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ORIGINAL ARTICLE

Comparison of Allergenic Properties of Salmon (*Oncorhynchus nerka*) between Landlocked and Anadromous Species

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ABSTRACT

Background: Salmon is one of the most widely consumed seafoods in Japan and many other countries around the world. Due to the confirmed cases of salmon-induced allergy, the food sanitation law in Japan stipulates salmon as one of the specific food items for which labeling is recommended when used as an ingredient of processed foods. However, trout, the landlocked form of anadromous salmon, is not subject to the allergen-labeling requirements, even though both populations belong to a single species. Since no supporting data have been demonstrated to make a clear distinction between these two populations in terms of allergenicity, we comparatively examined their allergenic properties using sera from patients allergic to fish.

Methods: Extracts of *Oncorhynchus nerka* from different habitats were obtained: kokanee (landlocked) and red salmon (anadromous). Control extracts were derived from four other species. This study focused on the (1) IgE-binding capacity of the fish extracts in patients' sera (n = 50), (2) ELISA inhibition test (n = 6), and (3) inhibition immunoblot test (n = 8) between the kokanee and red salmon.

Results: The extracts from kokanee and red salmon showed the highest correlation with each other in terms of the IgE-binding capacity, and showed complete (100%) reciprocal cross-inhibition in the ELISA inhibition test. On immunoblotting, there was no marked difference in the staining pattern between the two extracts, and each IgE-binding band gradually disappeared when the patients' sera were preincubated with the counterpart antigen in a dose-dependent manner.

Conclusions: These results suggest that kokanee has similar allergenic properties to red salmon.

KEY WORDS

allergenicity, ELISA, fish allergy, food allergy, IgE

INTRODUCTION

Most salmon are born in rivers, migrate to the sea, and return to the same rivers for spawning after spending several years at sea. However, some salmon do not migrate to the sea, but remain in the rivers. The former are called anadromous and the latter landlocked. As well-known examples, kokanee are landlocked and red salmon are anadromous in *Oncorhynchus nerka*, and rainbow trout are landlocked and steelhead are anadromous in *Oncorhynchus mykiss*. Thus, salmon and trout belong to a single species.

Salmon and trout are consumed worldwide, and are reportedly a cause of allergy.[1-5] In Japan, 24 and 8 cases of salmon-induced immediate food allergy were reported in 2001–2002 and 2005, respectively.[6] Currently, the Japanese Allergic Food Sanitation Law includes salmon in specified food ingredients, and the labeling of foods containing salmon is recommended. In contrast, labeling is not obligatory for trout, despite salmon and trout belonging to the same species living in different habitats.

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Table 1 Profile of 6 patients with allergic reactions to salmon and 2 patients with a high salmon-specific IgE level

Patient No.	Age (year)	Sex	Salmon-induced symptoms	Specific IgE (UA/ml, score)	
1	1	Male	Urticaria	2.25	2
2	1	Female	Urticaria	2.20	2
3	2	Male	Exanthema, itching	5.55	3
4	4	Female	Abdominal pain, vomit	75.5	5
5	5	Male	Abdominal pain, diarrhea	> 100	6
6	9	Female	Urticaria	6.17	3
7	1	Male	Avoidance	37.7	4
8	6	Male	Avoidance	24.4	4

There has been no report on the allergenicity of the two habitat types, and so there is no evidence to distinguish their allergenicity. We investigated the difference in allergenicity between the two habitat types based on binding with patients' IgE.

METHODS

Antigen extractions: Fish antigens were extracted from raw fish meat using 1 M KCl buffer, as previously reported.[7] Antigens were extracted from landlocked *Oncorhynchus nerka*, kokanee, and its anadromous type, red salmon, belonging to *Salmoniformes*, *Salmonidae*, *Oncorhynchus*. For controls, different species of the same genus, rainbow trout (*Oncorhynchus mykiss*) and silver salmon (*Oncorhynchus kisutch*), and species of a different order (*Perciformes*), Japanese jack mackerel (*Trachrus japonicus*) and bluefin tuna (*Thunnus thynnus*), popular foods in Japan, were selected.

ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA)

Each fish antigen-specific IgE was measured in sera of patients allergic to fish (n = 50), as follows:

The freeze-dried samples were dissolved (0.1 mg/ml) with PBS buffer and placed (0.1 ml/well) in each well of Nunc-Immuno Plate I (Nunc A/S, Roskilde, Denmark) for 1.5 hours at room temperature. Samples were discarded and SuperBlock Blocking Buffer in PBS (0.15 ml/well, Pierce, Rockford, IL, USA) was added and stored overnight at 4℃. Each well was washed with 0.2 ml/well of PBS-Tween and 0.1 ml/well of the serum diluted by SuperBlock Blocking Buffer (1 : 5) was added and stored overnight at room temperature. After being washed with PBS-Tween, Goat Anti-Human IgE BIOT (1 : 1,000, 0.1 ml/well, Vector Laboratories, Inc., Burlingame, CA, USA) was added for 1 hour at room temperature. This was washed well, and then streptavidin-HRP (1 : 5,000, 0.1 ml/well, Southern Biotechnology Associates, Birmingham, AL, USA) was added for 1 hour at room temperature. This was washed well, followed by incubation with 0.1 ml/well of TMB (ICN Biomedicals, Aurora, OH, USA) for 30 minutes under a light shield. The reaction was stopped by adding 0.1 ml/well of 1 N HCl, and measured with LSPLATE manager 2001 (Wako, Osaka, Japan).

