

blocked by curcumin (data not shown). These findings suggest that curcumin attenuates the transcription and secretion of IL-6 following *P. acnes* extracts stimulation.

TLR2 is known to be involved in IL-6 production in monocytes stimulated by *P. acnes*.³ In our present study, exposure of THP-1 cells to heat-killed *P. acnes* resulted in increased expression of TLR2 (Fig. 1a). Next, we investigated whether the curcumin mediated the suppression of IL-6 via TLR2. Pretreatment with curcumin significantly suppressed *P. acnes* extracts-induced upregulation of TLR2 mRNA expression (Fig. 1a). Moreover, co-treatment of THP-1 cells with curcumin and a TLR2 inhibitor, C29 (100 $\mu\text{mol L}^{-1}$), yielded greater suppression of the *P. acnes* extracts-induced IL-6 expression than did curcumin pretreatment alone (Fig. 1b). These results indicate that curcumin suppresses *P. acnes* extracts-induced IL-6 production by downregulating TLR2.

Activation of the NF- κ B signalling pathway is involved in TLR2-induced inflammatory cytokine production. NF- κ B p50, p65 and the inhibitor protein I κ B form an inactive ternary complex in the cytoplasm. When the NF- κ B signalling pathway is activated, I κ B α is phosphorylated and degraded, whereby NF- κ B p50/p65, released from I κ B α , translocates to the nucleus, binds to specific DNA-binding sites and regulates gene transcription.⁸ Our data in Figure 1(c) also displayed phosphorylation and degradation of I κ B α in THP-1 cells stimulated by *P. acnes* extracts, indicating activation of NF- κ B signalling. Furthermore, curcumin pretreatment for 2 h suppressed this *P. acnes* extracts-induced degradation and phosphorylation of I κ B α (Fig. 1c). Confocal microscopy revealed that the *P. acnes* extracts-induced translocation of NF- κ B p65 was blocked following curcumin pretreatment (Fig. 1d). Co-treatment of the cells with curcumin and an NF- κ B inhibitor (BAY 11-7082) yielded greater suppression of *P. acnes* extracts-induced IL-6 expression than did curcumin pretreatment alone (Fig. 1e). (Further figures are available on request from the authors.) However, there are two limitations of this study. Firstly, whether curcumin could affect other cytokines or pathways associated with acne was not investigated; secondly, its effects in an *in vivo* model need to be clarified.

In conclusion, curcumin exerts anti-inflammatory effects on heat-killed *P. acnes*-stimulated THP-1 cells by suppressing IL-6 production via TLR2 downregulation and inhibition of the TLR2–NF- κ B signalling pathway.

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

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DEAR EDITOR, Fish allergy (FA) is the second most frequent type of food allergy in Japan.¹ Fish allergens listed in the database of the World Health Organization 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 sensitization to allergens because of 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 summarizing the clinical details of the patients and controls is available on request from the authors. All the patients showed immediate-type responses, such as urticaria or anaphylaxis, upon eating fish and had positive results on 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 patient 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-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 pIs were excised (arrows, Fig. 1b), analysed by nano liquid chromatography–mass spectrometry (MS)/MS and all were identified as myosin heavy chain (MYHC).

Further, the cDNA of salmon MYHC was synthesized, over-expressed 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 control participants. Preincubation 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 preincubation 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 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. As our patients did not have an allergy to shrimp, the allergic epitopes of fish

