

# IgE Cross-reactivity between Fish Roe (Salmon, Herring and Pollock) and Chicken Egg in Patients Anaphylactic to Salmon Roe

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## ABSTRACT

**Background:** Salmon roe (SR) anaphylaxis has often been reported and SR-containing foods are designated as 'recommended for allergic labeling'; however, there have been no reports about its allergenicity, including its cross-reactivity. Because its cross-reactivity is controversial, clinicians are often confused concerning education regarding its dietary elimination. The purpose of this study was to examine the cross-reactivity between SR and other kinds of fish roe, salmon, or chicken egg (CE).

**Methods:** We measured the specific-IgE to SR, herring roe (HR), pollock roe (PR), salmon and CE using RAST in 27 patients with a fish allergy and 26 control subjects. Then, using the sera of 2 patients with SR anaphylaxis, an ELISA inhibition study was performed to examine the cross-reactivity between SR and HR, PR, salmon or CE. We then compared the IgE binding patterns to SR between the anaphylaxis patients and fish allergy patients with immunoblotting.

**Results:** There were positive correlations between SR and HR or PR, but none between SR and salmon or CE. In the ELISA study using sera from two patients with SR anaphylaxis, IgE-binding to SR was inhibited more than 50% only when the sera were pre-incubated with HR, inhibited almost 50% by PR in a dose-dependent manner, but not inhibited by CE or anisakis. Salmon inhibited the IgE binding to SR more than 50% in a SR-anaphylaxis patient. The IgE binding patterns to SR from anaphylaxis patients were almost identical and unlike those of patients with fish allergy.

**Conclusions:** There was a cross-reactivity between SR and HR, but no relationship between SR and CE.

## KEY WORDS

anaphylaxis, chicken egg, cross-reactivity, food allergy, salmon roe

## INTRODUCTION

There have been very few reports about allergy to fish roe. In western countries, there have been a few case reports, one reporting an anaphylaxis to caviar<sup>1</sup> and the other a severe IgE-mediated reaction to the roe of 2 species.<sup>2</sup> In Japan, even though salmon roe (SR) is listed as a 'recommended for allergic labeling' food because of some patient reports of anaphylaxis to SR, there have been no scientific reports on its al-

lergenicity. Generally, fish roe consists of yolk and vitelline, while there is no protein corresponding to the white of chicken eggs (CE). In fish, the yolk proteins originating from vitellogenin are classified as lipovitellin, phosvitin and a beta'-component. In a report using sera from several SR allergic patients, the beta'-component was identified as the main allergen.<sup>3</sup>

We were burdened with devising a diet for patients allergic to CE or fish if they asked to eat SR. We also have no answer as to whether SR anaphylactic pa-

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tients can eat different kinds of fish roe safely. The cross-reactivity among fish roe was investigated using a CAP-RAST system<sup>4</sup> and inhibition immunoblot,<sup>5</sup> but there have as yet been no articles demonstrating this. Moreover, the cross-reactivity between SR and CE is controversial.

In this study, we attempted to demonstrate the cross-reactivity between SR and other kinds of fish roe (herring roe : HR, Pollock roe : PR), salmon, or CE.

## METHODS

### EXTRACTS

Extracts of salmon (*Oncorhynchus kisutch*) and fish roe from salmon (*Oncorhynchus keta*), pollock (*Theragra chalcogramma*) and herring (*Clupea pallasii*) were obtained from Toyo Suisan Kaisha, Ltd. Raw or frozen salmon and fish roe were cleared of parasites and minced with a speed cutter (Matsushita Industry Company, Tokyo, Japan). Five grams each of salmon and fish roe were vortexed in 15 ml of 1M KCl-PBS in sterile centrifuge tubes, and placed overnight in a cold room (4°C). After the addition of a further 5 ml 1 M KCl-PBS, the samples were centrifuged at 13,000 rpm (20000xg). The supernatants were dialyzed against distilled water with a dialysis tube (cut off 6,000–8,000 of molecular weight) for 1–2 days in a cold room. The concentrates were lyophilized and stored at -20°C.

