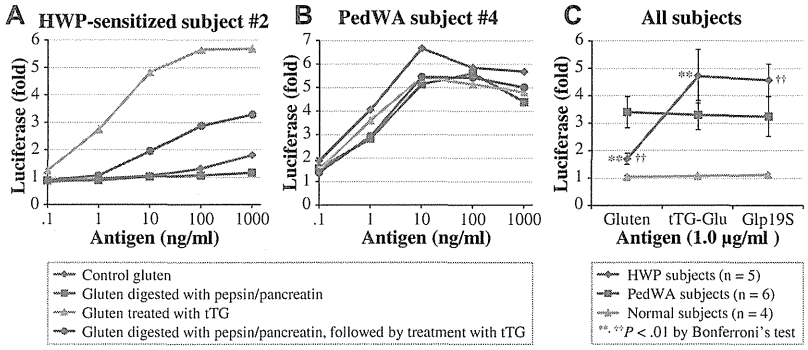

FIG 1. Typical EXiLE responses of serum IgE from HWP-sensitized subject **(A)** and patient with PedWA **(B)** to various treated glutens and EXiLE responses of all subjects (HWP-sensitized subjects, patients with PedWA, and healthy subjects) presented in Table E1 to gluten, gluten treated with tTG (tTG-Glu), and Glp19S **(C)**. Values are presented as mean ± SEMs. **, ††$P < .01$, n-way ANOVA and the *post hoc* test with the Bonferroni correction.

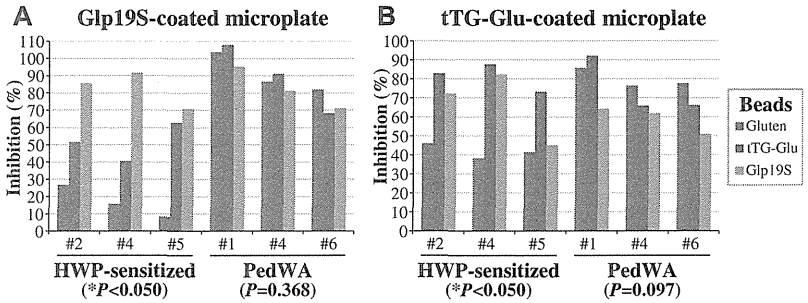

FIG 2. IgE ELISA inhibition with antigen-coated beads. Diluted sera from HWP-sensitized subjects and patients with PedWA were treated with antigen-coated beads for 2 hours, followed by centrifugation. The Glp19S-specific **(A)** or tTG-Glu–specific **(B)** IgE remaining in the supernatants was measured by using ELISA. Data are means of triplicate measurements and are shown as percentages of binding inhibition. *P* values of the Friedman test are shown.

luciferase expression (EXiLE).[3,4] This new method demonstrated that the IgE reactivity of the HWP-sensitized subjects to acid-hydrolyzed gluten differed from that of patients with pediatric wheat food allergy (PedWA).[5] HWP IgE was less reactive to native gluten but strongly reactive to hydrolyzed gluten, whereas IgE from patients with PedWA reacted to both. Binding of IgE of the HWP-sensitized subjects to gluten increased markedly after 30 minutes of 0.1 N hydrochloric acid treatment at 100°C, indicating neoepitope generation on the gluten molecules. These results suggest that orally ingested gluten might be metabolized *in vivo* to acquire cross-reactivity with the industrially generated acid HWP Glp19S.

We therefore compared the IgE reactivities of HWP-sensitized subjects and patients with PedWA using EXiLE assays with glutens treated with gastrointestinal enzymes, such as pepsin, pancreatin, and tissue transglutaminase (tTG). Gluten was treated with these enzymes according to the method of Palosuo et al,[6] with some modifications (see the Methods section in this article's Online Repository at www.jacionline.org). Clinical profiles of the study subjects are summarized in Table E1 in this article's Online Repository at www.jacionline.org. Three (60%) of the HWP-sensitized subjects had negative SPT responses to wheat, although all presented with systemic allergic reactions after wheat ingestion. Humanized rat RS-ATL8 mast cells[3-5] were sensitized with 1:100 dilutions of the subjects' sera overnight and stimulated for 3 hours with the treated glutens and Glp19S (Katayama Chemical Industries, Osaka, Japan) suspended in minimum essential medium supplemented with 10% FCS.

Pepsin and pancreatin digestion did not increase the reaction of the gluten with IgEs from either of the subject groups (Fig 1). HWP IgE showed low and no responses to intact and digested gluten, respectively, but IgE from patients with PedWA reacted strongly to both. Surprisingly, however, tTG treatment dramatically increased the reactivity of HWP IgE to intact and digested gluten without affecting the response of IgE from patients with PedWA. IgE immunoblotting also showed strong binding of HWP IgE to high-molecular-weight (HMW) components of tTG-treated glutens (tTG-Glu), with a characteristic smear pattern similar to that of Glp19S binding (see Fig E1 in this article's Online Repository at www.jacionline.org). Although the IgE of this patient (patient 2; gluten IgE, 36.2 UA/mL) recognized not only HMW components but also a 12- to 13-kDa undigested protein, which was considered to be an α-amylase inhibitor, she was less responsive to natural gluten, as revealed by using the EXiLE test (Fig 1, *A*). Considering that the EXiLE test can distinguish between binding and cross-linking of IgE with the antigen,[3,4] it appears reasonable to conclude that the IgE-responsive antigens were the HMW components rather than the 12- to 13-kDa proteins. These results indicate that tTG treatment generates neoepitopes on gluten molecules (even after digestion) in HWP-sensitized subjects, and these epitopes have sufficient affinity to cross-link IgE and induce mast cell activation.

Next, we performed inhibition ELISAs by using serum samples from selected patients and antigen-coated polystyrene beads

(see the Methods section in this article's Online Repository). Antigen-specific IgE was adsorbed to antigen-coated beads, and the remaining IgE in the supernatant was measured by using ELISA. When Glp19S was coated on an ELISA microplate, the binding of HWP IgE was strongly inhibited (71% to 92%) by the Glp19S-coated beads and moderately inhibited (41% to 63%) by the tTG-Glu–coated beads; however, untreated gluten-coated beads were less effective (<27%) at inhibiting the binding (Fig 2, *A*). In contrast, all the beads exhibited strong inhibition (68% to 108%) with IgE from patients with PedWA. However, when tTG-Glu was coated on the plates, the gluten- and tTG-Glu–coated beads inhibited the binding of HWP IgE moderately (38% to 46%) and strongly (78% to 88%), respectively (Fig 2, *B*). Glp19S-coated beads used in HWP-sensitized subjects and gluten/tTG-Glu/Glp19S–coated beads used in patients with PedWA (Fig 2, *B*) exhibited inhibition similar to that seen in Fig 2, *A*. These results indicate that tTG treatment of gluten generates IgE epitopes that cross-react with Glp19S, although tTG-Glu at least partially retains gluten-like structures. These results also suggest that IgE from patients with PedWA binds to tTG-insensitive structures in gluten, which are distinct from HWP IgE epitopes.

Many celiac disease studies have shown that digestion-resistant peptide fragments of wheat gluten can access the lamina propria and be deamidated by tTG.[7] However, it is also well known that gluten is deamidated during hydrolysis under acidic and heating conditions.[8] Therefore we performed limited proteolysis of gluten, tTG-Glu, and Glp19S with the endoproteinase Glu-C, which cleaves at glutamic acid residues in peptides (see the Methods section in this article's Online Repository). tTG treatment induced a mobility shift of gluten with a smear pattern, which was completely eliminated by Glu-C treatment (see Fig E2 in this article's Online Repository at www.jacionline.org). An increase in negatively charged glutamyl residues because of deamidation of glutamine residues might induce conformational changes in gluten, causing the observed mobility shift in SDS-PAGE.[8] The Glu-C–resistant components in the gluten and tTG-Glu were similar, suggesting that part of tTG-Glu remains unaffected by tTG treatment.

In Japan more than 1800 subjects have been confirmed as being sensitized to Glp19S-containing "Cha no Shizuku" soap as of February 2013. After recall of the soap by the distributor, gluten- and Glp19S-specific IgE levels have decreased in the sera of most patients.[9] However, many patients continue to experience allergic symptoms elicited by wheat ingestion, even after having negative serum gluten-specific IgE levels. We propose that patients with high Glp19S-specific IgE levels should avoid wheat ingestion, even if their gluten-specific IgE levels have decreased, because orally ingested wheat gluten can be deamidated by tTG after digestion, resulting in the generation of IgE epitopes that cross-react with Glp19S.