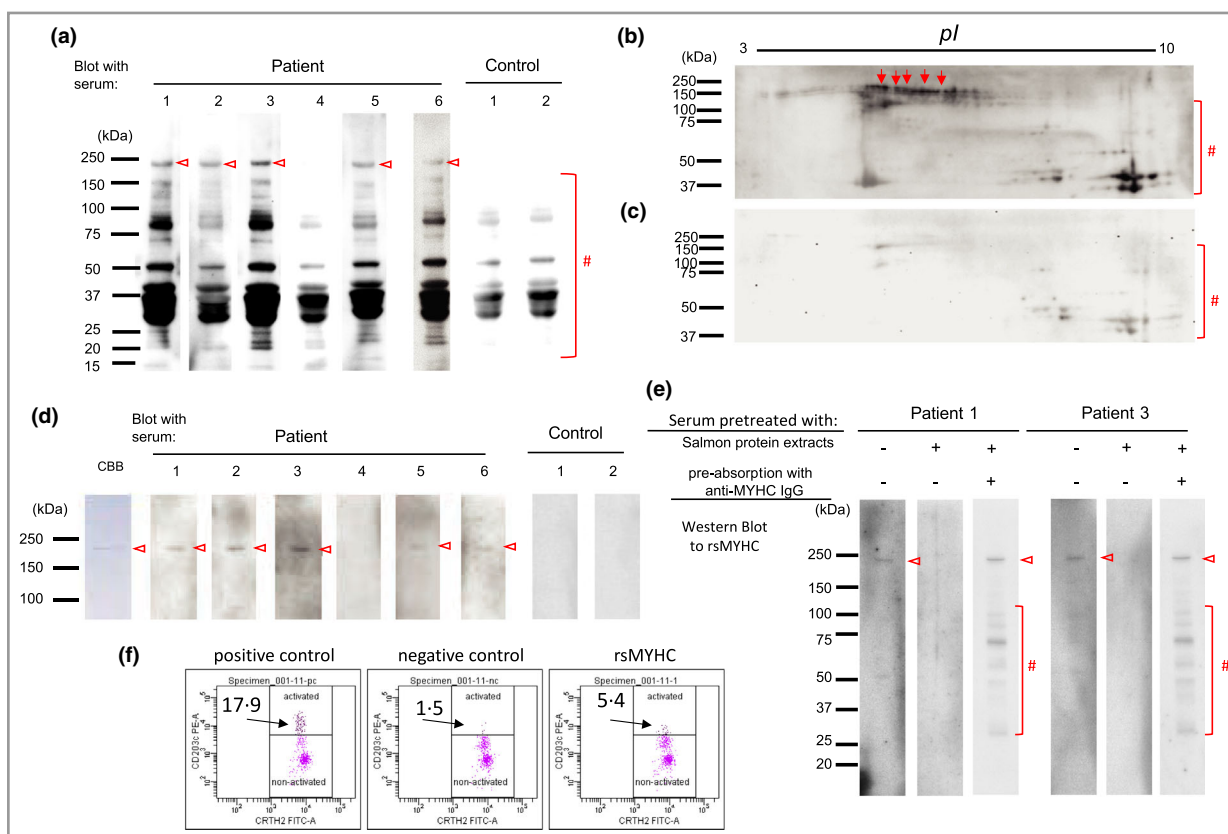


Fig 1. (a) Western blot (WB) using sera from patients with fish allergy and controls. Heat-untreated protein extracts from salmon were used for immunoblotting with sera from six patients and two controls, then were developed with anti-human IgE. Arrowheads, approximately 230-kD proteins; #, nonspecific bands presumably derived from the second antibody, even in the absence of serum (data not shown). (b, c) Heat-untreated protein extracts separated by two-dimensional-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 isoelectric points (pIs, red arrows) were isolated, then analysed by nano liquid chromatography–mass spectrometry (MS)/MS and all were identified as myosin heavy chain (MYHC). #, aforementioned nonspecific bands. (d) WB using recombinant salmon (rs)MYHC. 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 preincubated 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; #, nonspecific bands presumably derived from anti-MYHC IgG. (f) Basophil activation test. Peripheral blood from patient 6 was used. Positive control, anti-IgE; negative control, phosphate-buffered saline. rsMYHC, 10 µg mL⁻¹. Numbers indicate the percentage of CRTH2⁺CD203c⁺ cells (arrows).

MYHC might be distinct from those in shrimp. The antigenicity of MYHC was heat sensitive, as the immunoblot density was much reduced when the fish extract used was boiled (data not shown). This might be because of the relatively large size of MYHC, whose allergic epitope could be easily degraded by heat treatment. This also suggests that the sensitization to raw fish develops the allergy to MYHC.

As all of our patients with FA had AD with eczema on their hands either then or in the past, they might have been sensitized to MYHC during contact because of their increased skin barrier permeability. However, it is noteworthy that patients with AD do not necessarily show positive IgE reactivity for MYHC, as control 2 had severe AD but no FA (Fig. 1a). Surprisingly, the findings in a 7-year-old boy (patient 2), who first developed generalized urticaria at 1 year of age 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 (patient 3) and a 5-year-old girl (patient 6) who reacted to MYHC but not to parvalbumin, suggest a potential sensitization to fish allergens through AD skin early in 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 sensitization to MYHC in patients with AD.

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Does surgery of lymphatic malformations lead to an increase in superficial lymphangiectasia? A retrospective study of 43 patients

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DEAR EDITOR, Lymphatic malformations (LM) are rare congenital low-flow vascular malformations.¹ The goal of LM management is to maintain functionality, control associated symptoms and preserve aesthetic integrity. It requires a multidisciplinary approach, considering surgery, sclerotherapy, observation and new, promising medical treatments.^{2,3} Over time, except in the case of small well-circumscribed lesions, it is obvious that complete surgical removal of the LM is almost always impossible because of infiltrative lesions.^{4,5} Moreover, partial excision is classically considered to be associated with the risk of local recurrence.⁶ Worsening of LM after surgery, especially occurrence of superficial lymphangiectasia (SL), is sometimes discussed. SL are bothersome for patients because of intermittent swelling, haemorrhage and leakage of lymph from superficial vesicles. The aim with this retrospective study was to assess whether surgery might be a triggering factor for SL occurrence.

All patients with LM and surgery seen by the vascular malformations multidisciplinary clinic at Necker-Enfants Malades Hospital between 2011 and 2018 were studied. Data were collected from chart review, medical pictures and phone interviews with patients when necessary. The following information was retrieved: demographic data, the International Society of Study of Vascular Anomalies classification, location, details of surgery, presence of SL before and after surgery [no SL, stable SL (previously known and no exacerbation), occurrence of SL in the 6 months following surgery (two subgroups: few/scattered or diffuse SL)], and volume reduction assessment at last follow-up. We retrospectively studied the presence or not of SL in the patients seen in our clinic with LM who had not undergone surgery. Data were collected from medical charts and photographs.

In total, 43 patients (25 female and 18 male patients) were included. Clinical characteristics are summarized in Table 1. Surgical procedures included biopsies ($n = 5$), volume reduction surgery ($n = 35$) and limb amputation ($n = 3$). Sixteen patients (37%) do not have SL; 14 patients (33%) have SL and they remained stable; in seven patients, with mixed or microcystic LM, SL occurred within the first 6 months following surgery, among whom, four had diffuse SL and three only discrete SL. SL occurrence was observed on LM located on the limbs only.

All patients in the new onset of diffuse SL group were operated on before 1 year of age and were operated on between two and nine times. Twenty patients were operated on once. Only two of them developed discrete SL following surgery. The mean number of operations was 1.58 (range 1–5) in the no SL group. In the 33 patients who underwent debulking