Chicken egg extracts (egg white and egg yolk) were obtained in the same way as previously described.<sup>6</sup>

The protein concentrations of these samples were determined by a BCA protein assay (Pierce, Rockford, IL, USA).

### MEASUREMENT OF SPECIFIC IGE ANTIBODIES TO EXTRACTS FROM FISH, ROE AND CHICKEN EGG WHITE (CEW) USING THE RADIOALLERGOSORBENT TEST (RAST)

The freeze-dried samples were dissolved in 25 ml coupling buffer (0.1 M NaHCO<sub>3</sub>, 0.5 M NaCl) and centrifuged at 13,000 rpm (20000xg). Cyanogen bromide-activated paper disks were soaked in each fish extract solution and incubated with rotation at 4°C. After the supernatant was aspirated, 25 ml of blocking buffer (0.2 M glycine buffer) was added to the disks and incubated with rotation at room temperature (RT) for 5 hours. The disks were alternately washed with 0.1 M acetate buffer and blocking buffer 5 times. The disks were washed once with PBS-Tween and suspended in 40 ml of PBS-Tween. The disks in PBS-Tween were stored in a cold room (4°C).

These extract-conjugated paper disks were incubated for 5 hours with 25 µl of patient serum and 25 µl of PBS-Tween. After washing with PBS-Tween, 25 µl of <sup>125</sup>I-labeled anti-IgE (IgE-RIABEAD, DAINABOT

CO., LTD, Tokyo, Japan), approximately 2,200 Bq, and 25 µl of PBS-Tween were added and incubated overnight. After the free radioisotope was removed by rinsing with PBS-Tween, the bound radioisotope was measured in a gamma counter. Results were expressed as the percent binding of the total radioactivity added.

### ELISA (ENZYME-LINKED IMMUNOSORBENT ASSAY)

The freeze-dried samples were dissolved (100 µg/ml) with PBS buffer and placed (100 µl/well) in each hole on Nunc-Immuno Plate I (Nunc A/S, Roskilde, Denmark) for 1.5 hr at RT. Samples were discarded and SuperBlock Blocking Buffer in PBS (150 µl/well, Pierce, Rockford, IL, USA) was added and stored overnight at 4°C. Each well was washed with 200 µl/well of PBS-Tween and 100 µl/well of the serum diluted by SuperBlock Blocking Buffer (1 : 5) was added and stored overnight at RT. After being washed with PBS-Tween, Mouse Anti-Human IgE-BIOT (1 : 1,000, 100 µl/well, Southern Biotechnology Associates, Inc. Birmingham, AL, USA) was added for 1 hr at RT. This was washed well, then streptavidine-HRP (1 : 5,000, 100 µl/well, Southern Biotechnology Associates, Inc. Birmingham, AL, USA) was added for 1 hr at RT. This was washed well and followed by incubation with 100 µl/well of TMB (ICN Biomedicals, Inc. Aurora, OH, USA) for 30 minutes under a light shield. The reaction was stopped with 100 µl/well of 1N HCl and measured with LS-PLATE manager 2001 (Wako, Osaka, Japan).

### ELISA INHIBITION

Before addition to the ELISA plate that was pre-coated with extract of SR, salmon or chicken egg yolk (CEY), serum samples were pre-incubated with solutions containing extracts (SR, HR, PR, salmon, CEW, CEY and anisakis) of 5 different concentrations (0, 1, 10, 100, 1,000 and 10,000 µg/ml) as inhibitors at RT. The subsequent procedure was the same as that for ELISA described above.

