

Basophils from allergic rhinitis patients show allergen-specific upregulation of thymic stromal lymphopoietin receptor

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ARTICLE INFO

Article history:

Received for publication October 26, 2017.

Received in revised form November 24, 2017.

Accepted for publication December 11, 2017.

ABSTRACT

Background: An allergic rhinitis (AR) diagnosis is based on typical nasal symptoms and allergen sensitization testing. However, it is sometimes difficult to distinguish AR from non-AR, and it is especially difficult to identify the causal allergen for immunotherapy of patients with AR.

Objective: To identify differences among patients with AR, subjects with asymptomatic sensitization (AS), and subjects without sensitization (NS) that could facilitate the diagnosis of AR.

Methods: We used RNA sequencing to examine differential gene expression in unstimulated and allergen-stimulated peripheral basophils from participants with NS, AS, and AR. We selected genes that were upregulated after allergen stimulation and showed differences in expression in patients with AR compared with subjects with AS and NS. Basophil surface expression of 1 gene product was examined by flow cytometry. The usefulness of gene expression in the diagnosis of AR was examined with receiver operating characteristic curves.

Results: Expression of cytokine receptor-like factor 2 and its product, thymic stromal lymphopoietin receptor (TSLPR), was significantly increased in basophils of patients with AR after allergen stimulation. A significantly larger percentage of TSLPR-positive cells was observed after allergen-specific stimulation of basophils from patients with AR compared with subjects with AS. Basophil TSLPR expression was as good as or better than CD203c expression in discriminating between patients with AR and subjects with AS, as judged by receiver operating characteristic curves.

Conclusion: Our data suggest that TSLPR expression on basophils was specifically upregulated by allergen stimulation and might be useful for the identification of the causative allergen in patients with AR.

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Introduction

The diagnosis of allergic rhinitis (AR) is based on nasal symptoms, such as paroxysmal sneezing, nasal secretions, and nasal obstruction, and allergen testing. Allergen examinations include skin prick tests, measurements of specific immunoglobulin E (sIgE) in serum, and nasal allergen provocation tests. However, it is frequently difficult to distinguish AR from non-AR in clinical practice.¹ Although positive skin prick test reactions and the presence of sIgE in serum indicate sensitization with an allergen, they do not prove AR. Many asymptomatic subjects are sensitized to allergens but do not develop AR after allergen exposure,^{2–4} and it is not easy to dis-

criminate patients with AR from subjects with asymptomatic sensitization (AS). The nasal allergen provocation test can yield false-positive and false-negative results and must be performed with care. The quality and dose of the allergen used for provocation also must be carefully evaluated and the method should be standardized.^{5–7} In addition, nasal provocation tests are difficult to perform with young pediatric subjects.

A recent review of randomized controlled studies demonstrated that allergen-specific immunotherapy is efficacious and can change the natural course of AR.⁸ Immunotherapy for a certain allergen is effective only for patients who have AR induced by that allergen; therefore, confirmation of the causative allergen is essential.

Basophils express a high-affinity IgE receptor (FcεRI) on their cell surface, and the cells are activated by crosslinking of FcεRI-bound sIgE with the allergen. Activation induces secretion of preformed and newly synthesized mediators involved in the allergic inflammatory response.^{9–11} Recent studies have shown that basophils play active roles in the induction of T-helper cell type 2 responses, such as interleukin (IL)-4 production and antigen presentation.^{12–17} A previous study reported that basophils from subjects with AS are less

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Disclosures: Authors have nothing to disclose.

Funding Sources: This work was supported by a Project of City Area Grant and Japan's Agency for Medical Research and Development.

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<https://doi.org/10.1016/j.anaai.2017.12.005>

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reactive than cells from patients with AR, as measured by anti-IgE-triggered histamine release.¹⁸ The basophil activation test (BAT) is an ex vivo test that monitors upregulation of CD203c or CD63 expression at allergen exposure and thus measures the functional response of basophils.¹⁹ The BAT is useful for the diagnostic evaluation of patients with food and drug allergies and chronic urticaria.^{20–22} Although a correlation between the BAT and nasal provocation sensitivity in patients with AR has been reported,²³ the BAT has not been established as a standard method for AR diagnosis. Moreover, some patients with AR have been reported to be BAT nonresponders.^{19,21,24,25}

In this study, we sought to identify differences in basophils from patients with AR from subjects with AS that might contribute to the diagnosis of AR. We isolated peripheral basophils from 3 groups of individuals (patients with AR, subjects with AS, and subjects without sensitization [NS]), stimulated the cells with allergens in vitro, and examined gene expression by RNA sequencing. Then, we selected a differentially expressed candidate gene and determined the usefulness of changes in expression of the gene product for the diagnosis of AR induced by pollen and house dust mites (HDM).

Methods

Study Participants

Thirty-eight participants were classified into 3 groups: subjects with NS, subjects with AS, and patients with AR. Sensitization to Japanese cedar (JC) pollen (JCP) or HDM was considered positive if a serum JCP sIgE or HDM sIgE score was at least class 2 (0.7 UA/mL). The sensitization to JCP was tested outside the cedar pollen season using the ImmunoCAP method (Phadia AB, Uppsala, Sweden). Patients with AR and subjects with AS were recruited from our outpatient clinic. Volunteers were recruited for the NS group. All participants were tested using the nasal provocation test with JCP and HDM extract-containing disks (Torii, Tokyo, Japan). Patients with AR were sensitized to JCP and had typical symptoms of AR, such as paroxysmal sneezing, runny nose, nasal congestion, and nasal itching, that persisted during cedar pollen season (from the beginning of February to the end of April) for at least the past 3 years. This group, designated JCP/AR, also showed positivity for the nasal provocation test using a JCP extract-containing disk. Subjects in the JCP/AS group were sensitized to JCP but displayed none of the typical seasonal symptoms described earlier and showed negativity for the JCP nasal provocation test. The HDM/AR group was sensitized to HDM, showed persistent typical nasal symptoms, and showed positivity for the HDM nasal provocation test. The HDM/AS group was sensitized to HDM but displayed none of the persistent symptoms and showed negativity for the HDM provocation test. Subjects in the JCP/NS group were non-sensitized to JCP allergens, showed negativity for the JCP provocation test, and had no typical seasonal nasal symptoms. Subjects in the HDM/NS group were non-sensitized to HDM allergens, showed negativity for the HDM provocation test, and had no typical persistent nasal symptoms. This study was approved by the ethics committees of Chiba University (Chiba, Japan) and was performed in accordance with the principles expressed in the Declaration of Helsinki. Written informed consent was obtained from all study subjects.