Table 2 Correlation coefficient of IgE-binding rate (n = 50)

	Red salmon	Kokanee	Silver salmon	Rainbow trout	Jack mackerel
Kokanee	0.885				
Silver salmon	0.851	0.882			
Rainbow trout	0.706	0.746	0.849		
Jack mackerel	0.723	0.824	0.700	0.685	
Bluefin tuna	0.182	0.281	0.198	0.101	0.332

Using patients allergic to non-fish substances as controls (n = 30), the measured ELISA values of IgE antibodies against the fish antigens were compared with the mean control ELISA values. The values were divided by the SD of the control, and presented as Z scores. The IgE Z score against each fish meat was calculated using the equation below:

Z score against fish meat = (measured value of patients allergic to fish–mean measured value for control)/SD of the measured values of the control

The IgE antibody titers against the fish species were compared with regard to the Z score.

ELISA INHIBITION

Before addition to an ELISA plate precoated with extracts of red salmon or kokanee, serum samples were pre-incubated with solutions containing extracts (red salmon, kokanee, silver salmon, rainbow trout, Japanese jack mackerel, and bluefin tuna) at 4 different concentrations (0, 0.001, 0.01, 0.1, and 1.0 mg/ml) as inhibitors at room temperature. The subsequent procedure was the same as that for ELISA described above. To compare the rates of inhibition of IgE binding to the red salmon and kokanee antigens on the addition of each fish antigen, sera of 6 patients with salmon allergy (patients 1–6 in Table 1) were pooled and used.

TRANSFER AND IMMUNOBLOTTING

Sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed using a 4–20% Trisglycine precast gel (Tefco Corporation, Machida, Japan) according to the Laemmli method under reducing conditions. Each sample was separated at 120 V for 2 hours. After electrophoresis, proteins were transferred to Immobilon-P membranes (Millipore, Bedford, MA, USA), as previously reported.[8] For the detection of IgE bound to the protein bands, the blot was reacted with biotin-labeled anti-human IgE antibody used in ELISA (1 : 1,000) for 3 hours, washed,

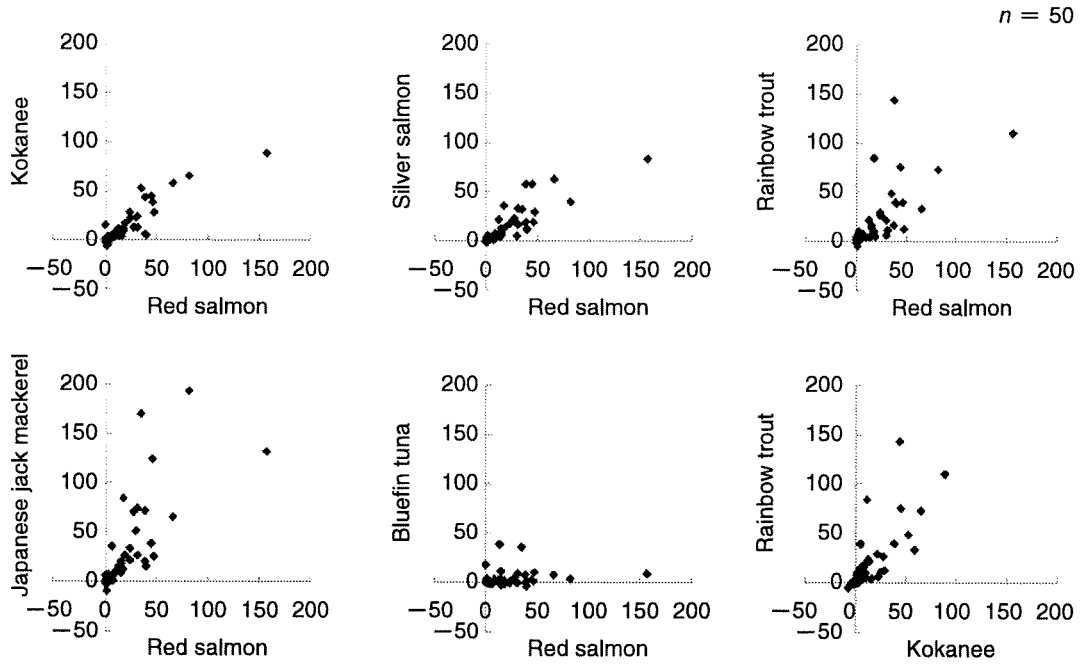

Fig. 1 Correlation of IgE binding to red salmon and kokanee antigens with that to other fish species antigens (n = 50). The IgE binding capacity of sera of 50 patients allergic to fish with each fish antigen was presented as the Z score and compared.

and reacted with streptoavidin-HRP (1 : 5,000) for 1 hour. After being washed, the blot was subjected to color development using the ECL™ Western Blotting Analysis System (GE Healthcare UK, Little Chalfont, UK).

Sera of the 6 patients allergic to salmon and 2 fish-allergic patients avoiding salmon ingestion because of a high salmon-specific IgE level, 8 sera in total (patients 1–8 in Table 1), were investigated. For the control, sera from patients allergic to non-fish substances were used.

IMMUNOBLOT INHIBITION

After each protein was transferred to the Immobilon-P membrane, pooled sera showing a high IgE antibody titer to multiple fish antigens preincubated with each extracted solution (0, 1, and 100 ug) as inhibitors were added. The detection of bound IgE was the same as described above.

The pooled serum of the 8 patients applied to immunoblotting was investigated.