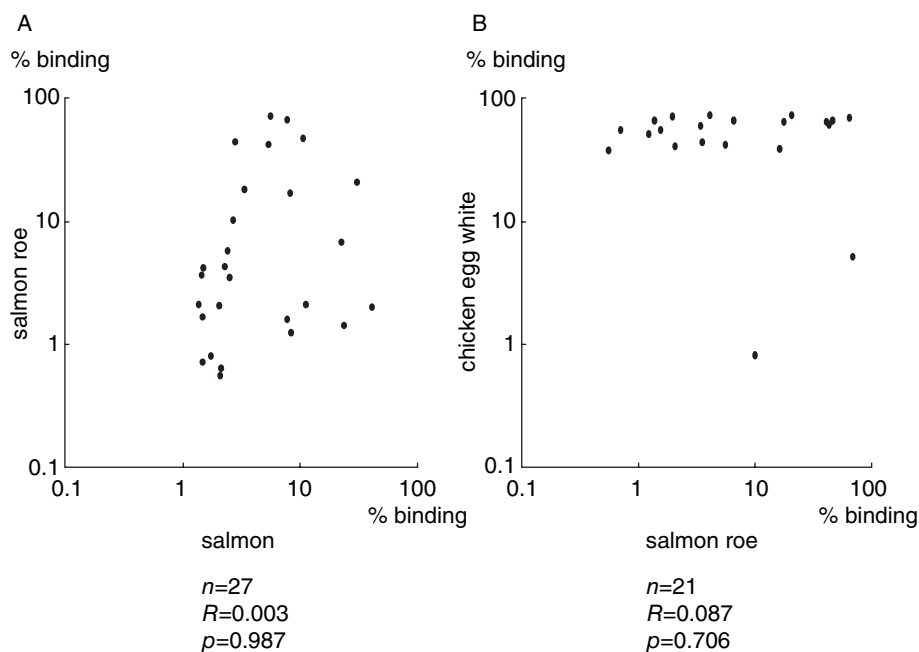
### TRANSFER AND IMMUNOBLOTTING

Sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed in a 4–20% Tris-glycine precast gel (Tefco Corporation, Machida, Japan) according to Laemmli under reducing conditions. Each sample was separated at 120 V for 2 h. Immunoblot of the proteins and detection of bound serum IgE were performed as previously reported.<sup>7</sup>

### N-TERMINAL AMINO ACID SEQUENCE

After blotting, Immobilon™-P membranes were stained with 0.1% amido black (Sigma Diagnostics, St. Louis, MO, USA) in 50% methanol and 10% acetic acid, destained with 40% methanol and 10% acetic acid and air-dried. Protein bands with IgE-binding activity

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**Fig. 1** Relationship of the specific IgE value between salmon and salmon roe (**A**) and between salmon roe and chicken egg white (**B**). IgE values of each extract were measured by radioallergosorbent test (RAST) in serum from 27 fish allergy patients and 26 non-fish allergy patients (control subjects). When the IgE values of both extracts were less than the mean + 2SD of the control subjects, the sample was excluded from this study. There were no significant relationships of IgE values between salmon and salmon roe ( $R=0.003$ ), and between salmon roe and chicken egg white ( $R=0.087$ ).

were excised and subjected to N-terminal amino acid sequencing via automated Edman degradation using an Applied Biosystems model 470 A protein sequencer.<sup>8</sup> The resulting phenylthiohydantion (PTH) amino acid derivatives were identified using a model 120A one-line PTH analyzer and the standard Applied Biosystems program. The N-terminal amino acid sequence of each protein was determined at least twice.

### HUMAN SERA

Twenty-seven patients (male : female = 20 : 7, range of age ; 6 months (m) to 11 years (y) ; mean  $\pm$  SD = 4y4 m  $\pm$  3 y 6 m,) allergic to fish were enrolled in the RAST study. Fish allergy was diagnosed based on at least one convincing report of a hypersensitive reaction to fish ingestion and positive results (more than class 2) to at least one item (salmon) of the fish-specific IgE using the CAP system (Pharmacia Diagnostics, Uppsala, Sweden). They had episodes of clinical allergic reactivity to one or more fish. Twenty-six children (male : female = 18 : 8, range of age ; 3 m to 11 y 9 m; mean  $\pm$  SD = 4 y 8 m  $\pm$  3 y 6 m ) were enrolled as control subjects for the RAST study. They had no histories of allergic reactions to fish ingestion and had negative IgE results (class 0) to at least five