Analysis of Basophil Gene Expression by RNA Sequencing

Blood samples (90 mL) were obtained from subjects in the 3 groups (JCP/NS: $n = 6$, 5 men, mean age $\pm$ SD 35.8 ± 4.5 years; JCP/AS: $n = 7$, 6 men, 37.9 ± 4.1 years; JCP/AR: $n = 9$, 6 men, 38.9 ± 4.6 years) outside the JCP dispersal season. They took no antiallergic medications such as antihistamines, anti-leukotrienes, and ste-

roids for at least 1 month before the blood sampling. Basophils were purified by negative selection using the EasySep Human Basophil Enrichment kit (Stemcell Technologies, Grenoble, France). The purified basophils were incubated for 4 hours with or without 0.1 ng/mL of JCP extract (Hayashibara, Okayama, Japan) in RPMI-1640 complete medium (Sigma-Aldrich, St Louis, Missouri). Total RNA was extracted using the TRIzol reagent (Life Technologies, Gaithersburg, Maryland) and prepared using a PureLink RNA Mini Kit (Invitrogen, Carlsbad, California). RNA sequencing (RNA-seq) libraries were prepared using a SureSelect Strand-Specific RNA Library Preparation kit (Agilent, Santa Clara, California). Sequencing was performed on a HiSeq 1500 system using a TruSeq Rapid SBS kit (Illumina, San Diego, California) in 50-base single-end mode. RNA-seq data analysis was performed with the CLC Genomics Workbench (Qiagen, Redwood City, California) using the same parameters as in the TopHat-Cufflinks analysis. Expression data were normalized using the fragments per kilobase of exon model per million mapped fragments (FPKM) values by quantiles. Genes differentially expressed in unstimulated and JCP-stimulated basophils from the 3 groups were evaluated using R 2.15.0 software (R Project, Vienna, Austria). Statistically significant differences in gene expression were analyzed using the Kruskal-Wallis test followed by the Steel-Dwass post hoc test and were expressed as fold change. A P value less than .05 was considered statistically significant. A change in mean FPKM value greater than 1.5 among the 3 groups was defined as differential expression.

Real-Time Polymerase Chain Reaction

Total RNA was extracted from purified basophils using the TRIzol reagent and reverse transcribed using a SuperScript VILO cDNA Synthesis Kit (Applied Biosystems, Foster City, California). Cytokine receptor-like factor 2 (*CRLF2*) expression levels were calculated using a sequence detection system (StepOnePlus, *CRLF2* assay ID Hs00845692_m1; Applied Biosystems), and cDNA levels were normalized to the expression of *ACTB* (assay ID Hs99999903_m1).

Detection of Thymic Stromal Lymphopoietin Receptor Expression by Flow Cytometry

For the experiment using purified basophils, cells were incubated for up to 48 hours with 0, 0.1, or 1 ng/mL of JCP extract or 1 μ g/mL of anti-IgE monoclonal antibody (Bethyl Laboratories, Montgomery, Texas) in RPMI-1640 complete medium at 37°C in 5% CO₂. For the experiment using basophils from whole blood, samples of heparinized whole blood (5 mL) were obtained from the subjects and 100 μ L of the samples was incubated for 24 hours with 0, 0.1, or 1 ng/mL of JCP extract or HDM extract (INDOOR Biotechnologies, Charlottesville, Virginia) in a 1-mL volume of complete medium. Erythrocytes were lysed with a lysing solution. For the 2 experiments, cells were incubated with Fc blocking reagent (Miltenyi Biotec, Bergisch Gladbach, Germany) and then stained with allophycocyanin-conjugated antihuman CD3 (BioLegend, San Diego, California), fluorescein isothiocyanate-conjugated anti-CD294 (Santa Cruz Biotechnology, Dallas, Texas), and phycoerythrin-conjugated anti-thymic stromal lymphopoietin receptor (TSLPR; BioLegend) antibodies. Data were collected using a FACSCalibur (BD Biosciences, Franklin Lakes, New Jersey) and analyzed using FlowJo software (TreeStar, Ashland, Oregon).

Basophil Activation Test

CD203c expression on basophils was examined using an Allergenicity kit (Beckman Coulter, Fullerton, California) according to the manufacturer's protocol. Briefly, heparinized whole blood (100 μ L) was incubated for 15 minutes at 37°C with an activation

solution and with 0, 0.1, or 1 ng/mL of JCP extract or HDM extract. Then, cells were stained with allophycocyanin-conjugated anti-CD3, phycoerythrin-conjugated anti-CD203c (Beckman Coulter), and fluorescein isothiocyanate-conjugated anti-CD294. The percentage of CD203c-positive cells within the CD294-positive population was analyzed using a FACSCalibur.

Statistical Analysis

Data are presented as mean $\pm$ SD. Analysis of 2-group comparisons was performed using the Mann-Whitney *U* and paired *t* tests, as appropriate. Correlations were assessed using the Spearman rank test. A *P* value less than .05 was considered statistically significant. The ability of each test to discriminate patients with AR from subjects with AS was evaluated by constructing receiver operating characteristics (ROC) curves in which the sensitivity vs 1 – specificity was plotted for each possible cutoff level. The area under the curve (AUC) was determined using GraphPad Prism 6.00 (GraphPad Software, La Jolla, California).

Results

Subjects

The demographics and characteristics of the study subjects are presented in Table 1.