RESULTS

COMPARISON OF RATES OF IgE BINDING TO THE FISH ANTIGENS (FIG. 1, TABLE 2)

On comparison of IgE binding shown in Table 2, the correlation between red salmon and kokanee, belonging to the same species, was the highest (r = 0.885), and that between red salmon and bluefin tuna, belonging to different orders, was the lowest (r = 0.182). As shown in Figure 1, the line representing the correlation between red salmon and kokanee was slightly sloped toward the red salmon side.

COMPETITION FOR IgE (FIG. 2)

In the ELISA inhibition test, the rates of IgE-binding inhibition caused by different species in the same genus, silver salmon and rainbow trout, were about 50%, and those by species belonging to a different order, Japanese jack mackerel and bluefin tuna, were 83 and 70%, respectively. In contrast, between red salmon and kokanee, IgE binding was 100% inhibited by the counterpart antigen.

IMMUNOBLOT AND IMMUNOBLOT INHIBITION (FIG. 3, 4)

On immunoblotting, there was no marked difference in the staining pattern between the two antigens; however, a 94-kDa band was only stained in kokanee in 4 patients (patients 4, 5, 7, and 8 in Fig. 3). Staining of 13-kDa bands of red salmon and kokanee was positive in all sera excluding the control serum. This protein band was confirmed as parvalbumin using the mouse monoclonal antiparvalbumin antibody clone PARV-19 (1 : 3,000; Sigma-Aldrich, St Louis, MO, USA, data not shown). Staining of 44-54-kDa protein bands of the two habitat types was equivalent in 5 patients (patients 2, 4, 5, 7, and 8). All IgE binding to 13- and 44-54-kDa proteins of red salmon and kokanee and the 94-kDa protein of kokanee was inhibited by the addition of the antigen of the other species in a concentration-dependent manner (Fig. 4). Twenty-

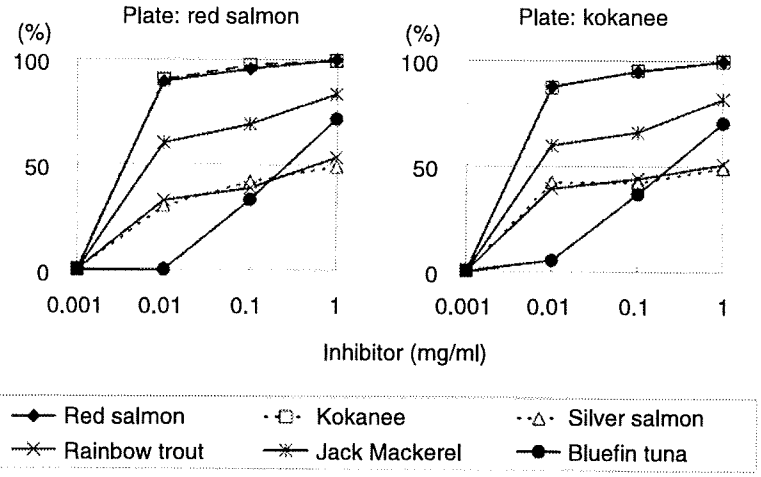

Fig. 2 ELISA inhibition with red salmon and kokanee antigens. Sera of 6 patients allergic to salmon were pooled and used. The rate of inhibition by other fish species antigens were 48-83%, but red salmon and kokanee antigens inhibited IgE binding to the counterpart by 100%.

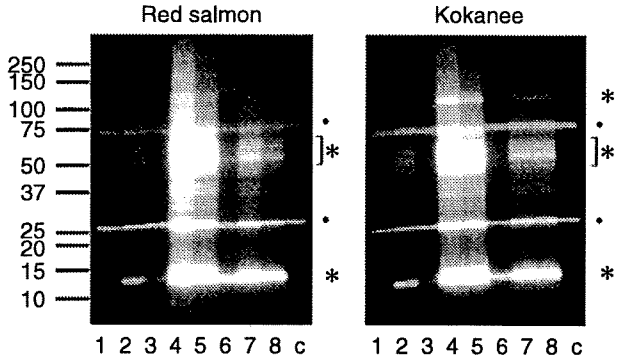

Fig. 3 Immunoblotting of red salmon and kokanee antigens. IgE of most patients' sera bound to the 13-kDa band common in red salmon and kokanee. Lanes 1-6: sera of 6 patients allergic to salmon, lanes 7 and 8: sera of 2 patients with a high CAP level, c: serum of patients allergic to non-fish substances. *: Specific binding, •: nonspecific binding.

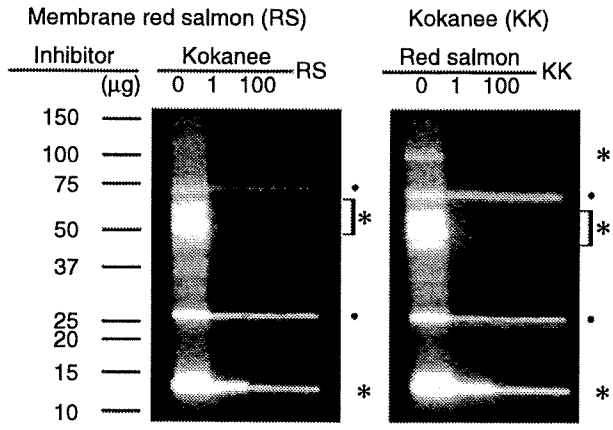

Fig. 4 Immunoblot inhibition between red salmon and kokanee. The pooled serum used in Fig.3 was subjected to the inhibition test between red salmon and kokanee. The specific IgE binding to 13- and 44-54-kDa proteins common in red salmon and kokanee and 94-kDa protein in kokanee was inhibited by the addition of the counterpart antigen at a low level. *: Specific binding, • : nonspecific binding.

five- and 75-kDa protein bands were reacted with the control serum, and the binding was not inhibited by the addition of the identical antigen, suggesting that the binding was nonspecific.

