Myosin heavy chain, a novel allergen for fish allergy in patients with atopic dermatitis

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Fish allergy (FA) is the second most frequent type of food allergy in Japan.[1] Fish allergens listed in the database of the WHO include parvalbumin, which is known to be the major allergen, β-enolase, aldolase A, vitellogenin and tropomyosin.[2] Patients with atopic dermatitis (AD) have an increased risk for sensitisation to allergens due to their increased skin barrier permeability.[3] Here, we enrolled six patients with FA, who had also been diagnosed with AD according to the diagnostic criteria of the Japanese guidelines.[4] A table summarising the clinical details of ~~these six cases~~ and controls is available ~~from the corresponding author on application~~. All of those patients showed immediate-type responses, such as urticaria or anaphylaxis, upon eating fish and had positive results of skin prick tests and/or serum IgE reactivity with CAP for fish allergens.

Western blot (WB) analysis using crude extracts from salmon revealed that a protein of approximately 230-kDa (arrowheads, Fig. 1a) was recognized by serum IgE from all six patients except #4. None of the sera from six control individuals reacted with the 230-kDa protein (two representative blots are shown as controls #1 and 2). We performed two-dimensional (2D) gel electrophoresis to separate proteins according to their isoelectric point (*pI*) followed by immunoblotting using sera from patient #1 and control #1 (Fig. 1b, c, respectively). The expected allergen recognized by the patient's IgE but not by the control IgE was a protein of approximately 230-kDa at a *pI* of 4.5-6.0 (Fig. 1b, c). The protein spots with the expected molecular weight at five different *pI*s were excised (arrows, Fig. 1b), analysed by nano LC-MS/MS and all were identified as myosin heavy chain (MYHC).

Further, the cDNA of salmon MYHC was synthesized, overexpressed in *Escherichia coli*, purified as recombinant salmon (rs) MYHC and then used for WB analysis. As expected, a protein of approximately 230-kDa was detected by serum IgE from patients

#1, 2, 3, 5 and 6 (arrowheads, Fig. 1d), but not by patient #4 or the controls. Pre-incubation of the sera from patients #1 and 3 with the salmon protein extract abrogated the IgE reactivity with rsMYHC (Fig. 1e), clearly indicating that the 230-kDa protein with which IgE reacted was MYHC. However, the IgE reactivity with rsMYHC remained after pre-incubation with the salmon extract in which MYHC had been absorbed by an anti-MYHC IgG antibody (arrowheads, Fig. 1e), further strengthening the IgE specificity for MYHC. Finally, basophil activation tests revealed the rsMYHC-dependent IgE reactivity in the peripheral blood of patient #6 (Fig. 1f).

MYHC, an approximately 225-kDa protein, forms complexes with myosin light chains, which is known to be an allergen for shrimp allergy.[2] However, no previous study has identified MYHC as a fish allergen. Based on a blast search, the amino acid sequence of salmon MYHC exhibits a homology of only about 47.3% to shrimp MYHC. Since our patients did not have an allergy to shrimp, the allergic epitopes of fish MYHC might be distinct from those in shrimp. The antigenicity of MYHC was heat-sensitive, since the immunoblot density was much reduced when the fish extract used was boiled (data not shown). This might be due to the relatively large size of MYHC, whose allergic epitope could be easily degraded by heat treatment. This also suggests that the sensitisation to raw fish develops the allergy to MYHC.

Since all of our FA patients had AD with eczema on their hands either at present or in the past, they might have been sensitised to MYHC during contact due to their increased skin barrier permeability. However, it is noteworthy that AD patients do not necessarily show positive IgE-reactivity for MYHC, since control #2 had severe AD but no FA (Fig. 1a). Surprisingly, the findings in a 7-year-old boy (#2), who first developed generalized urticaria at the age of 1 year and reacted to both parvalbumin and MYHC; a 10-month-

old infant patient (#4), who was reactive to parvalbumin but not to MYHC; and a 14-year-old girl (#3) and a 5-year-old girl (#6) who reacted to MYHC but not to parvalbumin, suggested a potential sensitisation to fish allergens through AD skin at an early time of life. Thus, MYHC should be on the list of FA allergens to allow us to make a correct diagnosis of FA, particularly in cases not determined by conventional screenings with parvalbumin as the representative allergen. Raw fish cuisine has become very popular not only in Japan but also outside of Japan. Touching raw fish might increase the risk of sensitisation to MYHC in AD patients.

References

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Figure legend

Fig. 1. (a) Western blot using sera from FA patients and controls. Heat-untreated protein

extracts from salmon were used for immunoblotting with sera from 6 patients and 2 controls, then were developed with anti-human IgE. Arrowheads, approximately 230-kD proteins; #, non-specific bands presumably derived from the second antibody, even in the absence of serum (data not shown). (b, c) Heat-untreated protein extracts separated by 2D-gel electrophoresis followed by IgE immunoblotting using sera from patient #1 (b) and control #1 (c). Five fractions of approximately 230-kD proteins with different *pI*s (red arrows) were isolated, then analyzed by nano LC-MS/MS and all were identified as MYHC. #, aforementioned non-specific bands. (d) Western blot using rsMYHC. CBB, Coomassie Brilliant Blue R-250; arrowheads, bands corresponding to rsMYHC. (e) Validation of IgE immunoreactivity to MYHC with an inhibition assay. Sera from patients #1 and #3 were pre-incubated with or without the salmon protein extract, then Western blotted on rsMYHC. The specificity of MYHC was confirmed by restoration of the IgE reactivity when salmon protein extracts were used after absorption of MYHC with an anti-MYHC IgG antibody. Arrowheads, bands corresponding to rsMYHC; Asterisks, non-specific bands presumably derived from anti-MYHC IgG. (f) Basophil activation test. Peripheral blood from patient #6 was used. Positive control, anti-IgE; negative control, PBS. rsMYHC, 10 μg/mL. Numbers indicate the percentage of $CRTH2^{+}CD203c^{+}$ cells (arrows).