RNA-Seq and Real-Time Polymerase Chain Reaction Analysis of Basophils

The RNA-seq detected the expression of approximately 40,000 genes in basophils. Heat maps were created of gene expression in the 3 groups, with 2-group comparisons between JCP-stimulated and unstimulated conditions. The JCP/AR group showed the largest number of significantly differentially expressed genes (2,060) between unstimulated and JCP-stimulated basophils followed by the JCP/AS (1,301) and JCP/NS (647) groups (Fig 1A). We identified 12 genes (*ADAM8*, *ARL9*, *BRAF*, *CRLF2*, *DUS1L*, *ETV7*, *IL13*, *IL3RA*, *OR4C46*, *PBX1*, *SWT1*, and *XAB2*) for which the allergen-specific change in expression was significantly larger in the JCP/AR group than in the JCP/NS group and larger in the JCP/AR group than in the JCP/AS group. RNA-seq FPKM values for 1 of the 12 genes, *CRLF2*, in unstimulated and JCP-stimulated basophils from the 3 subject groups are shown in Figures 1B and 1C. This gene was confirmed only by real-time polymerase chain reaction analysis to show a significantly larger allergen-specific upregulation in basophils from the JCP/AR group than from the JCP/NS group (Fig 1D).

TSLPR Expression on Basophils after Stimulation with JCP Extract

The *CRLF2* gene product is a subunit of TSLPR. TSLPR expression was confirmed on purified basophils after stimulation with JCP

extract for 12 to 48 hours, as measured by flow cytometry. Maximal expression was detected on JCP/AR basophils after 12 to 24 hours. Representative profiles of JCP/AR and JCP/NS cells are shown in Figure 2A. Representative profiles of TSLPR expression on basophils after JCP allergen (0.1 ng/mL) stimulation also were detected when 100 μ L of whole blood samples from the JCP/AR group were analyzed (Fig 2B). Therefore, whole blood samples were used for the detection of TSLPR expression on basophils in subsequent experiments.

JCP-Specific Upregulation of TSLPR on Basophils From JCP/AR Group

The levels of serum JCP-specific IgE in the JCP/NS, JCP/AS, and JCP/AR groups are shown in Figure 3A. There was a significant difference in the levels of JCP-specific IgE between the JCP/AS and JCP/AR groups. However, there were no significant differences among the groups in the baseline expression of TSLPR on unstimulated basophils (Fig 3B) and no significant correlations between TSLPR expression and levels of total serum IgE, JCP sIgE, or HDM sIgE. There was a significant increase in TSLPR-positive basophils in cultures of JCP/AR blood samples stimulated with JCP extract for 24 hours compared with unstimulated cells (Fig 3C). In addition, the JCP-stimulated increase in the percentage of TSLPR-positive cells was significantly higher for basophils from the JCP/AR group compared with the JCP/AS and JCP/NS groups (Fig 3C). ROC curve analysis showed that JCP/AR could be diagnosed with a sensitivity, specificity, and accuracy of 58.3%, 95.0%, and 81.3%, respectively, using a cutoff value of 1.5-fold change in TSLPR-positive basophils after stimulation with 0.1 ng/mL of JCP extract. No significant correlations were detected in the JCP/AS or JCP/AR group between levels of serum JCP-specific sIgE and changes in TSLPR expression after stimulation with 0.1 ng/mL (Fig 3D) or 1 ng/mL of JCP extract (data not shown). Among subjects in the JCP/AR group who also were sensitized, but not allergic, to HDM, there was no significant increase (>1.5-fold) in the percentage of TSLPR-positive basophils after HDM stimulation.

CD203c Expression on Basophils from JCP/NS, JCP/AS, and JCP/AR Groups

A significantly larger percentage of JCP-stimulated than unstimulated basophils was positive for CD203c in the JCP/AS and JCP/AR groups (Fig 4A). In addition, significantly more basophils from the JCP/AR group compared with the JCP/AS and JCP/NS groups were positive for CD203c after stimulation with 0.1 and 1 ng/mL of JCP extract (Fig 4A).

ROC Curve Analysis of Ability of TSLPR and CD203c Expression to Discriminate between JCP/AR and JCP/AS Groups

The ROC curves were constructed to determine whether JCP-stimulated changes in the percentage of TSLPR-positive and

Table 1
Demographics and Characteristics of Study Subjects

JCP group	n (women)	Age (y) ^a	JCP sIgE (UA/mL) ^a	With HDM/AR, n	With HDM/AS, n
JCP/AR	12 (3)	38.7 $\pm$ 6.2	65.4 $\pm$ 77.4 (<i>P</i> < .01 vs JCP/AS group)	4	4
JCP/AS	11 (5)	37.2 $\pm$ 6.0	11.2 $\pm$ 17.1	4	1
JCP/NS	9 (4)	36.3 $\pm$ 6.4	ND	2	1
HDM group	n (women)	Age (y) ^a	HDM sIgE (UA/mL) ^a	With JCP/AR, n	With JCP/AS, n
HDM/AR	11 (2)	38.7 $\pm$ 6.2	11.9 $\pm$ 11.8 (ns vs HDM/AS group)	4	3
HDM/AS	7 (4)	37.2 $\pm$ 6.0	11.5 $\pm$ 19.7	3	1
HDM/NS	9 (3)	36.3 $\pm$ 6.4	ND	1	1

Abbreviations: AR, allergic rhinitis; AS, asymptomatic sensitized; HDM, house dust mite; JCP, Japanese cedar pollen; ND, not detected (<0.35 UA/mL); NS, non-sensitized; ns, not significant; sIgE, specific immunoglobulin E.

^aData are presented as mean $\pm$ SD.