DISCUSSION

The Allergen Food Sanitation Law includes salmon in specified food ingredients, and the labeling of foods containing salmon is recommended. However, such labeling is not obligatory for trout, despite salmon and trout belonging to the same species living in different habitats, which may have been due to the numbers of reported cases, and not based on allergenicity. In our previous study on 38 patients with fish allergy, 12 were allergic to salmon, but only one, a 4-year-old infant allergic to salmon, was allergic to trout.[9] We in Japan have very few occasions to eat trout compared to salmon, which may be a reason for the small number of reported cases of trout allergy. However, if their allergenicity is identical, those allergic to salmon may develop allergy when they eat non-labeled foods containing trout, which should be prevented. Thus, we investigated the difference in antigenicity between salmon and trout using sera of patients with salmon allergy.

Using sera of patients allergic to fish, the correla-

tion of IgE binding to red salmon and kokanee with those to other fish species was investigated. The correlation was highest between the same species, and 100% inhibition was achieved on the ELISA inhibition test, suggesting that the antigenicity of red salmon and kokanee was almost the same. A high-level correlation of IgE binding with a different species, Japanese jack mackerel, was also noted, but the ELISA inhibition rate did not reach 100% even at the highest salmon antigen concentration, suggesting the presence of a specific allergen shared by red salmon, kokanee, and Japanese jack mackerel, other than the major antigens. No correlation with bluefin tuna was noted in IgE binding, suggesting that tuna show little common antigenicity, but the inhibition reached nearly 70% with an increase in the concentration, indicating that protein with a common antigenicity is present in tuna, although the content is low.

On immunoblotting, a strongly stained 94-kDa protein was detected in kokanee, and this may have emerged due to differences in the habitat, but IgE binding to kokanee was inhibited by red salmon from a low concentration on the ELISA inhibition test, and, consistently, IgE binding to the 94-kDa protein was inhibited by red salmon at a low concentration on immunoblot inhibition. Based on these findings, it is unlikely that the variation in the expression level of this protein leads to a difference in allergenicity between the habitat types.

The allergenicity of red salmon and kokanee may be equivalent, and the labeling of foods containing trout is also recommended.

ACKNOWLEDGEMENTS

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REVIEW ARTICLE

Diagnosis of Food Allergy Based on Oral Food Challenge Test

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ABSTRACT

Diagnosis of food allergy should be based on the observation of allergic symptoms after intake of the suspected food. The oral food challenge test (OFC) is the most reliable clinical procedure for diagnosing food allergy. The OFC is also applied for the diagnosis of tolerance of food allergy. The Japanese Society of Pediatric Allergy and Clinical Immunology issued the 'Japanese Pediatric Guideline for Oral Food Challenge Test in Food Allergy 2009' in April 2009, to provide information on a safe and standardized method for administering the OFC. This review focuses on the clinical applications and procedure for the OFC, based on the Japanese OFC guideline.

KEY WORDS

food hypersensitivity, guideline, immunoglobulin E, oral food challenge, tolerance

ABBREVIATIONS

OFC, oral food challenge test; IgE, immunoglobulin E; Japanese OFC Guideline, Japanese Pediatric Guideline for Oral Food Challenge Test in Food Allergy 2009; DBPCFC, double-blind placebo-controlled food challenge; GI, gastrointestinal; SPT, skin prick test; HRT, basophil histamine-releasing test; FPIES, food protein-induced enterocolitis syndrome.

INTRODUCTION

Food allergies affect 12.8% of infants, 5.1% of 3-year-olds[1] and 1.3-2.6% of school-age children in Japan. These allergies are associated with numerous social problems in nurseries, kindergartens and schools, particularly in terms of providing lunches to the affected children,[2] and in preparing for unexpected severe reactions after accidental ingestion of allergic foods.[3]

In 2008, the Japanese Society of School Health issued a guideline for the management of allergic diseases in schools (http://www.hokenkai.or.jp/). This guideline emphasized the importance of proper medical diagnosis for appropriate management of allergic students, especially with food allergy.

Definitions and diagnosis of food allergy should be based on the presence of clinical manifestations after ingestion of the offending food.[4] Proof of an immunological mechanism, typically as the detection of allergen-specific immunoglobulin (Ig)E antibodies, should be associated with the diagnosis, but proof of sensitization itself without provocation is not diagnostic of food allergy.[5]

Clinical testing to detect allergen-specific IgE antibodies (ImmunoCAP FEIA®, Phadia KK., Tokyo) is widely used in Japanese pediatric practice, particularly for patients with infantile atopic dermatitis, to determine the allergic background of the eczema. Examinations have sometimes been performed before the introduction of solid foods to babies, not only for the management of eczema,[6] but also to avoid unexpected anaphylactic reactions at the first intake of foods to which the baby might already have been sensitized through breast milk.[7] Transient elimination of sensitized foods may help to control the allergic conditions of infants, but proper diagnosis of food allergy should follow.[8]

Diagnosis of food allergy should be based on a convincing history of allergic reactions or on the result of an oral food challenge test (OFC).[9] The OFC has been covered by public medical insurance in Japan

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since 2006, but too few institutions can provide the OFC to meet the needs of patients, and a standardized protocol for the OFC has been absent.[10]

The Japanese Society of Pediatric Allergy and Clinical Immunology issued the 'Japanese Pediatric Guideline for Oral Food Challenge Test in Food Allergy 2009' (Japanese OFC Guideline, available only in Japanese) in April 2009, providing for the first time information about a safe and standardized method for administering the OFC.[11] This review focuses on the role of and practical methods for the OFC in the diagnosis and management of food allergy, based on the Japanese OFC Guideline.