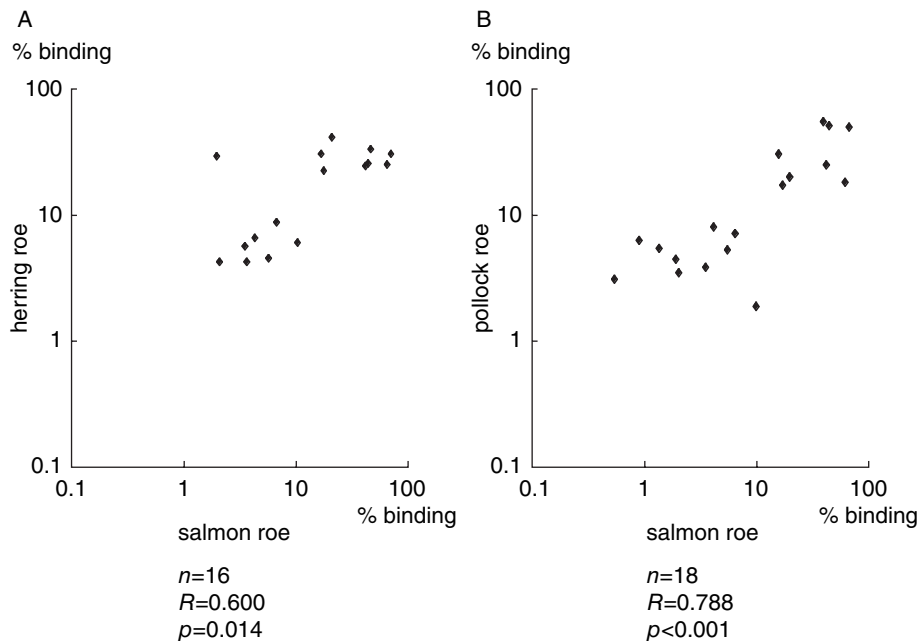
items from fish in a commercial CAP-system.

As samples for the ELISA inhibition and immunoblot study, we choose sera from 3 patients. Two patients had obvious hypersensitive episodes (anaphylaxis) to SR and another patient had no episodes of hypersensitive reaction because of their avoidance of SR due to its high-IgE values (class 2) to salmon, SR and CEW. All samples were stored at  $-20^{\circ}\text{C}$  until use.

## RESULTS

### THE RELATIONSHIP OF THE IgE VALUES BETWEEN SALMON AND SR (Fig. 1A), AND BETWEEN SR AND CEW IN THE RAST STUDY (Fig. 1B)

We measured the IgE value to each extract in the serum of fish allergy patients and control subjects. When the IgE values to both extracts were less than the mean + 2SD of the control subjects, the sample was excluded from this study. Finally, 27 patients were enrolled, as shown in Figure 1A, and 21 samples were used, as shown in Figure 1B. There were no relationships in the IgE values between salmon and SR ( $R = 0.003$ ) or between SR and CEW ( $R = 0.087$ ).



**Fig. 2** Relationship of the IgE value between salmon roe and herring roe (A) or pollock roe (B). Each sample was measured in the same way as described in Figure 1. There were positive correlations between salmon roe and herring roe ( $R=0.600$ ) and between salmon roe and pollock roe ( $R=0.788$ ).

### RELATIONSHIP OF IgE VALUES BETWEEN SR AND HR OR PR (Fig. 2)

The samples were measured in the same way as described above and the results are shown in Figure 2. Sixteen (A) and eighteen (B) samples were matched in each study. There were positive correlations between SR and HR ( $R = 0.600$ ), and between SR and PR ( $R = 0.788$ ).

### ELISA INHIBITION

As shown in Figures 3A, B, the binding of IgE from two anaphylaxis patients to SR was inhibited more than 50% by pre-incubation of the serum with HR and almost 50% by those with PR in a dose dependent manner; however, there was no efficient inhibition with CEY and anisakis. CEW also did not efficiently inhibit the binding of IgE to SR (data not shown). Because the binding of IgE to salmon and CEY was quite low, the assessment of inhibition of these two allergens was not possible. Inhibition of the IgE binding to SR was achieved more than 50% by salmon in patient A. In another patient (B), inhibition of the IgE to SR was achieved nearly 50% by salmon inhibitor at one level below the maximum concentration. Because the solution of salmon inhibitor was very sticky, testing the maximum concentration (10,000  $\mu\text{g}/\text{ml}$ ) was not possible.