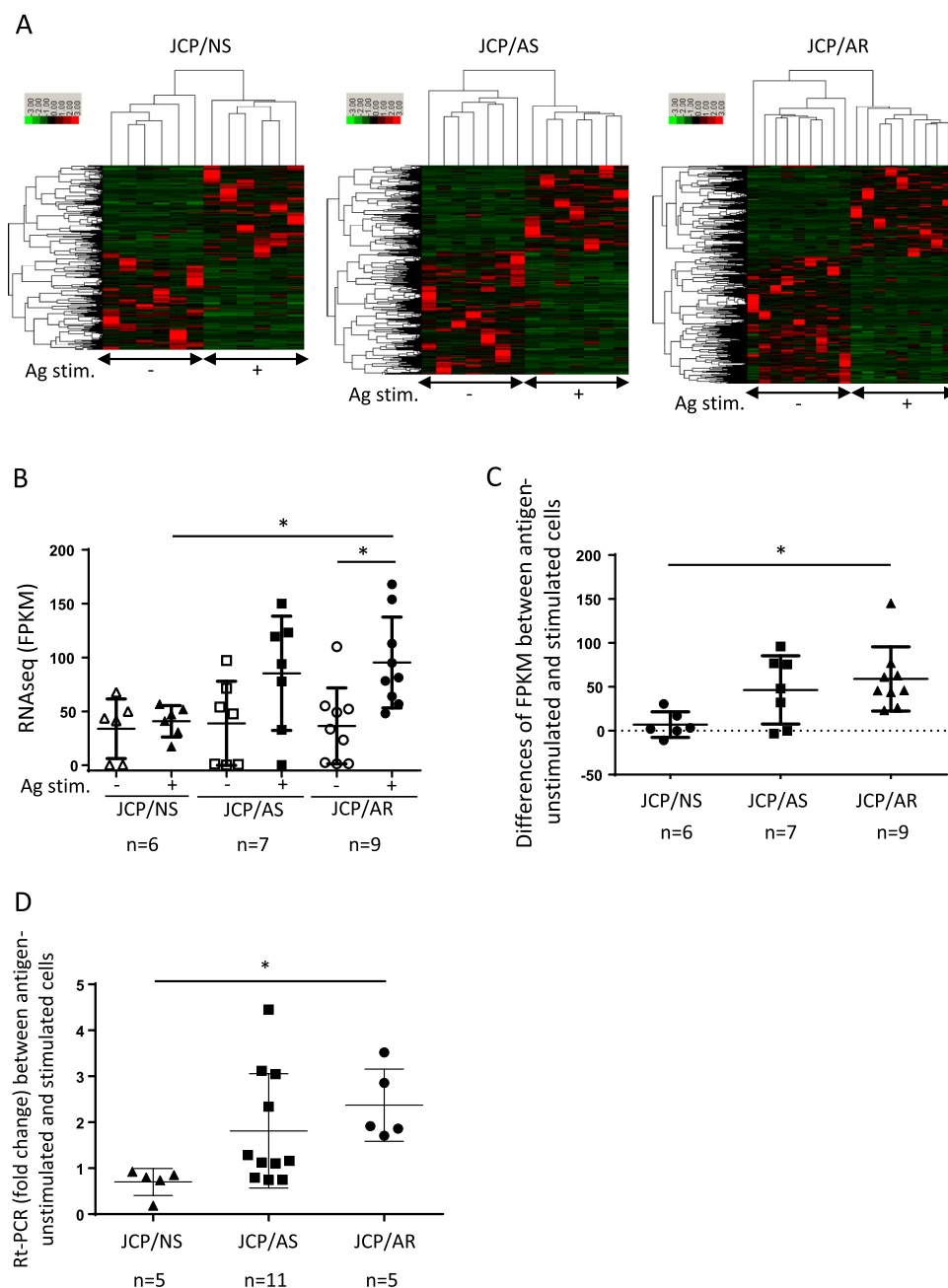


Figure 1. *CRLF2* gene expression by basophils. (A) The heat maps in the 3 groups (JCP/NS, JCP/AS and JCP/AR) were created with a 2-group comparison with and without 0.1 ng/mL of JCP extract. (B) RNA-seq FPKM values for *CRLF2* expression in JCP-stimulated and unstimulated basophils from the 3 study groups. (C) Differences in RNA-seq FPKM values for *CRLF2* expression between JCP-stimulated and unstimulated basophils. (D) The fold change in *CRLF2* expression in JCP-stimulated and unstimulated basophils evaluated by RT-PCR. * $P < .05$. Ag, antigen; AR, allergic rhinitis; AS, asymptomatic sensitization; FPKM, fragments per kilobase of exon model per million mapped fragments; JCP, Japanese cedar pollen; NS, not sensitized; RNA-seq, RNA sequencing; RT-PCR, real-time polymerase chain reaction.

CD203c-positive basophils could distinguish between the JCP/AR and JCP/AS groups (Fig 4B, C). The highest AUC value (0.84) was obtained for the change in TSLPR-positive basophils after stimulation with 0.1 ng/mL of JCP extract.

HDM-Specific Upregulation of TSLPR on Basophils From HDM/AR Group

We observed no significant differences in the levels of HDM-specific serum IgE between the HDM/AS and HDM/AR groups (Fig 5A). Changes in the percentage of TSLPR-positive basophils after stimulation with HDM extract are shown in Figure 5B. Significant

upregulation of TSLPR was observed in HDM-stimulated compared with unstimulated basophils from the HDM/AR group and in HDM-stimulated basophils from the HDM/AR group compared with the HDM/AS and HDM/NS groups (Fig 5B). Using a cutoff value of a 1.5-fold change in TSLPR-positive basophils after stimulation with 0.1 ng/mL of HDM extract, patients in the HDM/AR group could be identified with a sensitivity, specificity, and accuracy of 81.8%, 93.8%, and 88.9%, respectively. No significant correlations were detected between the levels of serum HDM-specific sIgE and changes in TSLPR expression after stimulation with 0.1 or 1 ng/mL of HDM extract for the HDM/AS or HDM/AR group (data not shown). Of patients in the HDM/AR group who were sensitized, but not allergic,