CHARACTERISTICS AND CROSS-REACTIVITIES OF FOOD ALLERGENS THAT AFFECT THE OCCURRENCE OF FOOD ALLERGY

Hen's eggs, cow's milk and wheat are the three major food allergens accounting for 70% of patients who required treatment for acute reactions in 2008 in Japan. Peanut, salmon roe, shrimp and buckwheat are the next most common food allergens.[12]

Reactivity of food allergens or allergenic components of the foods can be highly modified by cooking methods. Hen's egg allergens, particularly ovalbumin, are sensitive to denaturing by heating, resulting in loss of IgE-binding capacity. Ovomucoid, on the other hand, is relatively resistant to heating[13] and protease digestion.[14] As a result, some patients with egg allergy can tolerate extensively heated egg products, and IgE antibody to ovomucoid can offer a good diagnostic marker to predict whether a child can eat heat-treated eggs.[15]

Caseins constitute 76-86% of whole milk proteins, and among these, αs1-casein is the major milk allergen.[16] This protein does not contain disulfide bonds and shows no tertiary structure. This characteristic structure explains why most IgE-binding epitopes are sequential (linear) and not susceptible to heat denaturation.[17] Conversely, another milk allergen, β-lactoglobulin, is highly conformational, and extensive heating may decrease the reactivity of milk for some patients.[18]

Wheat allergens can be divided into two fractions: a water-salt soluble fraction (albumins and globulins); and gluten (gliadin and glutenin). Wheat and other cereal grains share a number of homologous proteins, mostly in the water-salt soluble fraction,[19] whereas gluten is a component exclusive to wheat. The fact that most patients with wheat allergy can consume other cereals, such as rice or corn, suggests that the dominant wheat allergens and IgE epitopes exist in components that are not cross-reactive with other cereals. Specific IgE testing for recombinant ω-5 gliadin can offer a good marker of immediate-type wheat allergy or anaphylaxis in children,[20] as well as wheat-dependent exercise-induced anaphylaxis in adults.[21]

Allergen components of peanut have been extensively characterized, and recombinant allergens are ready for use in research.[22] However, no single recombinant allergen is satisfactory for the diagnosis of peanut allergy in terms of sensitivity and specificity.[23] Cross-reactivity to homologous proteins in soybeans, Gly m 5 (vs. Ara h 1) and Gly m 6 (vs. Ara h 3),[24] and other tree nut allergens[25,26] requires more extensive study, particularly in terms of the relationship with clinical manifestations.

Taken together, knowledge of food allergens is required to interpret the results of allergen-specific IgE testing,[27] but no single in vitro test represents an alternative to a convincing history of allergic symptoms or the OFC.

ORAL FOOD CHALLENGE TEST

DEFINITION OF THE OFC

The general methodology for the OFC is to administer the suspected food in gradually increasing doses under a medical setting.[28] A single trial with intake of a small amount of the suspected food at home or in the office may help in the introduction of eliminated foods, but is not defined as an OFC, because it is not diagnostic of food allergy.

An open challenge refers to an OFC in which the patient can recognize the target food without blinding. The results can be definitive if the challenge yields either negative results or positive results with objective symptoms. This approach may be appropriate for most infants or young children, because psychological claims of symptoms are negligible at those ages. However, if the patient complains only of subjective symptoms such as abdominal pain or pruritus, particularly when the patient displays anxiety about the challenge, interpreting challenge result is difficult.

A single-blind challenge means that the patient does not know whether the food contains the suspected allergen, but the observer knows.[29] A masking effect sometimes helps to reduce psychological effects or difficulty eating in small children, but a single-blind challenge without placebo is essentially similar to an open challenge.

A double-blind placebo-controlled food challenge (DBPCFC), in which both the patient and observer are blinded to the challenge material, remains the gold standard for diagnosing food allergy for both clinical and scientific purposes.[30] A provocation kit containing dried powder[31] of each food (whole egg, cow's milk, wheat and soybean) and a masking material (strawberry puree) is provided through the Food Provocation Network in Japan by the National Food Allergy Research Group (Fig. 1).

AIMS AND INDICATIONS

The OFC is generally carried out for two purposes:

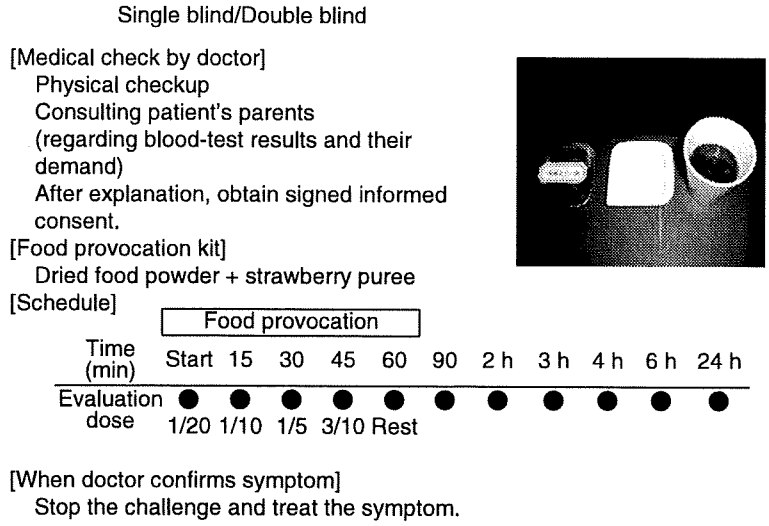

Fig. 1 Provocation kit and the protocol for blind food challenge.

diagnosis of food allergy; or determination of tolerance to the allergic food.