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REFERENCES

1. Fukutomi Y, Itagaki Y, Taniguchi M, Saito A, Yasueda H, Nakazawa T, et al. Rhinoconjunctival sensitization to hydrolyzed wheat protein in facial soap can induce wheat-dependent exercise-induced anaphylaxis. J Allergy Clin Immunol 2011;127:531-3.
2. Chinuki Y, Kaneko S, Dekio I, Takahashi H, Tokuda R, Nagao M, et al. CD203c expression–based basophil activation test for diagnosis of wheat-dependent exercise-induced anaphylaxis. J Allergy Clin Immunol 2012;129:1404-6.
3. Nakamura R, Uchida Y, Higuchi M, Nakamura R, Tsuge I, Urisu A, et al. A convenient and sensitive allergy test: IgE crosslinking-induced luciferase expression in cultured mast cells. Allergy 2010;65:1266-73.
4. Nakamura R, Ishiwatari A, Higuchi M, Uchida Y, Nakamura R, Kawakami H, et al. Evaluation of the luciferase assay-based in vitro elicitation test for serum IgE. Allergol Int 2012;61:431-7.
5. Nakamura R, Nakamura R, Adachi R, Itagaki Y, Fukutomi Y, Teshima R. Evaluation of allergenicity of acid-hydrolyzed wheat protein using an in vitro elicitation test. Int Arch Allergy Immunol 2013;160:259-64.
6. Palosuo K, Varjonen E, Nurkkala J, Kalkkinen N, Harvima R, Reunala T, 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 2003;111:1386-92.
7. Caja S, Mäki M, Kaukinen K, Lindfors K. Antibodies in celiac disease: implications beyond diagnostics. Cell Mol Immunol 2011;8:103-9.
8. Yildiz F, editor. Advances in food biochemistry. Boca Raton (FL): CRC Press; 2009.
9. Japanese Society of Allergology Meeting Report of the Special Committee for the Safety of Protein Hydrolysates in Cosmetics: 6th meeting (Oct. 8, 2012). Available at: http://www.jsaweb.jp/modules/en/index.php?content_id=11. Accessed March 11, 2011.

Available online August 30, 2013.
http://dx.doi.org/10.1016/j.jaci.2013.07.017

Acid-suppressive drug use in pregnancy and the toddler's asthma risk: A crossover, case-control study

To the Editor:

Acid-suppressive drugs (ASDs) are considered effective and safe to use during pregnancy to treat gastroesophageal reflux disease. Three recent studies reported a causal relation between prenatal exposure to ASDs and the development of childhood asthma, but unmeasured confounding could not be ruled out.[1-3] We applied a bidirectional case-crossover design to minimize time-invariant confounding influences using siblings from the same mother as control subjects and compared our findings with those of a case-control design.

The studies were performed with data from a pregnancy database as part of the University Groningen IADB.nl pharmacy prescription database (for more information, see the Methods section in this article's Online Repository at www.jacionline.org).[4] Because case-crossover designs can be vulnerable to time trends, we first examined the time trend in exposure to ASDs (Anatomical Therapeutic Chemical [ATC] code A02B) among all registered singleton pregnancies in the database between 1995 and 2006. For both the bidirectional case-crossover and case-control designs, the study population was restricted to toddlers of singleton birth between 1995 and 2006 who could

REVIEW

Pediatric allergy and immunology in Japan

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Abstract

The Japanese Society of Pediatric Allergy and Clinical Immunology (JSPACI) was started in 1966 and currently has 3613 members as of August 1, 2012. The number of pediatricians specializing in allergies who have been certified by the Japanese Society of Allergology is 817. Among these, there are 125 training directors and training facilities for allergy and clinical immunology. The JSPACI first published an asthma guideline specific for children in 2000, and this has been revised every 3 yrs, contributing to better control of pediatric asthma. Food allergy management guidelines were first developed in 2005, which have helped to improve the care of food allergy patients. Among 514 pediatric training programs by the Japanese Society of Pediatrics, there are 312 facilities routinely performing oral food challenges. Among these, there were already 53 facilities performing oral immunotherapy at the end of 2011, treating 1400 cases of food allergy. The prevalence of pediatric allergic diseases has increased in Japan over the past 50 yrs. A number of International Study of Asthma and Allergies in Childhood surveys have been conducted in the past at specific times. The prevalence of wheezing among children aged 13–14 yrs in 2002 was 13.0%. Multi-year surveys found a 1.5- to 2-fold increase every 10 yrs until 2002. However, according to the latest data in 2012, asthma prevalence seems to have slightly decreased in Japan. Food allergy mainly associated with infantile atopic eczema among infants younger than 1 yr of age is the most common form as with other developed countries. The estimated food allergy prevalence based on data from several surveys is 5–10% among infants (0–6 yrs) and 1–2% among schoolchildren (6–15 yrs). A variety of patients suffering from primary deficiency syndrome have been actively analyzed. Previously, antibody defects and well-defined syndromes with immunodeficiency were analyzed, but recent research is focusing on not only acquired immune disorders but also on innate immune disorders. In contrast to the widespread use of oral immunotherapy, one immediate issue is to develop and reassess subcutaneous and sublingual immunotherapies for mite and Japanese cedar pollen antigens that have been disused in Japan since the 1990s.

Historical background of the Japanese Society of Pediatric Allergy and Clinical Immunology

The Japanese Society of Pediatric Allergy and Clinical Immunology (JSPACI) was started in 1966 under the name of 'Pediatric Allergy Meeting' as a sectional meeting of the Japanese Society of Pediatrics (Table 1). It was renamed JSPACI in 1986 and commenced biannual publication of a domestic journal in 1987. The journal was published four times a year from 1991 and then subsequently increased to five times a year in 2001. 'Japanese Pediatric Guideline for the Treatment and Management of Asthma (JPGL)' was published in 2000; it has been revised nearly every 3 yrs (1) and the latest version is JPGL2012. 'Japanese Pediatric Guideline for Food Allergy' was first published in 2005 (2) and revised in 2011. 'The General Guideline for Pediatric Allergic Diseases', a synthesized guideline for bronchial asthma, allergic rhinitis, atopic dermatitis, and food allergy, was published in 2011.

The first academic meeting was held in 1966 in Tokyo, and the 50th anniversary meeting will be in Yokohama in October 2013. The sixth and present JSPACI President is Professor N. Kondo (Gifu University); the first president was T. Matsumura, followed in succession by Y. Nakayama, M. Baba, S. Nishima, and A. Morikawa.

Table 1 Japanese Society of Pediatric Allergy and Clinical Immunology chronology

			Journal		Annual meeting	
Year	Events	President	JSPACI journal	Guideline, etc.		Place
1966	Pediatric Allergy Association was founded	Matsumura T			1st	Tokyo
1975		Nakayama Y			12th	Fukuoka
1981		Baba M			18th	Yokohama
1986	Renamed as JSPACI				23rd	Kitakyushu
1987			2 Journals/yr		24th	Tokyo
1991			4 Journals/yr		28th	Saitama
1994	Otsuka academic article award started				31st	Utsunomiya
1997		Nishima S			34th	Tokyo
1998	Office relocation (Tokyo to Fukuoka)				35th	Osaka
2000				JPGL2000	37th	Maebashi
2001			5 Journals/yr		38th	Kitakyushu
2002	The Web site of JSPACI was established			JPGL2002	39th	Morioka
2004	The best article award of JSPACI started				41st	Tokyo
2005	The clinical research encouraging award was established	Morikawa A		JPGL2005 JPG for Food Allergy 2005	42nd	Fukui
2006	Office relocation (Fukuoka to Gunma)				43rd	Chiba
2008		Kondo N		JPGL2008	45th	Yokohama
2009	Office relocation (Gunma to Gifu)			JPG for OFC in Food Allergy 2009	46th	Fukuoka
2011	Office relocation (Gifu to Tokyo) Joint congress with 16th APAPARI			Pediatric Allergy Guideline 2011 JPGL2012 JPG for Food Allergy 2012 Brochure for Allergic children during disaster	48th	Fukuoka
2013					50th	Yokohama

JSPACI: Japanese Society of Pediatric Allergy and Clinical Immunology; JPGL: Japanese Pediatric Guideline for the Treatment and Management of Asthma; APAPARI: Asia Pacific Association of Pediatric Allergy, Respirology & Immunology; JPG: Japanese Pediatric Guideline.