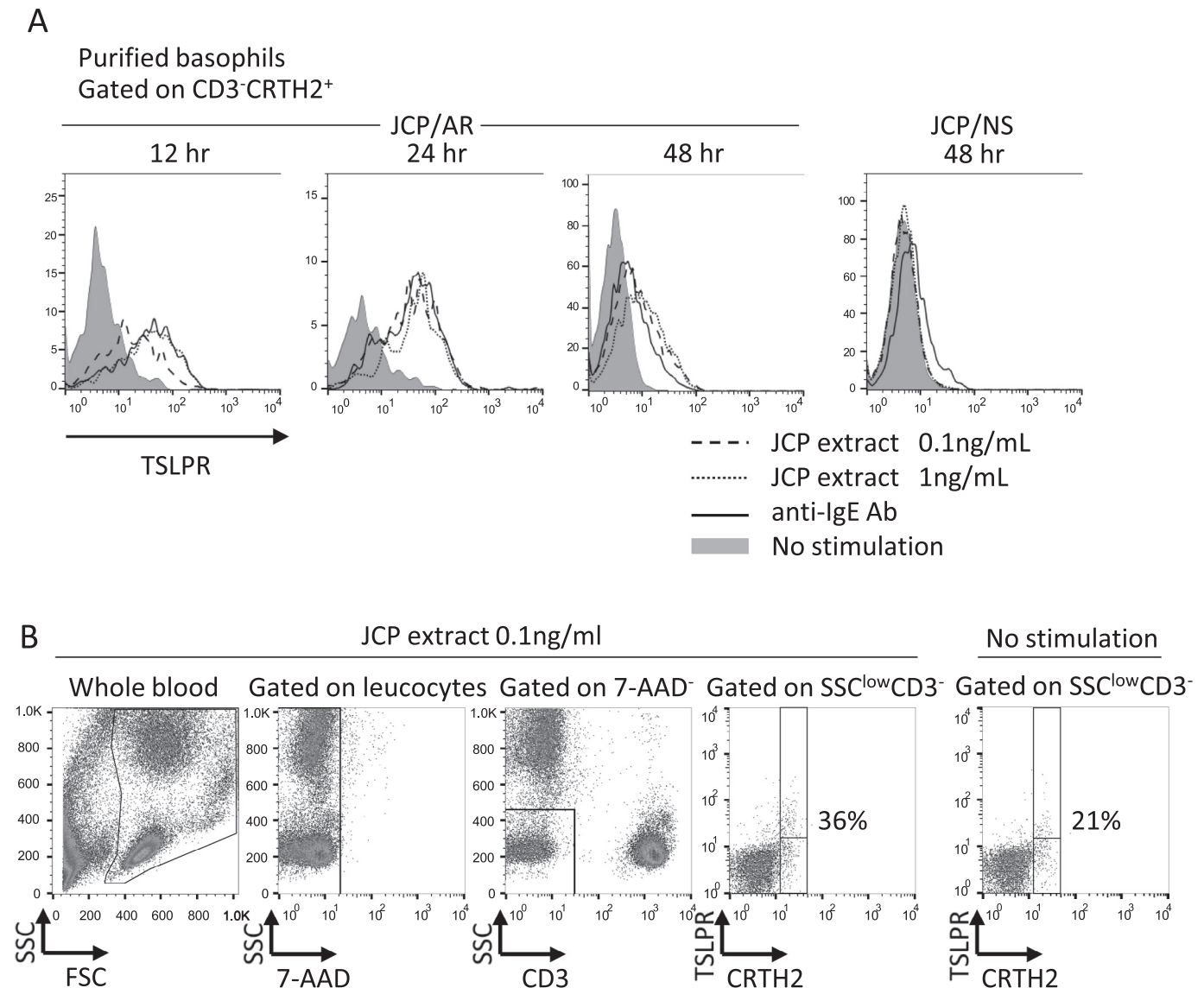


Figure 2. TSLPR expression on basophils. (A) Flow cytometric detection of TSLPR expression on basophils purified from the JCP/AR and JCP/NS groups and incubated without (gray histograms) or with 0.1 or 1 ng/mL of JCP extract for 12 to 48 hours. (B) Representative plots showing upregulation of TSLPR on basophils in whole blood from a patient in the JCP/AR group after in vitro stimulation with 0.1 ng/mL of JCP extract. 7-AAD, 7-aminoactinomycin D; Ab, antibody; AR, allergic rhinitis; AS, asymptomatic sensitization; FSC forward scatter; IgE, immunoglobulin E; JCP, Japanese cedar pollen; NS, not sensitized; SSC, side scatter; TSLPR, thymic stromal lymphopoietin receptor.

to JCP, there was no significant increase in TSLPR-positive basophils after JCP stimulation.

CD203c Expression on Basophils from HDM/NS, HDM/AS, and HDM/AR Groups

A significantly larger percentage of HDM-stimulated than unstimulated basophils was positive for CD203c in the HDM/AS and HDM/AR groups (Fig 6A). Significantly more basophils in the HDM/AR group compared with the HDM/AS and HDM/NS groups were positive for CD203c after stimulation with 1 ng/mL of HDM extract (Fig 6A).

ROC Curve Analysis of Ability of TSLPR and CD203c Expression to Discriminate between HDM/AR and HDM/AS Groups

The ROC curves evaluating the ability of HDM-stimulated changes in the percentage of TSLPR-positive and CD203c-positive basophils to differentiate between the HDM/AR and HDM/AS groups are shown in Figures 6B and 6C. The highest AUC (0.86) was obtained for the change in TSLPR-positive basophils after stimulation with 0.1 ng/mL of HDM extract.

phils to differentiate between the HDM/AR and HDM/AS groups are shown in Figures 6B and 6C. The highest AUC (0.86) was obtained for the change in TSLPR-positive basophils after stimulation with 0.1 ng/mL of HDM extract.