Diagnostic OFC is typically used in three situations. First, if a patient is suffering from chronic allergic conditions such as atopic dermatitis or persistent gastrointestinal (GI) symptoms, and elimination of the suspected food ameliorates the symptoms, an OFC to confirm the recurrence of symptoms is considered to establish an accurate diagnosis. Second, if a patient is suffering from acute allergic symptoms after eating multiple foods, and a precise history and/or in vitro diagnostic testing indicates some suspected foods, definitive diagnosis of the offending food may be achieved using the OFC. Third, and most frequently, is with the introduction of a sensitized food as confirmed by the presence of specific IgE antibody or positive results from a skin prick test (SPT), for the first time in life. This scenario is mostly the case in infants with atopic dermatitis, but patients and their family with known food allergy tend to avoid highly allergenic foods such as peanuts, buckwheat and shrimp, particularly if they have ever shown positive specific IgE titers. Careful setting of the OFC may be needed in this case, because introduction of a highly sensitized food for the first time in life can sometimes induce severe reactions.

Diagnosis of the achievement of tolerance (outgrowing the allergy) is another important indication for the OFC. Most infants with egg,[32] milk,[33] wheat[34] or soybean allergies tend to outgrow these allergies during childhood. Information on symptoms following accidental exposure helps determine an indication for the OFC. If the patient has experienced a severe reaction recently within 1 year, the OFC is not indicated. Patients with strict avoidance of the allergic food for more than 1 year may be considered for an OFC. Information about daily consumption of foods containing small amounts of the suspected component is also helpful to determine indications and procedures for the OFC.

Allergies to peanut,[35] tree nuts,[36] buckwheat or shrimp, especially in older children or adults, are thought to continue throughout life. An OFC to those foods may not be indicated unless loss of sensitization is confirmed by negative results from an SPT or specific IgE test.

DECIDING ON THE CHALLENGE PROTOCOL

Selection of a challenge protocol should be based on the safety and accuracy of the OFC.[37] The total provocation dose may be large enough compared to daily consumption of the suspected food for the proper diagnosis of food allergy, but is sometimes considered too high for a highly sensitized patient with a history of severe reaction, in terms of safety. Using step-wise procedures in the OFC may be an option, with challenge using a small amount preceding a full-dose challenge.

The challenged food should be standardized for diagnosis of the food allergy. However, processed food may be an option for patients with known food allergy. Introduction of extensively heated foods,[38] partially digested foods or fermented food such as "miso", "shoyu" or "natto", which are traditional Japanese soy products,[39] may be tolerated and even effective for the induction of tolerance in some patients. Although allergenic activities of these foods are generally decreased, OFC should be considered before introduction, because some patients experience severe reactions to these foods.

Precise information on the history of the patient, which has already been mentioned, and immunological laboratory data are essential for deciding on the indications and procedure for OFC.

1) Sampson[41] (U_A/ml)

Specific IgE	Egg white	Milk	Peanut	Fish
Diagnostic decision points	7	15	14	20

2) Komata[39]

Age (years)	<1	1	2≤
Egg white	13.0	23.0	30.0
Milk	5.8	38.6	57.3

3) Ando[14]

Food	Raw egg white		Heated egg white	
Specific IgE	Egg white	Ovomucoid	Egg white	Ovomucoid
Positive decision points	7.38	5.21	30.7	10.8

Fig. 2 Positive decision points for allergen-specific IgE titers to diagnose food allergy without food challenge.

Positive decision points for specific IgE antibodies, which indicate IgE titers with over 95% probability for positive challenge, have been proposed for some allergens (Fig. 2).[40] Patients with specific IgE titers above this point may be advised to continue a restricted diet without undergoing an OFC.[41] Probability curves for specific IgE titers are also helpful to predict the probability of positive challenge.[42] Even so, OFC might be performed for highly sensitized patients to identify the threshold amount of suspected food inducing allergic symptoms, and to provide the patient with advice on safe levels of the food. Emphasis is required on the fact that specific IgE titers do not always correlate to threshold amounts of food or the severity of allergic symptoms.

SPT also indicates sensitization to the suspected food,[43,44] sometimes in patients with negative results for specific IgE in serum. Results from an SPT help to predict a positive challenge in patients with negative or low specific IgE titers to milk or egg,[45] but false positive results are also common.

The basophil histamine-releasing test (HRT) is also commercially available in Japan.[46] High scores (Class 4) in HRT for egg white, milk and wheat suggest more than 90% probability for positive challenge, particularly in patients who have experienced anaphylaxis.[47] Decreased HRT titers in patients maintaining high specific IgE titers sometimes indicate the achievement of tolerance to the food.

SETTING AND PROCEDURES

All institutes at which OFCs are performed have to be fully equipped for access to emergency treatment. The site may be in-hospital, but an outpatient office or clinic may also be suitable for some patients in whom severe reactions are not predicted. A safe, clean and comfortable environment, hopefully free from contact with other patients with infectious diseases, needs to be provided for patients to spend a long period. Well-trained doctors or nurses should keep in touch with the patient throughout the procedure, and the contribution of a dietitian helps a great deal.[48]

The risks and benefits of OFC should be discussed with the patient and parents, and written informed consent needs to be obtained in most cases.