Overview of JSPACI

JSPACI aims to help the science and medicine of pediatric allergy and related areas to advance and to disseminate information. The society also aims to promote pediatric health. Studies are conducted in a wide variety of areas: allergic diseases such as bronchial asthma, atopic dermatitis, food allergy, drug allergy, allergic rhinitis, and anaphylaxis, as well as immune disorders such as autoimmune disease, hereditary immunodeficiency disease, infectious disease, and respiratory disease. Its constituent members are general practitioners interested in the area of pediatric allergy, clinical doctors specializing in pediatric allergy, clinical and basic researchers, and paramedical staff. Through basic research, clinical research, and epidemiologic studies, the society makes efforts to explicate the causes and states of diseases; to develop systems of diagnosis, therapeutic management, and prognostic factors, as well as precautions; to cooperate with nurses, pharmacists, and dieticians; and to communicate with patients. It also takes part in preventive medicine such as vaccinations, in maternal and child health, and in school health.

To achieve its goals, the society performs the following activities:

1 Holding an academic meeting once a year,
2 Publishing journals (five times a year),
3 Holding research meetings, short courses, and lectures,
4 Facilitating communication between members,
5 Coordinating with related organizations, both domestic and abroad, and
6 Performing other tasks necessary to achieve its goals.

The society had 3613 members as of August 1, 2012, and the number of members has been increasing annually. The organization consists of a board of directors, a board of councilors, and the general assembly. To promote its tasks efficiently, ten committees have been established: the Editorial Committee, Rules and Regulations committee, Epidemiology Committee,

Health Insurance Committee, Food Allergy Committee, Pharmaceutical Affairs Committee, Guideline Committee for Therapeutic Management of Asthma, Research Promotion Committee, International Exchange Committee, and Conflict of Interest Committee. In addition, the society has set up formal working groups to correspond quickly and appropriately to occasional situations, such as a working group to cope with the new influenza strain in 2009, another group responsible for drawing up 'The General Guidelines for Pediatric Allergic Diseases' in 2010, and another for coping with the Great East Japan Earthquake in 2011. This last working group not only provided support for the victims of the earthquake but also created a pamphlet titled 'The Treatment of Pediatric Allergic Diseases in Times of Disasters (Japanese and English version)' (JSPACI: http://www.jspaci.jp/). The editor in chief at PAI (Prof. U Wahn) also generously expressed sympathy over this tragedy, and we were deeply impressed by his message (3). The secretariat moved from Gifu to Tokyo, and at present, there are only a few staff members.

The number of pediatricians specializing in allergies who have been certified by the Japanese Society of Allergology (JSA) is 817 in Japan, and among these, there are 125 training directors in allergy. There are 126 training facilities in Japan, and the distribution of these training facilities and specialists in pediatric allergy is shown in Fig. 1. The present population of Japan is about 127 million. The figure shows that specialists and training facilities are centered around large cities, and the distribution of allergy training programs is closely related to population distribution. Pediatric board-certified doctors become eligible to be allergy specialists after 3 yrs' training at allergy training facilities. JSA also requires a board examination qualification after the training. JSA board-certified pediatric allergy specialists are required to renew their certification every 5 yrs by collecting continuing medical education credits.

Allergy practice in Japan

The Japanese medical system and the position of allergists

The system of public health insurance for the whole nation has been maintained in Japan for 50 yrs and is effective in the restraint of medical expenses. The ratio of medical expenses to the total gross domestic product in Japan is low in rank among member nations of the Organization for Economic Cooperation and Development. The fee a doctor receives is fixed regardless of doctor's length of experience. Thus, doctors are being unfairly compensated to achieve control of medical costs. Although private practice is customary in Europe and the United States, it is seldom found in Japan except in the field of cosmetic surgery. While 4000 or more allergy specialists are authorized by JSA, insurance institutional financial incentives to medical specialists do not exist in Japan. Even without a referral from a primary physician, a patient is able to consult an allergist freely; this is called a free-access system. It enables quite recently medical specialists to advertise the possession of specialist medical qualifications. In Japan, when doctors start their own clinic, they are allowed to advertise freely their practice in any field of medicine other than anesthesiology. Some practitioners advertise as allergists, and patients are consulting these doctors without realizing that they lack specialty training and proper qualification. Because JSA and JSCAPI have prepared guidelines for the treatment of a variety of allergic diseases,

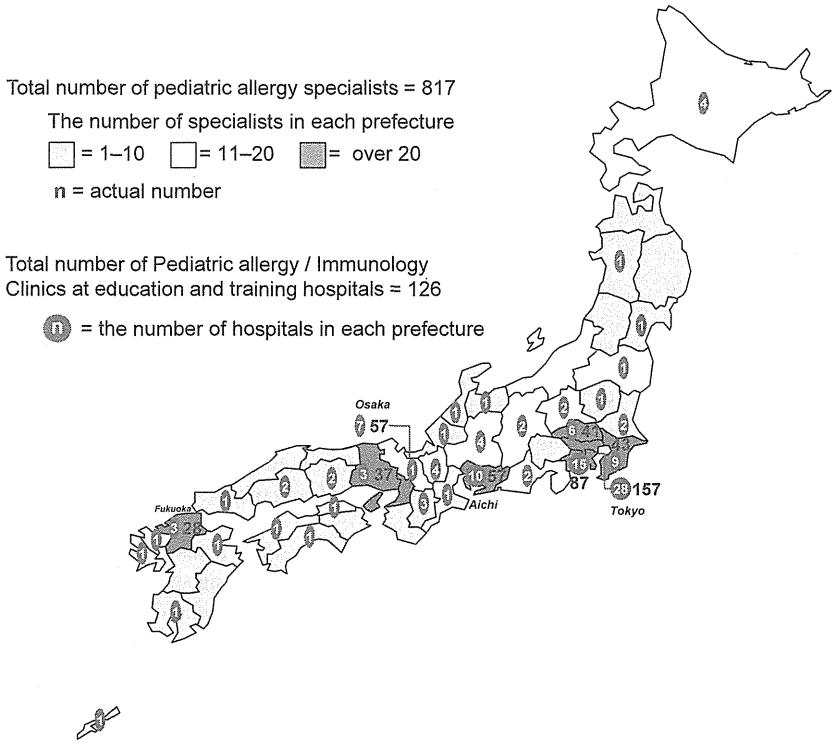

Figure 1 Number of training programs and specialists. The number of pediatricians specializing in allergies in Japan who have been certified by the Japanese Society of Allergology is 817. There are 126 training facilities in the whole country. The distribution of training facilities and specialists in pediatric allergy in Japan is shown.

over the past 10 yrs, general practitioners have been able to treat allergic diseases to some extent.

Allergy diagnosis and allergen-specific immunotherapy

By global standards, a doctor who diagnoses allergic disease correctly and performs allergen-specific immunotherapy is usually regarded as an allergist, but not necessarily in Japan. In Japan, allergenic extracts for diagnostic and treatment purposes have been manufactured and sold since 1963 by only one company (TORII Pharmaceutical Co., Ltd. Tokyo, Japan) in the domestic supply system. Because a wide range of antigen-specific IgE antibody tests is covered by health insurance, these tests are more commonly conducted than skin prick tests. The targets of antigen-specific subcutaneous immunotherapy (SCIT) are atopic asthma (caused by house dust mites) and allergic rhinitis (caused by house dust mites and Japanese cedar pollen) in Japan, and immunotherapy has been widely used from the 1960s to the middle of the 1980s. The development of oral anti-allergic drugs prospered in the 1980s in Japan, led by disodium cromoglycate to treat bronchial asthma. Allergen-specific immunotherapy gradually went out of use in the mid-1980s, although the medical cost of the therapy remained low for many years and the therapy required regular injections. After the 1990s, topical steroids such as inhaled corticosteroids (ICS), second-generation antihistamine drugs, and leukotriene receptor antagonists (LTRA) were recommended by professional society guidelines and the use of SCIT waned. Although oral immunotherapy (OIT) for food allergies is widely performed in Japan (see section 'Current Treatment Approaches'), the efficacy of SCIT for aeroallergens should be re-evaluated; furthermore, sublingual immunotherapy (SLIT) is still emerging.

History

Bronchial asthma

From a historical point of view, the treatment for pediatric bronchial asthma in Japan has greatly changed over time. In the 1960s, asthma prevalence began to rise, and its causes were mainly attributed to its relationship with air pollution or psychosocial aspects. Treatment was mostly directed at alleviating symptoms with the inhalation of a short-acting β-agonist or oral administration, injection, or intravenous infusion of aminophylline. Subcutaneous immunotherapy (SCIT) at low doses for house dust was used as a long-term treatment for bronchial asthma. At that time, there was the first epidemic of asthma deaths in developed countries, including Japan.