Discussion

Allergen sensitization is usually considered positive when the sIgE score is at least class 1; however, the sIgE values or classes do not reflect the absence or presence of symptoms or the severity of AR. Some subjects with AS had sIgE scores of class 2 or above 4 and sometimes even class 5 or 6. JCP is one of the most important causes of AR in Japan. In our previous study of middle-age subjects in Japan, approximately 40% of subjects sensitized to JCP had JCP-induced AR and approximately 12% of HDM-sensitized subjects had HDM-induced AR.²⁶

In this study, we sought to identify a marker that could distinguish patients with AR from those with non-AR including subjects

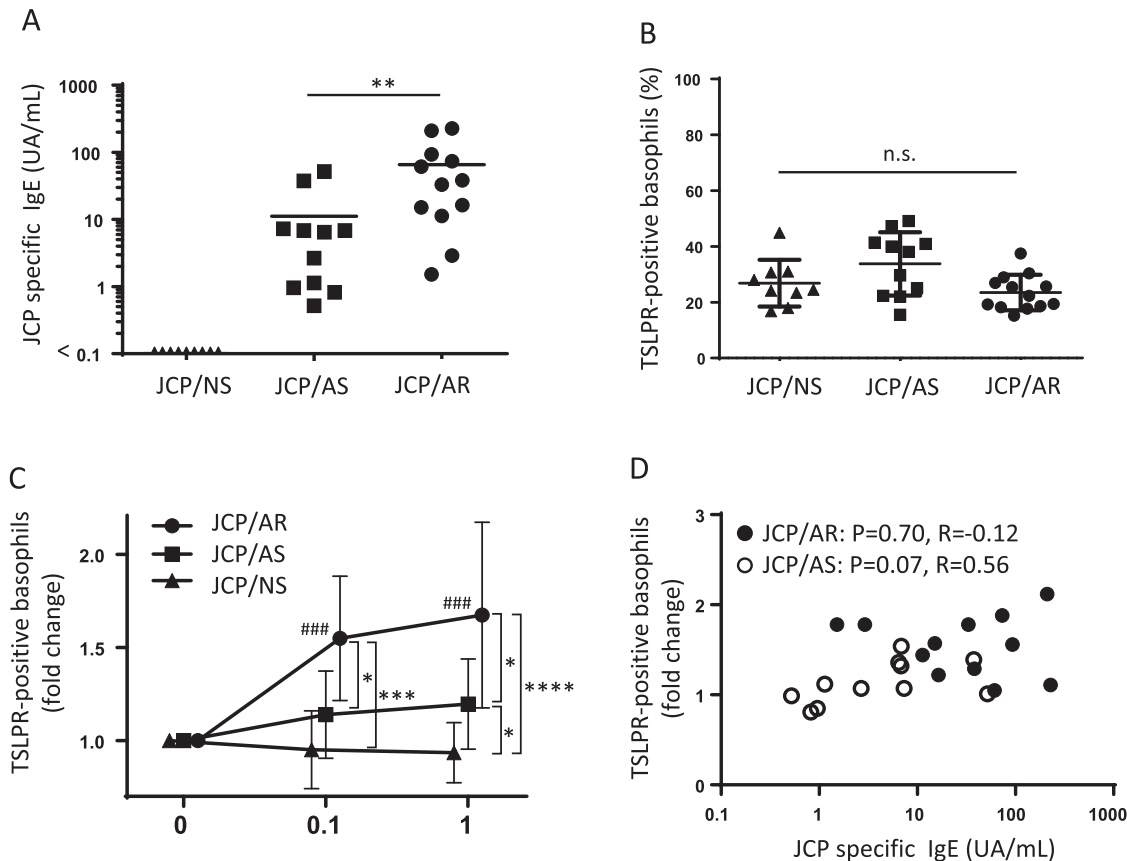


Figure 3. Serum JCP-specific IgE levels at baseline and TSLPR expression on basophils from the JCP/NS, JCP/AS, and JCP/AR groups. (A) Serum JCP-specific IgE levels in the 3 subject groups at baseline. (B) Percentage of TSLPR-positive basophils at baseline (unstimulated). (C) Fold change in the percentage of TSLPR-positive basophils after stimulation with 0.1 or 1 ng/mL of JCP extract. * $P < .05$, *** $P < .001$, **** $P < .0001$ for the indicated comparisons; ### $P < .001$ vs 0 ng/mL of JCP extract. (D) Correlation of fold change in percentage of TSLPR-positive basophils and serum JCP-specific IgE levels between the JCP/AS and JCP/AR groups. AR, allergic rhinitis; AS, asymptomatic sensitization; IgE, immunoglobulin E; JCP, Japanese cedar pollen; NS, not sensitized; TSLPR, thymic stromal lymphopoietin receptor.

with AS. We identified 12 genes for which the allergen-specific change in expression was significantly greater in the JCP/AR group than in the JCP/NS group. Of these, the *CRLF2* gene was confirmed only by real-time polymerase chain reaction analysis to show a significantly larger allergen-specific upregulation in basophils from the JCP/AR group than from the JCP/NS group.

CRLF2 encodes a member of the type I cytokine receptor family that, together with interleukin-7 α , forms the heterodimeric TSLPR.^{27–29} In this study, TSLPR expression was increased on basophils from the JCP/AR group after stimulation with JCP in vitro. Maximal expression, which was detected after 12 to 24 hours of incubation, was significantly higher for JCP-stimulated basophils from the JCP/AR group than from the JCP/AS group. Similarly, TSLPR expression on basophils was higher for HDM-stimulated basophils from the HDM/AR group than from the HDM/AS group. Moreover, the effect on TSLPR expression was allergen specific and was not upregulated on HDM-stimulated basophils from the JCP/AR group, regardless of whether the subjects were sensitized or allergic to HDM. TSLPR upregulation was detected not only on purified basophils but also on basophils in a small amount (100 μ L) of whole blood samples. Therefore, increased TSLPR expression on basophils could be a useful in vitro diagnostic marker for detecting the causal allergens in patients with AR.

Epithelium-derived cytokines such as TSLP, interleukin-25, and interleukin-33, which have been reported to be produced or released after mucosal damage, stimulate T-helper cell type 2 responses and are involved in the pathogenesis of atopic dermatitis and asthma.³⁰ Other studies have reported increased production

of TSLP from the nasal mucosa of patients with AR³¹ and increased expression of TSLPR by the local mucosal membrane and basophils from patients with allergic asthma.³² However, the significance of increased TSLP and TSLPR expression by basophils derived from patients with AR has not yet been defined. In this study, we found that peripheral blood basophils from patients with AR significantly upregulated TSLPR expression after allergen stimulation, suggesting that TSLPR could be involved in the aggravation of allergic inflammation and symptoms in patients with AR.