Before proceeding with the OFC, the patient needs to be stable in terms of allergic symptoms and free from any acute illness. Antihistamines should have been discontinued for >72 h and any other medications for the treatment or prevention of allergic diseases discontinued for an appropriate period based on the duration of action, except inhaled corticosteroids and topical corticosteroid ointments applied on small areas of skin lesions.

Typical challenge foods and total doses administered are listed in Table 1. The starting dose should be 1 g (1 ml) or less of the food.[49] The typical challenge scheme is to divide the total dose into 3-6 incremental doubling doses, such as 1, 2, 4, 8 and 16 g of boiled egg white or 1, 5, 10, 25, 50 and 100 ml of milk. A challenge with smaller doses should be considered for patients deemed to be at risk of severe reaction, such as 0.1 ml for the starting dose of milk.[50]

When processed food is used for a blind challenge, equivalent doses of allergen content should be considered and a standardized cooking method may be applied to minimize the variation of allergen activity.

Doses are generally given every 15-30 min over 1-2 h. A longer dosing interval might be applied for severe patients or for those who have experienced a late-onset allergic reaction after intake of the suspected food. If a sign of suspicious reaction appears, the next dose should be postponed to observe the progress of symptoms, or the same dose should be repeated to avoid overloading.

The patient may stay in hospital for more than 2 h after the final dose is given or the provoked symptoms disappear. Upon discharge, the patient needs to be instructed to observe the possibility of late-onset symptoms, even after a negative (passed) challenge.

SYMPTOMS AND TREATMENTS

The expected reactions during OFC involve cutaneous, mucosal, respiratory, GI, cardiovascular and neurological symptoms (Table 2). Parallel to the allergic reactions observed with accidental intake, cutaneous symptoms are most frequently observed in 80% of positive (failed) challenges, followed by respiratory (35%) and GI (25%) symptoms.[51]

Respiratory symptoms are common and need to be treated properly. Coughing might be divided into two categories: dry and staccato coughing estimated to be of laryngeal origin; and productive coughing associated with wheezing or asthma.[52]

Oral symptoms are frequently reported at the beginning of challenge, but sometimes disappear afterward. Distinguishing whether such symptoms are a

Table 1 Recommended protocol for open food challenge

Target foods	Challenge foods	Step†	Initial dose	Total dose	Scheme
Egg	Boiled egg yolk	1	1 g	15 g (1 egg yolk)	1-2-4-8 g
	Boiled egg white	2‡	0.1 g	2-4 g	0.1-0.2-0.5-1-2 g
		3	1 g	16-32 g (1 egg)	1-2-4-8-16 g
Milk	Raw milk	1	0.05-0.1 ml	15-30 ml	0.1-1-2-4-8-15 ml
		2	1-5 ml	100-200 ml	1-5-10-25-50-100 ml
Wheat	Udon noodle (boiled)	1	0.5 g	15-30 g	0.5-1-2-4-8-15 g
		2	1 g	50-100 g	1-2-5-15-25-50 g
Fish	Boiled or baked fish		1 g	30-60 g	1-2-4-8-15-30 g
Soy	Tofu (soy paste)		1 g	50-100 g	1-2-5-15-25-50 g

†A stepwise challenge protocol may be considered for high-risk patients.

‡Processed foods (cookies, cakes, etc.) are also available.

Table 2 Signs and symptoms observed in OFC

Cutaneous
- Pruritus, erythema, urticaria, angioedema

Oral
- Throat pain, itching of palate, tongue or lips, palatal redness or hives

Mucosal
- Eye swelling, tears, conjunctivitis

Upper respiratory
- Rhinorrhea, sneezing, and nasal obstruction

Lower respiratory
- Coughing, wheeze, dyspnea, stridor, hoarseness, chest tightness

Gastrointestinal
- Nausea, vomiting, diarrhea, abdominal pain or cramp

Cardiovascular
- Hypotension, light-headedness, cold extremities, cyanosis, syncope, collapse

Neurological
- Behavioral change, loss of activity, restlessness, dizziness, sleep

part of systemic reactions or an oral allergy syndrome induced by local absorption of water-soluble allergens is difficult, but may be important.

Neurological symptoms might be a sign of systemic reactions, particularly when a small child is violently frightened and crying, or suddenly turns quiet.[53] Overwhelming tiredness and sleepiness are sometimes observed in older children associated with GI symptoms, but without cardiovascular symptoms like hypotension or decreased oxygen saturations.

Grading symptoms is helpful for deciding on treatment strategies (Table 3). Treatment may not be necessary for localized skin or mild mucosal symptoms (Grade 1). Most skin and mucosal symptoms may be treated using antihistamines (oral or parenteral). Beta-agonist inhalation may be applied to mild respiratory symptoms, and oxygen should be administered if oxygen saturation falls below 95% (Grade 2, Step 1 treatment, Fig. 3).

When symptoms reach Grade 3, Step 2 treatment should be applied. Intramuscular adrenaline (0.01 mg/kg) is the first-line treatment in Step 2. Effects of adrenaline may be observed within 5 min, when most skin, respiratory, GI and even neurological signs tend to disappear. If the effect was insufficient or symptoms reappear after 10-15 min, repeat administration of intramuscular adrenaline may be considered, and additional treatments such as intravenous fluid, parenteral antihistamine or corticosteroids should be applied. Repeat inhalation of beta-agonists or adrenaline[54] may be an option for persistent but mild respiratory symptoms.

In cases of severe reactions accompanied by intractable hypotension or respiratory distress, full resuscitation with bolus rehydration (30 ml/kg normal saline), respiratory supports and catecholamine should be applied in the intensive care unit (Step 3).