As shown in Figure 3C, the binding of IgE to each extract in patients who avoided SR because of the high IgE values to salmon, SR and CEW, was not in-

hibited by any heterogeneity inhibitor.

### IMMUNOBLOTTING OF SR WITH SERA FROM 3 PATIENTS (Fig. 4)

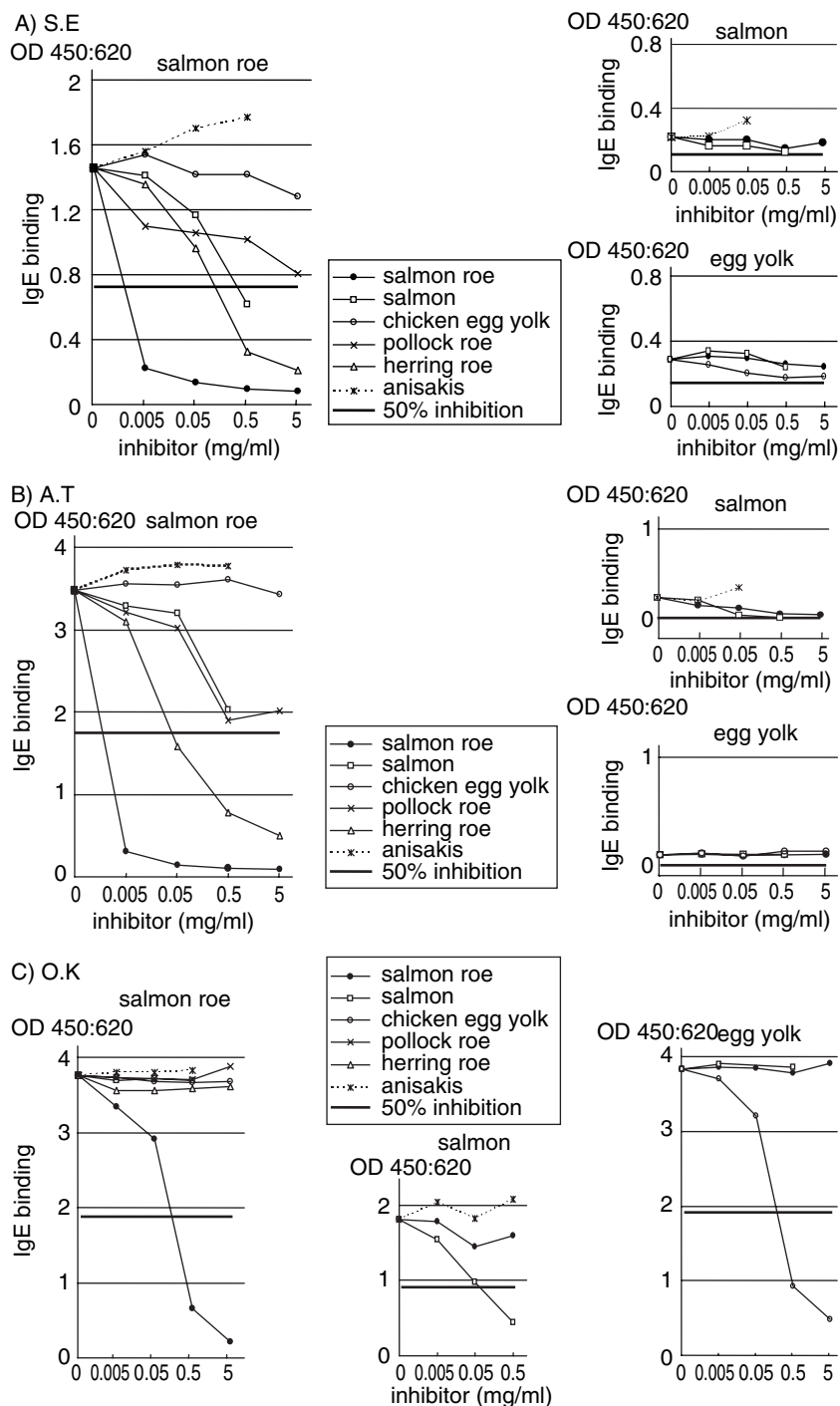
The IgE binding patterns to SR on the membrane were different between patients with anaphylaxis to SR and without any anaphylactic episodes to SR. The two anaphylactic patients reacted to the protein with relatively low molecular weight bands (15 and 17 kDa), while the patient with no episodes of hypersensitivity to SR reacted to that with a relatively high molecular weight protein band (21 kDa). Partial protein sequences of these bands were determined and screened for homology with sequences in the Swiss Prot Data Base. These protein bands were suspected as being fragments of vitellogenin, because of an almost complete identity with the amino-acid sequence of vitellogenin precursor (JC 4956) from rainbow trout.<sup>9</sup>