In the 1970s and 1980s, long-term hospitalization treatment expanded throughout the country for patients with severe asthma for whom treatment at outpatient clinics was difficult. Over 3000 schoolchildren received such treatment in hospitals. The mainstream treatment was SCIT at low doses for house dust, inhalation of disodium cromoglycate, slow release of theophylline, and oral administration of an anti-allergic drug that is unique to our country. After the second epidemic of asthmatic deaths around 1990 (4), ICS began to be used. After publication of JPGL in 2000 (5), ICS became established as the first-choice treatment. As a result, the number of hospitalizations due to asthma attack and the number of asthmatic deaths decreased dramatically, coupled with the appearance of LTRA on the market and its increased use.

Food allergy

In addition to pediatric asthma, issues related to food allergy were also taken up at the Pediatric Allergy Meetings from the beginning of its establishment in 1966. However, bronchial asthma was the central subject of this society from those days until the early 2000s, and food allergy remained in the background with only a small number of doctors seeing patients in their own way. Antigen-specific IgE antibody measurements came to be covered by insurance in the 1980s. Because this test was positive for various food antigens, many doctors advised avoiding these foods without confirmation of efficacy. The turning point in food allergy practice in our country occurred at the beginning of the 21st century. This was the result of many activities such as a nationwide survey of immediate types of food allergy, the start of food allergy labeling in 2001 (6), the establishment of an oral food challenge (OFC) network supported by a research grant from the Ministry of Health, Labor, and Welfare (MHLW), the publication of food allergy management guideline in 2005 by a research grant from MHLW (7), and the approval of OFC by the national health insurance system. Since the 2000s, the practice, but not research on food allergy diagnosis and treatment in our country, has become one of the most advanced in the world.

Pediatric clinical immunology

Primary immunodeficiency syndromes (PID) are now classified into eight disease categories by the International Union of Immunological Societies (IUIS) (8). A variety of patients suffering from primary deficiency syndrome have been actively analyzed in Japan. In the past, antibody defects and well-defined syndromes with immunodeficiency such as Bloom syndrome and ataxia-telangiectasia syndrome were analyzed (9–11). However, recent research is focusing not only acquired immune disorders but also innate immune disorders such as IRAK4 deficiency, anhidrotic ectodermal dysplasia with immunodeficiency (EDA-ID), and autoinflammatory disorders.

Epidemiology

Bronchial asthma

Prevalence

Asthma prevalence has been rapidly increasing in recent years. The International Study of Asthma and Allergies in Childhood survey was conducted in Fukuoka City and Tochigi Prefecture and across Japan to examine the prevalence of asthma at specific times (12). The prevalence in Japan was comparable with or slightly lower than the prevalence in the United Kingdom, Australia, New Zealand, Canada, and USA (e.g., Fukuoka City, 13%; Tochigi Prefecture, 19%). According to a survey conducted over several years, in which the same

physicians used the same protocol in subjects with the same background, a 1.5- to 2-fold increase was reported every 10 yrs (Fig. 2) (12). However, according to the latest data, asthma prevalence seems to be decreasing, as shown in Fig. 2. Regarding other allergic diseases, the prevalence of atopic dermatitis is also decreasing among school children. Conversely, the prevalence of allergic rhinitis and allergic conjunctivitis is increasing, and the reason for the increase can be attributed to a sharp increase in pollinosis prevalence.

Asthma death

The incidence of childhood death due to asthma has decreased dramatically in the past 10 yrs (Fig. 3). In 2012, there were only three deaths of about 16.7 million children (aged 0–14 yrs) (the death rate was 0.02 per 100,000). The main reasons for the phenomenon are thought to be as follows: (i) the regularly published JPGL contributes to equal accessibility of medical treatment; (ii) the use of controllers, mainly ICS and LTRA, has become widespread, especially as early treatment; (iii) an emergency medical treatment system during nighttime and holidays has been provided throughout the country; (iv) medical expenses for children are free or reduced.

Food allergy

Prevalence

Food allergy is common among infants younger than 1 yr of age and decreases with aging, which indicates that tolerance develops with aging. The estimated prevalence in Japan is 5–10% among infants and 1–2% among schoolchildren (7).

Causative food allergens

We prospectively investigated the immediate-type food allergy cases in collaboration with more than 2000 doctors between 2001 and 2002 (6). The contributing doctors included those working in hospitals with more than 200 beds as well as allergy specialists working in clinics. Contributing doctors were asked to respond to a questionnaire every 3 months for 2 yrs from 2001 to 2002 and report immediate-type food allergy cases by mail. By only focusing on the immediate-type food allergy, cases were restricted to those in which symptoms occurred within 60 min after ingestion of the suspected food. A total of 3882 cases were reported within the 2-yr period (Table 2). The patients ranged in age from 0 to 80 yrs, with 50% (1969) of them <2 yrs of age. The most common cause of food allergy was hens' eggs (38.3%), followed by cows' milk (15.9%), wheat (8%), shellfish (6.2%), fruits (6%), buckwheat (4.6%), fish (4.4%), and peanuts (2.8%). Notably, the cause of food allergy differed greatly among age groups.

Food-induced anaphylaxis was seen in 10.9% of the reported cases. Hens' eggs, cows' milk and dairy products, wheat, buckwheat, and peanuts were the major causes of food-induced anaphylaxis in Japan. This prospective investigation into immediate-type food allergies has been repeated every 3 yrs as a means of monitoring the condition of food allergies in Japan.

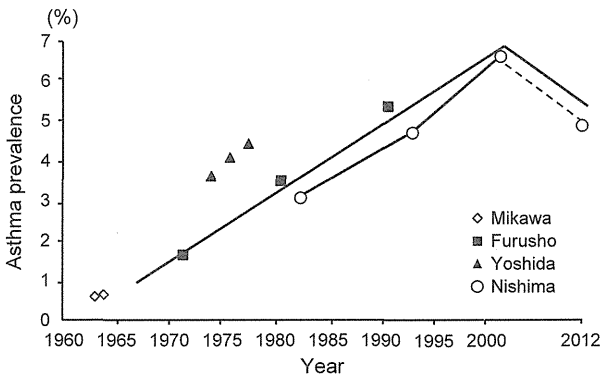

Figure 2 Changes over years in the prevalence of bronchial asthma in Japanese elementary school children reported by four investigators. According to a multi-year survey in which the same physicians used the same protocol in subjects with the same background, a 1.5- to 2-fold increase was reported every 10 yrs. According to the latest data in 2012, asthma prevalence seems to be decreasing.

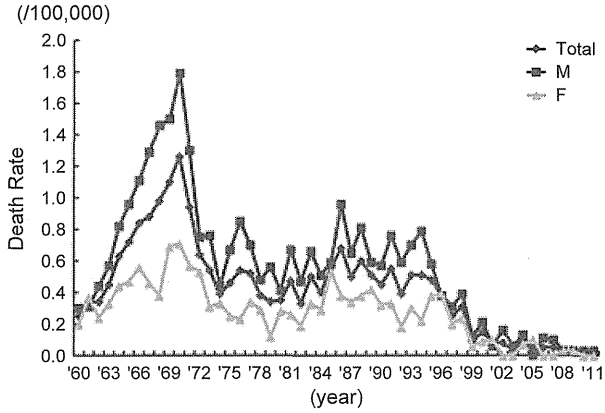

Figure 3 Mortality from asthma in children of 10–14 yrs of age from 1960 to 2011. The incidence of childhood death due to asthma has decreased dramatically in the past 10 yrs. In 2012, there were only three deaths of about 16.7 million children (age 0–14 yrs) (the death rate was 0.02 per 100,000).

The prevalence of other allergic diseases in elementary school

Total population questionnaire surveys were conducted 15 times from 1975 to 2006 for all children attending public elementary schools in Japan, with the number of subjects ranging from 460,000 to 900,000. The lifetime prevalence of atopic dermatitis increased to 24% by 1993 and then decreased. The prevalence of rhinitis increased to 25% by 2003, whereas the prevalence of non-seasonal symptoms plateaued from 1993 onwards at 11% and vernal symptoms increased. The prevalence of itchy eyes continued to increase to 21% in 2006, and vernal symptoms increased sharply (13). The most common cause of seasonal rhinitis is Japanese cedar pollen (vernal), and its prevalence exceeds 30% of total population.