In this study, no significant correlation was observed between TSLPR expression and serum sIgE concentration, suggesting that TSLPR expression did not simply depend on the serum sIgE level. Other factors, such as levels of other specific immunoglobulins, affinity and reactivity of sIgE, signal transduction through other Fc γ receptors, or the presence of soluble suppressive factors, could influence antigen-specific signal transduction in basophils.^{33,34} In consequence, crosslinking of sIgE by allergen or Fc ϵ RI-mediated intracellular signal transduction in basophils might be decreased in subjects with AS or increased in patients with AR. A combination of these factors might better reflect the physiologic response to allergen in patients with AR and thus show a better association with AR symptoms than sIgE levels alone. Further studies are needed to clarify the mechanisms underlying the differential response of basophils from patients with AR and subjects with AS.

The BAT measures the upregulation of CD203c or CD63 on basophils at the time of activation,^{35–37} and the efficacy of the BAT as an in vitro tool for the diagnosis of allergy has been studied. The BAT has high specificity and sensitivity in diagnosing food allergy²²

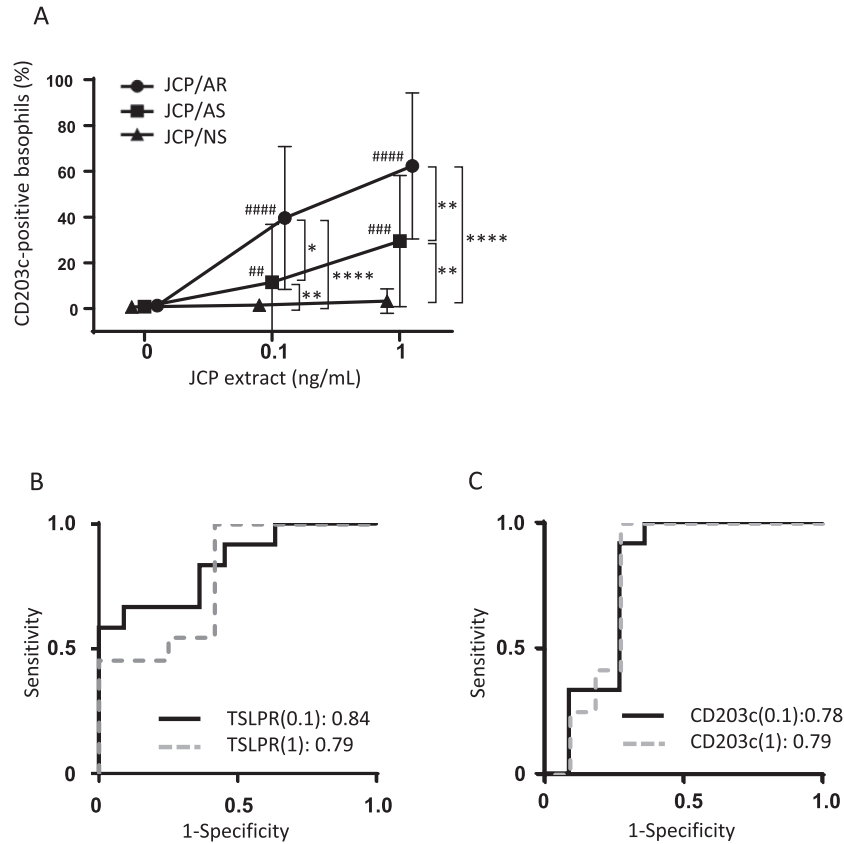


Figure 4. CD203c expression on basophils after stimulation with JCP and the ability of TSLPR and CD203c expression to discriminate between the JCP/AR and JCP/AS groups. (A) Fold change in the percentage of CD203c-positive basophils after stimulation of whole blood with 0.1 or 1 ng/mL of JCP extract. * $P < .05$, ** $P < .01$, **** $P < .0001$ for the indicated comparisons; ## $P < .01$, ### $P < .001$, #### $P < .0001$ vs 0 ng/mL JCP extract. Receiver operating characteristics curves evaluating the power of JCP-stimulated changes in the percentage of basophils expressing TSLPR (B) and CD203c (C) to discriminate between the JCP/AR and JCP/AS groups. Numbers indicate the areas under the receiver operating characteristics curves after stimulation with 0.1 or 1 ng/mL of JCP. AR, allergic rhinitis; AS, asymptomatic sensitization; IgE, immunoglobulin E; JCP, Japanese cedar pollen; NS, not sensitized; TSLPR, thymic stromal lymphopoietin receptor.

and correlates with the nasal provocation titer in patients with AR.^{23,38} In the present study, CD203c expression on basophils from subjects with AS was upregulated after allergen stimulation, but to a significantly lesser degree than in cells from patients with AR. However, some BAT nonresponders were observed in the HDM/AR group. BAT nonresponders have been reported to compose 6% to 17% of the patient population, and basophils from nonresponders are unresponsive to stimulation through FcεRI.^{20,21,24} We found that

the AUC of the change in TSLPR expression was equal to or greater than the change in CD203c expression on basophils from the same subjects. The observed differences in AUC and accuracy between CD203c and TSLPR might represent interpatient differences in the threshold or intensity of early- and late-phase responses of basophils to antigen stimulation.