### DISCUSSION

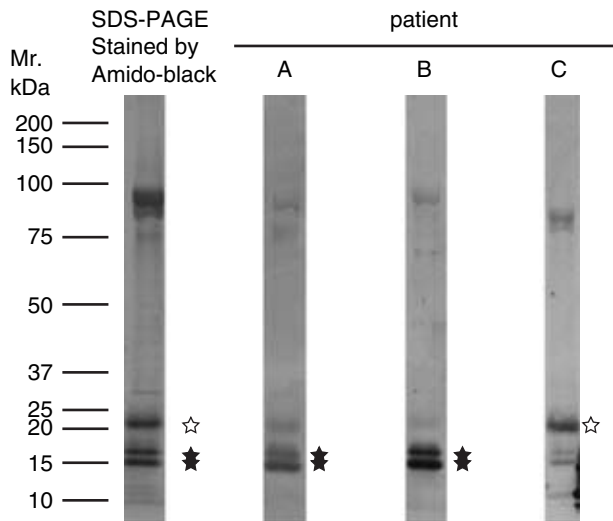
SR allergy is well known to cause severe anaphylaxis, and it was thus recommended to be labeled as allergenic. Despite its nature, there were no articles about its allergenicity, including cross-reactivity. In clinical work we were at a loss to account for whether a patient with egg allergy can eat SR safely or a fish allergic patient can eat SR.

Regarding the cross-reactivity between SR and CEW, Ito *et al.* recently described that there was no

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**Fig. 3** ELISA inhibition between salmon roe (SR) and various antigen (salmon, herring roe: HR, pollock roe: PR or chicken egg yolk: CEY) using sera from two patients who had anaphylactic reactions to salmon egg (**A** and **B**), and sera from a patient with fish allergy who had no allergic reactions to SR but avoided SR because the IgE value of salmon, SR, chicken egg white were high (**C**). In the two sera from patients with anaphylaxis to SR (**A**, **B**), the binding of IgE to SR was inhibited more than 50% by pre-incubation of the serum with HR and almost 50% by those with PR in a dose dependent manner; however, no efficient inhibition of IgE to SR was seen by pre-incubation with CEY or anisakis. Salmon inhibited the IgE binding to SR more than 50% in patient **A**. On the other hand, in the sera from patient **C** the IgE binding to each extract was not inhibited by pre-incubation of the serum with any heterogeneity extract.



**Fig. 4** Immunoblot of salmon roe with sera from the same patients described in Figure 3.

The IgE binding patterns were different between the patients with anaphylaxis to salmon roe (A and B) and fish allergy without anaphylactic episodes to salmon roe (C). Both IgE from anaphylaxis patients bound to relatively low molecular weight protein bands, while IgE from the fish allergy patient reacted with the relatively high molecular weight protein band. Outlined and solid stars indicate the protein bands of salmon roe that were strongly bound by the patient's IgE.

correlation between them based on measuring the specific IgE with the CAP-RAST system.<sup>10</sup> The same results were seen in our RAST study. Moreover, Tanaka *et al.* reported in the minutes of the Japanese Society of Allergology (2001) that there were no cross-reactivities between SR and CEW or CEY in their inhibition immunoblot study.<sup>5</sup> These results corresponded with those of our ELISA inhibition study. These data suggested that there were no cross-reactivities between SR and CEW or CEY; however, our examinations were performed only in a small number of samples and further investigations are needed to clear this important problem.