Table 2 Causative food allergens in each age group in 2001 and 2002 survey

Causative foods	Total cases (%)	0 year	1 year	−3 year	−6 year	−19 year	≥20 year
Eggs	1486 (38.3)	789 (62.1)	312 (44.6)	179 (30.1)	106 (23.3)	76 (15.2)	24 (6.6)
Milk products	616 (15.9)	255 (20.1)	111 (15.9)	117 (19.7)	84 (18.5)	41 (8.2)	8 (2.2)
Wheat	311 (8.0)	90 (7.1)	49 (7.0)	46 (7.7)	24 (5.3)	48 (9.6)	54 (14.8)
Fruits	232 (6.0)	40 (3.1)	30 (4.3)	30 (5.1)	40 (8.8)	45 (9.0)	47 (12.8)
Buckwheat	179 (4.6)	4 (0.3)	23 (3.3)	45 (7.6)	27 (5.9)	54 (10.8)	26 (7.1)
Fish	171 (4.4)	21 (1.7)	32 (4.6)	22 (3.7)	18 (4.0)	37 (7.4)	41 (11.2)
Shrimp	161 (4.1)	4 (0.3)	10 (1.4)	20 (3.4)	29 (6.4)	59 (11.8)	39 (10.7)
Peanuts	110 (2.8)	4 (0.3)	22 (3.1)	31 (5.2)	28 (6.2)	22 (4.4)	3 (0.8)
Soybean	76 (2.0)	22 (1.7)	16 (2.3)	9 (1.5)	8 (1.8)	9 (1.8)	12 (3.3)
Meat	71 (1.8)	13 (1.0)	6 (0.9)	7 (1.2)	7 (1.5)	19 (3.8)	19 (5.2)
Others	469 (12.1)	28 (2.2)	88 (12.6)	88 (14.8)	83 (18.3)	89 (17.8)	93 (25.4)
Total	3882	1270	699	594	454	499	366

Current treatment approaches

Japanese pediatric guidelines for the treatment and management of asthma (JPGL)

JPGL was published in 2000 and revised in 2002, 2005, 2008, and 2012. A summary of the 2008 edition of the guidelines can be read in English in 'Japanese Guideline for Allergic Disease' (1). Compared with the severity classifications of adults and of foreign countries, the JPGL version has differed by one rank since 2002 (1, 5, 14): For example, the mild persistent type of asthma in Japanese children is equivalent to the intermittent type of asthma in the GINA guidelines, and the moderate persistent type in Japan is equivalent to the mild persistent type. This is because the goal of treatment for achieving a level of control is relatively high in Japan and treatment with a long-term controller is commenced earlier than other countries.

In Japan, for a severe asthma attack in which hospitalization management is needed, continuous inhalation of isoproterenol is given under the monitoring of heart rate and SpO2, together with systemic steroid and inhaled oxygen (15, 16). This common treatment contributes to the remarkable decrease in the number of cases of tracheal intubation with mechanical ventilation.

Treatment plans involving a long-term controller are not so different from the guidelines around the world. Some minor differences are related to the frequency of medication of LTRA for asthma exacerbations induced by viral infection. Anti-IgE antibody treatment is not available yet, but it will be released soon upon the completion of clinical trials.

Food allergy

Guidelines for food allergy

In 2005, as a result of research activity supported by grants from the MHLW, the 'Food Allergy Management Guidelines 2005' was posted on the Internet (7). These guidelines were created to help general practitioners improve their diagnosis and treatment of food allergies and to improve the quality of life of patients with food allergies. To encompass food allergy from infancy to adulthood, the project committee included not only pediatricians but also internists, dermatologists, and otolaryngologists. After the release of the guidelines, OFC tests were approved as a medical examination on hospital admission by the national health insurance system. In 2008 and 2011, the 'Food Allergy Management Guideline' was revised to include recent advances (7).

'The Japanese Pediatric Guideline for Food Allergy' was also published in 2005 (2) and then fully revised in 2011 (Japanese Pediatric Guideline for Food Allergy 2012) to have partial unification with 'Food Allergy Management Guideline 2011'. JSPACI also published guidelines for conducting oral food challenges in 2009.

Oral food challenges and oral immunotherapy

The MHLW research group and JSPACI together play an important role in the progression of food allergy practice. As shown in Fig. 4, among 514 pediatric training programs approved by the Japanese Society of Pediatrics, there are 312 facilities (58.3%) performing OFC, which means that OFC is already part of the routine diagnostic procedure for food allergy in our country. Based on another survey on OIT by the MHLW research group, there were already 53 facilities performing OIT either in an inpatient setting or in an outpatient setting by the end of 2011, treating 1400 cases of food allergy (mostly hens' eggs, cows' milk, wheat, and peanuts). There are a variety of protocols for OIT. The target subjects' age is usually above 3 yrs, and a majority are 5 yrs and older. We have realized that most of the food allergy patients can be 'desensitized' as far as they can ingest causative foods periodically and that OIT for cows-milk allergy is the most difficult among them.

Other allergic diseases

Atopic dermatitis guidelines

Given the importance of appropriate diagnosis and appropriate assessment of cutaneous symptoms in treatment of atopic dermatitis, the basics of treatment in these guidelines are composed of (i) investigation and countermeasures of causes and exacerbating factors, (ii) correction of skin dys-

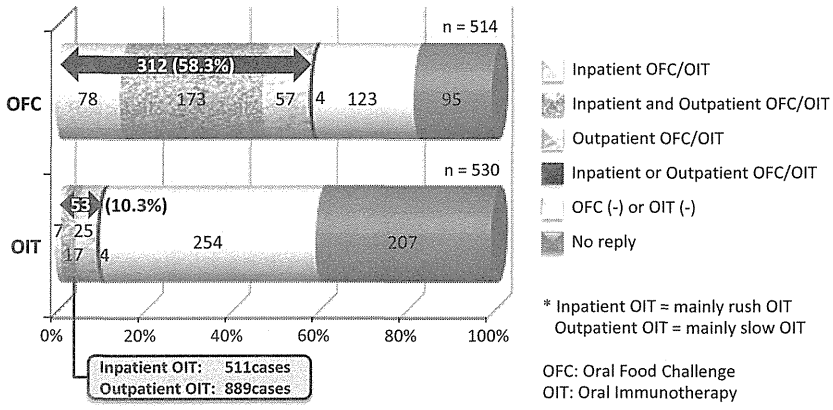

Figure 4 Number of facilities performing OFC and OIT. Among 514 pediatric training programs by the Japanese Society of Pediatrics, there are 312 facilities (58.3%) performing OFC. Based on another survey on OIT, there were already 53 facilities performing OIT either in an inpatient setting or in an outpatient setting by the end of 2011, treating 1400 cases of food allergy (mostly hens' eggs, cows' milk, wheat, and peanuts).

functions (skin care), and (iii) pharmacotherapy, as three mainstays (17).

Allergic rhinitis guidelines

The most common pharmacotherapy is non-sedative antihistamine with nasal spray steroids. Allergen avoidance is recommended, but sometimes impossible. Subcutaneous allergen immunotherapy for Japanese cedar is available, but not so widely used (18).

Pediatric clinical immunology

First, several diagnostic approach methods were established in Japan. Recently, rapid screening methods were developed for severe combined immunodeficiency (SCID), hyper-IgE syndrome (HIES), familial hemophagocytic lymphohistiocytosis (FHL), chronic granulomatous disease (CGD), innate immune defects, and cryopyrin-associated periodic syndrome (CAPS) using real-time PCR or flow cytometer. The measurements of T-cell receptor excision circles (TREC) and immunoglobulin k-deleting recombination excision circles (KREC) are used as screening method for antibody deficiency syndromes (19). The cytokine production measurement from mitogen-stimulated blood cells is useful for diagnosis of HIES, AD-CMC, and innate immune defects such as IRAK4 deficiency and EDA-ID (20–23). The detection of LPS-induced monocyte cell death is useful for rapid diagnosis of CAPS (24). Intracellular staining is a useful tool for rapid diagnosis of FHL and CGD (25, 26).

Secondly, for the suspected patients of PID and the common physician treating PID patients, PID Japan (PIDJ) was organized, and a genetic analysis center for pathogenic genes of PID was started in 2008 in collaboration with RIKEN Research Center for Allergy and Immunology (http://pidj.rcai.riken.jp/). Precise diagnosis and the accumulation of disease phenotypes may contribute to the developments of future novel treatments for each specific disease. For example, in innate immune defects, because IRAK4 deficiency and MyD88 deficiency showed the immune-deficient tendency against narrow species of pyogenic bacteria only in infancy, defense against bacterial infection is important only in infancy, but not in adulthood. On the other hand, EDA-ID has more severe phenotypes than IRAK4 deficiency, and hematopoietic stem cell transplants have been tried. Now, in Japan, many disease groups for rare diseases have been investigated in conjunction with the MHLW. The results of these research studies have been or will be published elsewhere (27, 28).