Using a cutoff of a 1.5-fold change in basophil TSLPR expression after JCP or HDM stimulation, the sensitivity and specificity

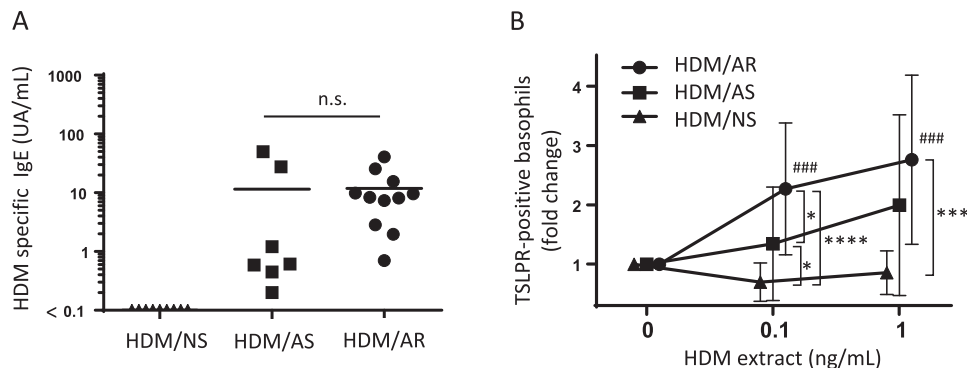


Figure 5. Serum HDM-specific IgE levels at baseline and TSLPR expression on basophils from the HDM/NS, HDM/AS and HDM/AR groups. (A) Serum HDM-specific IgE in the 3 subject groups at baseline. (B) Fold change in the percentage of TSLPR-positive basophils after stimulation with 0.1 or 1 ng/mL of HDM extract. * $P < .05$, *** $P < .001$, **** $P < .0001$ for the indicated comparisons; ### $P < .001$ vs 0 ng/mL of HDM extract. AR, allergic rhinitis; AS, asymptomatic sensitization; IgE, immunoglobulin E; HDM, house dust mites; n.s., not significant; NS, not sensitized.

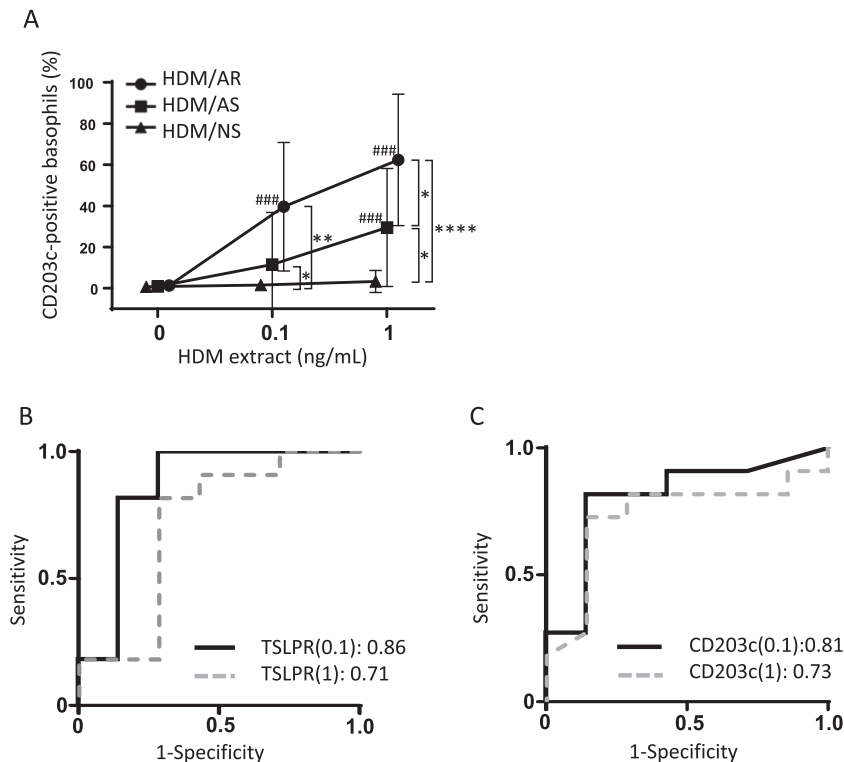


Figure 6. CD203c expression on basophils after stimulation with HDM and the ability of TSLPR and CD203c expression to discriminate between the HDM/AR and HDM/AS groups. (A) Fold change in the percentage of CD203c-positive basophils after stimulation with 0.1 or 1 ng/mL of HDM extract. * $P < .05$, ** $P < .01$, **** $P < .0001$ for the indicated comparisons; ### $P < .001$ vs 0 ng/mL HDM extract. Receiver operating characteristics curves evaluating the power of HDM-stimulated changes in the percentage of basophils expressing TSLPR (B) and CD203c (C) to discriminate between the HDM/AR and HDM/AS groups. Numbers indicate the areas under the receiver operating characteristics curves after stimulation with 0.1 or 1 ng/mL of HDM extract. AR, allergic rhinitis; AS, asymptomatic sensitization; IgE, immunoglobulin E; HDM, house dust mites; NS, not sensitized; TSLPR, thymic stromal lymphopoietin receptor.

for AR diagnosis were high and accuracy was greater than 80%. Thus, measurement of basophil TSLPR expression might be useful for identifying the causative allergen in patients with AR. Studies with larger patient cohorts will be needed to validate our results.

In conclusion, the expression of *CRLF2* was significantly increased in specific allergen-stimulated peripheral basophils from patients with AR. Peripheral blood basophils from patients with AR expressed the *CRLF2* gene product, TSLPR, and the percentage of TSLPR-positive basophils increased significantly after causative allergen stimulation. The ROC AUC for TSLPR expression was as good as or better than that for CD203c expression in distinguishing patients with AR from subjects with AS and had high sensitivity and specificity. Collectively, our data suggest that expression of TSLPR on basophils might be useful for the identification of the causative allergen in patients with AR.

Acknowledgments

We thank Osamu Ohara (Kazusa DNA Research Institute) and Ken Nonaka (DNA Chip Research Inc) for their support with the RNA sequencing assay. JCP extract-containing disks were kindly provided by Drs Yuma Fukutomi and Akie Saito (Sagamihara National Hospital). We thank Heizaburo Yamamoto (Wakaba Clinic) for his support with the development of the experimental protocol. We thank all staff who made contributions to this study in the Department of Otorhinolaryngology, Head and Neck Surgery, Graduate School of Medicine, Chiba University. We thank Anne M. O'Rourke, PhD, from Edanz Group (www.edanzediting.com/ac) for editing a draft of this article.

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