With regard to the cross-reactivity between SR and salmon, a recent study has described that there was no significant relationship between them.<sup>10</sup> That was also observed in our RAST data. Additionally, there was no cross-reactivity in the inhibition immunoblot study.<sup>5</sup> However, we think that it is undeniable that there is cross-reactivity between SR and salmon. Shibata *et al.* described in their discussion the need for careful attention when feeding fish roe to patients with fish allergy, because the patients with anaphylaxis to fish roe often have fish allergy.<sup>11</sup> Actually, in our ELISA inhibition study using the serum from two patients having anaphylaxis to SR without allergy to

salmon, salmon extract inhibited more than 50% of IgE binding to SR in one patient, while in another patient salmon was inhibited by almost 50%. Neglecting the effect of anisakis on ELISA inhibition between salmon and SR, we used anisakis extract as an inhibitor and confirmed there was no inhibition of the IgE binding to salmon and SR. We also confirmed there was no contamination of SR to salmon using a patient who had no history of hypersensitive reactions to SR but high IgE values to salmon, SR and CEW, as shown in Figure 3C. Our data were different from previous data.<sup>5</sup> This difference may account for the reason why the analysis of the inhibition with the immunoblot was qualitative, while the ELISA inhibition was quantitative. Our data suggested that there is cross-reactivity between SR and salmon in some cases.

Cross-reactivity between SR and different kinds of fish roe was suspected by Watanabe *et al.* using the CAP-RAST system only in abstract form.<sup>4</sup> In our RAST examination, there were positive correlations between SR and HR or PR. In another report, using an inhibition immunoblot study, HR and PR inhibited the IgE binding to SR.<sup>5</sup> In our ELISA inhibition, using two sera from patients who had anaphylaxis to SR, the binding of IgE to SR was inhibited by both kinds of roe (HR and PR) to different degrees. This means that HR inhibited the IgE binding to SR more than 50%, while PR inhibited it to almost, but less than 50%. Those results suggested that there is a significant cross-reactivity between SR and HR. On the other hand, we suspected a partial cross-reactivity between SR and PR, because PR inhibited the IgE binding to SR in a dose-dependent manner, but less than 50%. We have been unable to come to a conclusion regarding the cross-reactivity between SR and PR, because of the small number of samples in our study.

Between two anaphylactic patients and a patient without anaphylactic episodes to SR there were differences in the IgE binding pattern to SR. Two anaphylaxis patients' IgE binding patterns were very similar, with reactions to protein bands of 15 and 17 kDa, while the pattern of IgE binding from another patient who had no experiences of anaphylaxis to SR reacted mainly to the relative higher molecular weight protein band (21 kDa) in Figure 4. The N-terminal amino acid sequences of these proteins were almost identical to the vitellogenin precursor of rainbow trout (*Oncorhynchus mykiss*). Two protein bands of 15 and 17 kDa had the same amino acid sequence and they were thought of as fragments from vitellogenin. The difference in molecular weight between them might have been due to a modification by carbohydrates or a different breaking point at the C-terminal. The 21 kDa protein had a different sequence to those of 15 and 17 kDa and was thought of as another fragment of vitellogenin. The smaller fragment was suspected to be the beta'-component and the large fragment was

speculated to be lipovitellin, due to the sequence similarity and molecular weight, respectively. These results were similar to Kubo's report<sup>3</sup> that the beta'-component has a relationship with the symptoms.

Moreover, recent research on molecular analysis has verified the presence of multiple vitellogenins in at least some fish species.<sup>12</sup> From these data we can say that vitellogenin exists in many kinds of fish roe, fish liver and even in chicken yolk with various mutations, and those mutations increase with species change. Symptoms of anaphylaxis to SR may develop in patients whose IgE is bound on a certain epitope of vitellogenin, especially on the amino acid sequence of the beta'-component; however, we can not state conclusively that the cross-reactivity was caused by vitellogenin because of insufficient data to demonstrate this.

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