Internationally reported research results from Japan in the field of pediatric allergy and immunology

This section presents the internationally reported human research results in pediatric allergy and immunology field over the past 5 yrs. As many other research results from Japan have been reported in the international journal of JSA (Allergol Int), please refer to the journal Web site (http://ai.jsaweb.jp/).

In the rapidly progressing area of genome research, genetic polymorphisms relevant to allergy or asthma are reported by genomewide association studies and functional analysis research (29–33). In basic research, the pathogenesis of virus infections of the respiratory tract epithelium cells (34) and eosinophil activation by *Staphylococcus aureus* (35) have been studied. The molecules of innate immune system, pathogenesis of autoinflammatory reactions, and molecular mechanisms of MyD88 in TLR4 signaling have been clarified (36). Five Japanese patients with four kinds of gene variations in NLRP3 were given a diagnosis of cryopyrin-associated periodic syndrome or juvenile idiopathic arthritis (37). A rapid screening method to detect autosomal-dominant ectodermal dysplasia with immune deficiency syndrome was reported (23). It should be noted that several pathogenic genes were first identified in Japan, such as IgG2 selective deficiency (CT2), hyper-IgE syndrome (STAT3 and Tyk2), autosomal-dominant chronic mucocutaneous candidiasis (STAT1), and autoinflammatory disorder with lipodystrophy (PSMB8) (38–41). Furthermore, the existence of somatic gene mosaicism of NEMO and NLRP3 has been established (24, 42). Kanegane et al. (27) described the clinical characteristics and outcomes of Japanese patients with X-linked lymphoproliferative syndrome (XLP) type 1 and suggested rapid and accurate diagnosis of XLP with the combination of flow cytometric assay and genetic analysis was important.

Research results on the pathogenesis of infantile asthma (43) and respiratory syncytial virus-associated lung diseases have

been reported (44), including the possibility of early intervention for asthma by suplatast tosilate (45). Concerning the management of asthma, the results of a survey of 34,699 children were published by Okabe et al. (46), showing that obesity was associated with asthma in preschool children. A nighttime sleep diary was a useful instrument to monitor daily asthma status in infants and young children with asthma (47). The pandemic H1N1 influenza viral infection can easily induce a severe asthma attack in atopic children without any history of either an asthma attack or an asthma treatment (48). A study showing the close association between rhinitis and nocturnal cough in young children has also been published by Higuchi et al. (49).

Regarding research on atopic dermatitis, the importance of TARC (thymus and activation-regulated chemokine)/CCL17 was reported (50), and coenzyme A contained in breast milk was reported to be associated with its pathogenesis (51).

In Japan, research on the pathogenesis of food allergy, its diagnosis, and treatment has been undertaken by many organizations and facilities for research, and the results have been reported worldwide. The mechanisms of neonatal and infantile non-IgE-mediated digestive food allergies have been reported (52, 53). Regarding the diagnosis of food allergy, using the advantage of OFC as a routine procedure, the probability and component-resolved diagnosis for hens' eggs (54), cows' milk (54), wheat (55–57), soybeans (55, 58), and peanuts (59) have been reported. In addition, studies have also shown the usefulness of basophil activation tests such as the histamine-releasing test and measurement of CD203c (60, 61).

Aside from these reports, there have been many other papers from Japan. Determination of risk factors for the development of allergic sensitization, asthma, and atopic dermatitis is in progress (62–67), and studies have shown the prevalence rates of allergic symptoms in Japanese children (13).

Social countermeasures for pediatric allergy

Japanese regulations for the labeling of food allergenic ingredients

According to a national survey of food allergy cases, the food-labeling system for specific allergenic ingredients (i.e., eggs, milk, wheat, buckwheat, and peanuts) in Japan was mandated under law in 2002 (6). In addition, the ministerial notification recommends the labeling of any food that contains the following 18 ingredients: abalone, squid, salmon roe, orange, kiwifruit, beef, walnut, salmon, mackerel, soybean, chicken, banana, pork, matsutake mushroom, peach, yam, apple, and gelatin. To the best of our knowledge, Japan is the first country to set up mandatory food allergy labeling and regulate it under national law. Additional labeling of shrimp/prawn and crab has also been mandatory since 2008. To monitor the validity of the labeling system, the Japanese government announced official methods for the detection of allergens in a 2002 ministry notification. The details of Japanese food allergen labeling are described in the review article (6).

Countermeasures for allergy in schools

The Research Study Committee on Allergic Diseases of the Ministry of Education, Culture, Sports, Science, and Technology reported the prevalence rates of various allergic diseases among approximately 12 million schoolchildren in elementary, junior high, and senior high schools throughout Japan in 2004. According to this report, the prevalence was 5.7% for bronchial asthma, 5.5% for atopic dermatitis, 2.6% for food allergies, 0.14% for anaphylaxis, 9.2% for allergic rhinitis, and 3.5% for allergic conjunctivitis (7).

Although it is presumed that these prevalence rates failed to cover mild cases of atopic dermatitis and allergic rhinitis or conjunctivitis, children who have some type of allergic disease seem to account for more than 20% of all schoolchildren, even when overlapping cases of multiple allergic diseases are discounted. The number of schoolchildren with allergic diseases is increasing, and various considerations are necessary to secure the safe school lives of such children. Medical specialists and school officials have developed a school life management certificate for children with allergic diseases, which can serve as a tool for communication between medical facilities and schools in cases where such children need special care (7). Children with asthma require special attention during exercise, in dusty environments, when in contact with animals, and during out-of-school activities involving overnight stay. Concerns for children with atopic dermatitis include stimulants that worsen skin eczema, such as perspiration, chlorine in swimming pools, and ultraviolet rays. School lunches are the most serious concern for children with food allergies. Even classes involving the handling of foodstuffs may cause health hazards, thus necessitating caution in this arena. Food allergies are the most frequent cause of anaphylaxis, but it should be noted that anaphylaxis may also occur after a combination of food and exercise or even after exercise alone. It is important that schools are aware of children needing reliever drugs for anaphylaxis and asthmatic attacks.

Future issues of pediatric allergy immunology in Japan

Regarding asthma, the curative effect is increased by the recent development of long-term controllers and the publication of the guidelines for therapeutic management, which have brought about sharp decreases in asthma deaths, serious cases, and emergency room visits. While long-term controllers control asthma symptoms, the prevention of onset, early correspondence (diagnosis and treatment), and the derivation of remission have not yet been controlled. One immediate issue is to develop and reassess subcutaneous and sublingual immunotherapies for mite antigen that have been disused in our country. We also need to reassess the efficacy of SCIT for Japanese cedar pollen and to develop SLIT for the allergen. Although our standards of medical practice for food allergy are thought to be advanced compared with the world standards, all of the expectations from patients with food allergies have not yet been met. Another urgent issue is to fill the gap on allergy practice between advanced medical institutions and less progressive ones. Countermeasures against anaphylaxis and the enhancement of

correspondence for allergies in nursery schools and grade schools will be important as well. JSPACI will make every effort to continue taking responsibility for public health measures.

Future issues in pediatric allergy are the prevention of onset, early correspondence (diagnosis and treatment), the prevention of aggravation, and the derivation of remission. It is necessary to further improve medical services and promote research. The standards by which a doctor is determined to be an allergy specialist are important because allergies are very common. The Japanese system of allergy specialists is currently at a turning point. JSPACI, in cooperation not only with Japanese professionals but also with international organizations and practitioners, will work on these issues for patients with allergies all over the world.