DIET MANAGEMENT BASED ON RESULTS OF THE OFC

Based on the total dose and symptoms provoked in the OFC, patients should be instructed about restrictions or re-introduction of the challenge food. Even after a negative challenge, the amount of food intake at home may not exceed that of the total dose at least several times to confirm safety.

Positive challenge does not always suggest a need for complete elimination of the food from the diet.[55] Patients may introduce small amounts of the target food within the appropriate safety range, at 1-10% of the threshold level in general, or the processed food in which decreased allergic reactivity is expected.

Repeated follow-up visits are needed to confirm the benefits of the OFC, particularly when re-introduction of the eliminated food is in progress. In many cases, the patient and parents are anxious about the occurrence of allergic symptoms even after a negative challenge, or may actually experience some mild symptoms after eating the target food. Providing instructions to the patient's school about restrictions to the

Table 3 Grading of symptoms observed with oral food challenge

Grade	Skin	Gastrointestinal	Respiratory mucosal	Cardiovascular	Neurological
1	Faint rash Wheals (<3) Pruritus	Nausea Oral/pharyngeal discomfort, itch	–	–	–
2	Localized rash Wheals (3-10) Worsening of eczema Increased scratch	Vomiting/diarrhea (1-2) Transient colic	Sneeze Rhinorrhea/nasal obstruction Scratch nose/eyes Cough (<10)	–	Loss of activity
3	Systemic rash/wheals Severe itch Angioedema	Vomiting/diarrhea (≥3) Persistent colic	Cough (≥10) Wheeze Husky voice/Barking cough Difficulty swallowing	Increased heart rate (≥15 bpm) Pallor	Fatigue・sleep or irritability
4	As above	Vomiting/diarrhea with dehydration	Dyspnea Weak respiration Cyanosis	Arrhythmia Mild hypotension Cold extremities Sweat skin	Dizziness Distraction
5	As above	As above	Respiratory arrest	Severe bradycardia Severe hypotension Cardiac arrest	Loss of consciousness

Grading should be based on the most severe symptom.

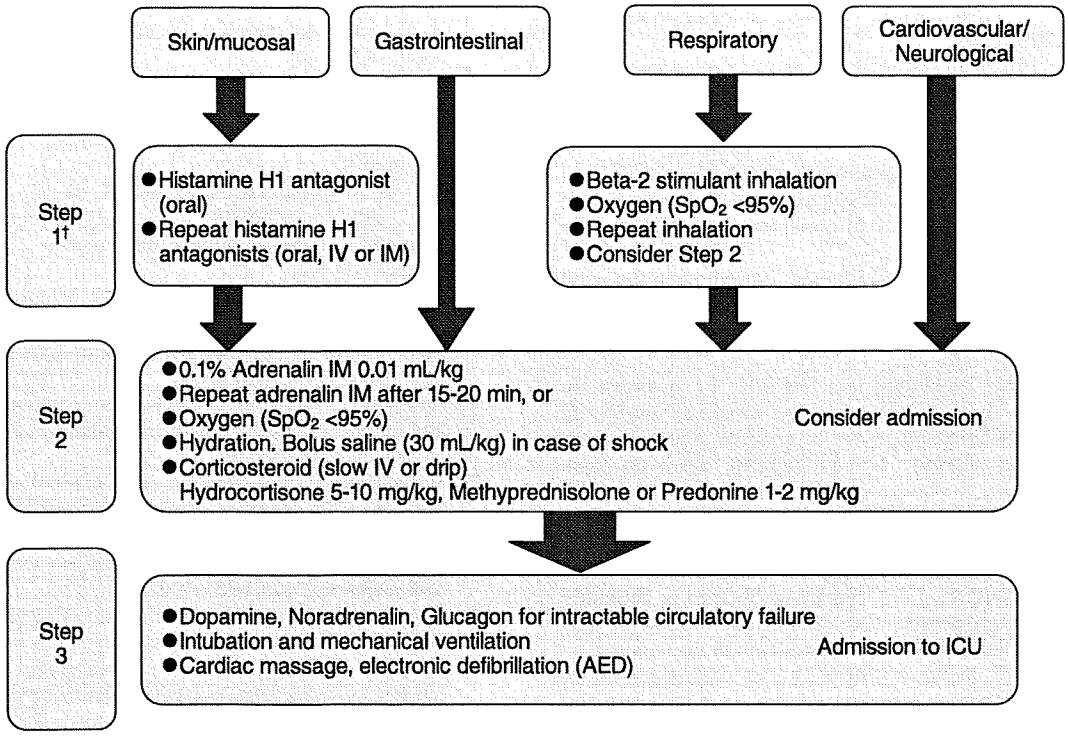

Fig. 3 Treatment plan for allergic symptoms. †Consider oral corticosteroid to prevent late reactions.

lunch menu is an important social activity to improve quality of life and safety of the patient.

FUTURE PROSPECTS

The Japanese OFC Guideline principally deals with the diagnosis of immediate food hypersensitivity. Diagnostic food challenge for non-IgE-mediated allergic reactions including food protein-induced enterocolitis syndrome (FPIES)[56] and late-onset worsening of eczema,[57] both of which are thought to be cell-mediated

immunological disorders, is not described in the guideline, because insufficient evidence is available to establish a standardized protocol at this time. Indirect food challenges such as provocation through breast milk after giving the target food to the lactating mother,[58] or labial food challenge[59] are also not dealt with.

The guideline does not recommend a single universal procedure, but places emphasis on users arranging their own protocol to meet the conditions of their institute and patient needs. In any case, safety remains the most important consideration, and the key safety point might be that OFC is conducted by experienced staff who are present throughout the procedure, continuously interacting with the patient.

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