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References

1. Nishimuta T, Kondo N, Hamasaki Y, Morikawa A, Nishima S. Japanese guideline for childhood asthma. *Allergol Int* 2011: **60**: 147–69.
2. Mukoyama T, Nishima S, Arita M, et al. Guidelines for diagnosis and management of pediatric food allergy in Japan. *Allergol Int* 2007: **56**: 349–61.
3. Wahn U, Matricardi PM, Kollmann D. Allergy epidemics, solidarity and support to countries through Pediatric Allergy and Immunology. *Pediatr Allergy Immunol* 2011: **22**: 349.
4. Tanihara S, Nakamura Y, Matsui T, Nishima S. A case-control study of asthma death and life-threatening attack: their possible relationship with prescribed drug therapy in Japan. *J Epidemiol* 2002: **12**: 223–8.
5. Nishima S, Furusho K, Japanese Society of Pediatric A, Clinical I. New pediatric guideline for the treatment and management of bronchial asthma in Japan. *Pediatr Int* 2003: **45**: 759–66.
6. Akiyama H, Imai T, Ebisawa M. Japan food allergen labeling regulation–history and evaluation. *Adv Food Nutr Res* 2011: **62**: 139–71.
7. Ebisawa M. Management of food allergy in Japan "food allergy management guideline 2008 (revision from 2005)" and "guidelines for the treatment of allergic diseases in schools". *Allergol Int* 2009: **58**: 475–83.
8. Al-Herz W, Bousfiha A, Casanova JL, et al. Primary immunodeficiency diseases: an update on the classification from the international union of immunological societies expert committee for primary immunodeficiency. *Front Immunol* 2011: **2**: 54.
9. Kasahara Y, Kaneko H, Fukao T, et al. Hyper-IgM syndrome with putative dominant negative mutation in activation-induced cytidine deaminase. *J Allergy Clin Immunol* 2003: **112**: 755–60.
10. Fukao T, Kaneko H, Birrell G, et al. ATM is upregulated during the mitogenic response in peripheral blood mononuclear cells. *Blood* 1999: **94**: 1998–2006.
11. Kaneko H, Isogai K, Fukao T, et al. Relatively common mutations of the Bloom syndrome gene in the Japanese population. *Int J Mol Med* 2004: **14**: 439–42.
12. Nishima S, Chisaka H, Fujiwara T, et al. Surveys on the prevalence of pediatric bronchial asthma in Japan: a comparison between the 1982, 1992, and 2002 surveys conducted in the same region using the same methodology. *Allergol Int* 2009: **58**: 37–53.
13. Yura A, Kouda K, Iki M, Shimizu T. Trends of allergic symptoms in school children: large-scale long-term consecutive cross-sectional studies in Osaka Prefecture, Japan. *Pediatr Allergy Immunol* 2011: **22**: 631–7.
14. Morikawa A, Nishima S, Japanese Society of Pediatric A, Clinical I. New Japanese pediatric guidelines for the treatment and management of bronchial asthma. *Pediatr Int* 2007: **49**: 1023–31.
15. Iikura Y, Matsumoto T, Fujita K, et al. Continuous isoproterenol inhalation therapy in children with severe asthmatic attack. *Int Arch Allergy Immunol* 1997: **3**: 370–2.
16. Sakamoto Y, Yamaguchi K, Furusho J, Fujita K, Iikura Y. Isoproterenol continuous inhalation therapy in an infant with severe bronchiolitis lifesaving therapy. *Pediatr Int* 1999: **41**: 94–6.
17. Katayama I, Kohno Y, Akiyama K, et al. Japanese guideline for atopic dermatitis. *Allergol Int* 2011: **60**: 205–20.
18. Okubo K, Kurono Y, Fujieda S, et al. Japanese guideline for allergic rhinitis. *Allergol Int* 2011: **60**: 171–89.
19. Nakagawa N, Imai K, Kanegane H, et al. Quantification of kappa-deleting recombination excision circles in Guthrie cards for the identification of early B-cell maturation defects. *J Allergy Clin Immunol* 2011: **128**: 223–5, e2.
20. Minegishi Y, Saito M, Nagasawa M, et al. Molecular explanation for the contradiction between systemic Th17 defect and localized bacterial infection in hyper-IgE syndrome. *J Exp Med* 2009: **206**: 1291–301.
21. Takezaki S, Yamada M, Kato M, et al. Chronic mucocutaneous candidiasis caused by a gain-of-function mutation in the STAT1 DNA-binding domain. *J Immunol* 2012: **189**: 1521–6.
22. Takada H, Yoshikawa H, Imaizumi M, et al. Delayed separation of the umbilical cord in two siblings with Interleukin-1 receptor-associated kinase 4 deficiency: rapid screening by flow cytometer. *J Pediatr* 2006: **148**: 546–8.
23. Ohnishi H, Miyata R, Suzuki T, et al. A rapid screening method to detect autosomal-dominant ectodermal dysplasia with immune deficiency syndrome. *J Allergy Clin Immunol* 2012: **129**: 578–80.
24. Saito M, Nishikomori R, Kambe N, et al. Disease-associated CIAS1 mutations induce monocyte death, revealing low-level mosaicism in mutation-negative cryopyrin-associated periodic syndrome patients. *Blood* 2008: **111**: 2132–41.
25. Murata Y, Yasumi T, Shirakawa R, et al. Rapid diagnosis of FHL3 by flow cytometric detection of intraplatelet Munc13-4 protein. *Blood* 2011: **118**: 1225–30.
26. Wada T, Muraoka M, Toma T, et al. Rapid detection of intracellular p47phox and p67phox by flow cytometry; useful screening tests for chronic granulomatous disease. *J Clin Immunol* 2013: **33**: 857–64.
27. Kanegane H, Yang X, Zhao M, et al. Clinical features and outcome of X-linked lymphoproliferative syndrome type 1 (SAP deficiency) in Japan identified by the combination of flow cytometric assay and genetic analysis. *Pediatr Allergy Immunol* 2012: **23**: 488–93.
28. Hoshina T, Takada H, Sasaki-Mihara Y, et al. Clinical and host genetic characteristics of Mendelian susceptibility to mycobacterial diseases in Japan. *J Clin Immunol* 2011: **31**: 309–14.

29. Noguchi E, Sakamoto H, Hirota T, et al. Genome-wide association study identifies HLA-DP as a susceptibility gene for pediatric asthma in Asian populations. *PLoS Genet* 2011: **7**: e1002170.
30. Harada M, Hirota T, Jodo AI, et al. Thymic stromal lymphopoietin gene promoter polymorphisms are associated with susceptibility to bronchial asthma. *Am J Respir Cell Mol Biol* 2011: **44**: 787–93.
31. Hitomi Y, Ebisawa M, Tomikawa M, et al. Associations of functional NLRP3 polymorphisms with susceptibility to food-induced anaphylaxis and aspirin-induced asthma. *J Allergy Clin Immunol* 2009: **124**: 779–85, e6.
32. An Y, Ohnishi H, Matsui E, et al. Genetic variations in MyD88 adaptor-like are associated with atopic dermatitis. *Int J Mol Med* 2011: **27**: 795–801.
33. Suzuki Y, Hattori S, Mashimo Y, et al. CD14 and IL4R gene polymorphisms modify the effect of day care attendance on serum IgE levels. *J Allergy Clin Immunol* 2009: **123**: 1408–11, e1.
34. Tadaki H, Arakawa H, Mizuno T, et al. Double-stranded RNA and TGF-alpha promote MUC5AC induction in respiratory cells. *J Immunol* 2009: **182**: 293–300.
35. Hosoki K, Nakamura A, Nagao M, et al. Staphylococcus aureus directly activates eosinophils via platelet-activating factor receptor. *J Leukoc Biol* 2012: **92**: 333–41.
36. Ohnishi H, Tochio H, Kato Z, et al. Structural basis for the multiple interactions of the MyD88 TIR domain in TLR4 signaling. *Proc Natl Acad Sci USA* 2009: **106**: 10260–5.
37. Ohnishi H, Teramoto T, Iwata H, et al. Characterization of NLRP3 variants in Japanese cryopyrin-associated periodic syndrome patients. *J Clin Immunol* 2012: **32**: 221–9.
38. Tashita H, Fukao T, Kaneko H, et al. Molecular basis of selective IgG2 deficiency. The mutated membrane-bound form of gamma2 heavy chain caused complete IGG2 deficiency in two Japanese siblings. *J Clin Invest* 1998: **101**: 677–81.
39. Minegishi Y, Saito M, Tsuchiya S, et al. Dominant-negative mutations in the DNA-binding domain of STAT3 cause hyper-IgE syndrome. *Nature* 2007: **448**: 1058–62.
40. Liu L, Okada S, Kong XF, et al. Gain-of-function human STAT1 mutations impair IL-17 immunity and underlie chronic mucocutaneous candidiasis. *J Exp Med* 2011: **208**: 1635–48.
41. Arima K, Kinoshita A, Mishima H, et al. Proteasome assembly defect due to a proteasome subunit beta type 8 (PSMB8) mutation causes the autoinflammatory disorder, Nakajo-Nishimura syndrome. *Proc Natl Acad Sci USA* 2011: **108**: 14914–9.
42. Nishikomori R, Akutagawa H, Maruyama K, et al. X-linked ectodermal dysplasia and immunodeficiency caused by reversion mosaicism of NEMO reveals a critical role for NEMO in human T-cell development and/or survival. *Blood* 2004: **103**: 4565–72.
43. Yoshihara S, Yamada Y, Abe T, Linden A, Arisaka O. Association of epithelial damage and signs of neutrophil mobilization in the airways during acute exacerbations of paediatric asthma. *Clin Exp Immunol* 2006: **144**: 212–6.
44. Kim CK, Callaway Z, Koh YY, Kim SH, Fujisawa T. Airway IFN-gamma production during RSV bronchiolitis is associated with eosinophilic inflammation. *Lung* 2012: **190**: 183–8.
45. Yoshihara S, Ono M, Yamada Y, Fukuda H, Abe T, Arisaka O. Early intervention with suplatast tosilate for prophylaxis of pediatric atopic asthma: a pilot study. *Pediatr Allergy Immunol* 2009: **20**: 486–92.
46. Okabe Y, Adachi Y, Itazawa T, et al. Association between obesity and asthma in Japanese preschool children. *Pediatr Allergy Immunol* 2012: **23**: 550–5.
47. Yoshihara S, Kanno N, Fukuda H, et al. Development and validation of a nighttime sleep diary in asthmatic children. *Pediatr Allergy Immunol* 2011: **22**: 667–70.
48. Hasegawa S, Hirano R, Hashimoto K, Haneda Y, Shirabe K, Ichiyama T. Characteristics of atopic children with pandemic H1N1 influenza viral infection: pandemic H1N1 influenza reveals 'occult' asthma of childhood. *Pediatr Allergy Immunol* 2011: **22**: e119–23.
49. Higuchi O, Adachi Y, Itazawa T, et al. Relationship between rhinitis and nocturnal cough in school children. *Pediatr Allergy Immunol* 2012: **23**: 562–6.
50. Fujisawa T, Nagao M, Hiraguchi Y, et al. Serum measurement of thymus and activation-regulated chemokine/CCL17 in children with atopic dermatitis: elevated normal levels in infancy and age-specific analysis in atopic dermatitis. *Pediatr Allergy Immunol* 2009: **20**: 633–41.
51. Higashi T, Shimojo N, Suzuki S, et al. Coenzyme A contained in mothers' milk is associated with the potential to induce atopic dermatitis. *Int Immunol* 2011: **23**: 741–9.
52. Nomura I, Morita H, Hosokawa S, et al. Four distinct subtypes of non-IgE-mediated gastrointestinal food allergies in neonates and infants, distinguished by their initial symptoms. *J Allergy Clin Immunol* 2011: **127**: 685–8.
53. Miyazawa T, Itahashi K, Imai T. Management of neonatal cow's milk allergy in high-risk neonates. *Pediatr Int* 2009: **51**: 544–7.
54. Komata T, Soderstrom L, Borres MP, Tachimoto H, Ebisawa M. The predictive relationship of food-specific serum IgE concentrations to challenge outcomes for egg and milk varies by patient age. *J Allergy Clin Immunol* 2007: **119**: 1272–4.
55. Komata T, Soderstrom L, Borres MP, Tachimoto H, Ebisawa M. Usefulness of wheat and soybean specific IgE antibody titers for the diagnosis of food allergy. *Allergol Int* 2009: **58**: 599–603.
56. Ebisawa M, Shibata R, Sato S, Borres MP, Ito K. Clinical utility of IgE antibodies to omega-5 gliadin in the diagnosis of wheat allergy: a pediatric multicenter challenge study. *Int Arch Allergy Immunol* 2012: **158**: 71–6.
57. Ito K, Futamura M, Borres MP, et al. IgE antibodies to omega-5 gliadin associate with immediate symptoms on oral wheat challenge in Japanese children. *Allergy* 2008: **63**: 1536–42.
58. Ito K, Sjolander S, Sato S, et al. IgE to Gly m 5 and Gly m 6 is associated with severe allergic reactions to soybean in Japanese children. *J Allergy Clin Immunol* 2011: **128**: 673–5.
59. Ebisawa M, Moverare R, Sato S, Maruyama N, Borres MP, Komata T. Measurement of Ara h 1-, 2-, and 3-specific IgE antibodies is useful in diagnosis of peanut allergy in Japanese children. *Pediatr Allergy Immunol* 2012: **23**: 573–81.
60. Sato S, Tachimoto H, Shukuya A, et al. Basophil activation marker CD203c is useful in the diagnosis of hen's egg and cow's milk allergies in children. *Int Arch Allergy Immunol* 2010: **152** (Suppl. 1): 54–61.
61. Sato S, Tachimoto H, Shukuya A, et al. Utility of the peripheral blood basophil histamine release test in the diagnosis of hen's egg, cow's milk, and wheat allergy in children. *Int Arch Allergy Immunol* 2011: **155** (Suppl. 1): 96–103.
62. Sugiyama M, Arakawa H, Ozawa K, et al. Early-life risk factors for occurrence of atopic dermatitis during the first year. *Pediatrics* 2007: **119**: e716–23.
63. Tadaki H, Arakawa H, Sugiyama M, et al. Association of cord blood cytokine levels with wheezy infants in the first year of life. *Pediatr Allergy Immunol* 2009: **20**: 227–33.
64. Kamemura N, Tada H, Shimojo N, et al. Intrauterine sensitization of allergen-specific IgE analyzed by a highly sensitive new allergen microarray. *J Allergy Clin Immunol* 2012: **130**: 113–21, e2.
65. Kondo N, Suda Y, Nakao A, et al. Maternal psychosocial factors determining the concentrations of transforming growth

factor-beta in breast milk. *Pediatr Allergy Immunol* 2011: **22**: 853–61.
66. Miyake Y, Sasaki S, Tanaka K, Hirota Y. Maternal B vitamin intake during pregnancy and wheeze and eczema in Japanese infants aged 16–24 months: the Osaka Maternal and Child Health Study. *Pediatr Allergy Immunol* 2011: **22** (Pt. 1): 69–74.
67. Kusunoki T, Mukaida K, Morimoto T, et al. Birth order effect on childhood food allergy. *Pediatr Allergy Immunol* 2012: **23**: 250–4.

特集 ◆日常生活とアレルギー

(旧)茶のしずく石鹸によるアレルギー

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はじめに

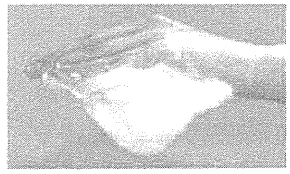
近年，加水分解コムギ末(グルパール 19S)を含む(旧)茶のしずく石鹸で洗顔することによって，グルパール 19S が経皮的・経粘膜的に吸収され，コムギアレルギーのなかった人にグルパール 19S に対する IgE 抗体を作らせ，これと交差反応するコムギ摂取時にアナフィラキシー反応を起こす重大な問題が生じた[1~3]．

本稿では疫学調査結果，診断基準，確定診断に必要な検査の 1 つである ELISA 法と，予後や発症のメカニズムについて概説する．最後に，このアレルギーが発症した背景としての，化粧品の安全性の問題を考察する．

(旧)茶のしずく石鹸とそのアレルギーの特異性

(旧)茶のしずく石鹸はグリチルリチン酸ジカリウムを有効成分として，その他の成分に泡立ちをよくする水解コムギ末と表示された加水分解コムギ(グルパール 19S)を含む医薬部外品である薬用石鹸として，2004 年 3 月から 2010 年 9 月まで 6 年 7 か月，466 万 7,000 名に合計 4,650 万 8,000 個販売された人気の商品であった(図 1)．加水分解コムギ末は，化粧品原料として従来汎用されていたが，これまでにグルパール 19S 以外の加水分

医薬部外品



泡立ちが非常によい

有効成分：グリチルリチン酸２K

その他の成分：石けん用素地，茶エキス-1，オウゴンエキス，カモミラエキス-1，アロエエキス-2，黒砂糖，ユキノシタエキス，ホホバ油，シア脂，ベントナイト，**水解コムギ末**，グリセリン，ファンゴ，ヒドロキシエタンジホスホン酸 4 Na，フェノキシエタノール，黄酸化 Fe，群青，香料，BG

2004/03 グルパール 19 S（片山化学工業研究所） 2010/09/26 プロモイス WG-SP（成和化成） 2010/12/07 加水分解シルク液 2011/6/19 ▶

現在は加水分解タンパク質は含んでいない

図 1 茶のしずく石鹸

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