

The usefulness of measuring tear periostin for the diagnosis and management of ocular allergic diseases

Hiroshi Fujishima, MD,^{a,*} Naoko Okada, PhD,^{b,*} Kenji Matsumoto, MD, PhD,^b Kazumi Fukagawa, MD, PhD,^{c,d} Ayako Igarashi, BS,^e Akio Matsuda, PhD,^b Junya Ono, BS,^f Shoichiro Ohta, MD, PhD,^g Hideki Mukai, MD, PhD,^h Mamoru Yoshikawa, MD, PhD,ⁱ and Kenji Izuhara, MD, PhD^j

Yokohama, Tokyo, Ichikawa, Sagami-hara, and Saga, Japan

Background: Chronic ocular allergic diseases such as vernal keratoconjunctivitis (VKC) and atopic keratoconjunctivitis (AKC) are accompanied by serious comorbidities; however, the underlying pathogenesis remains obscure. Furthermore, diagnosing conjunctival lesions in patients with atopic dermatitis and estimating the severity in AKC are important for the treatment of ocular allergic diseases.

Objective: We addressed whether periostin, a novel mediator and biomarker in allergic inflammation, is involved in the pathogenesis of ocular allergic diseases and whether periostin can be a biomarker for these diseases.

Methods: We investigated tear periostin in patients with seasonal allergic conjunctivitis (SAC), VKC, and AKC and allergic patients without conjunctivitis and compared it with tear IL-13 and serum periostin. Furthermore, in patients with AKC, we measured tear periostin before and after topical treatment with tacrolimus.

Results: Tears from patients with ocular allergic disease showed significantly high periostin levels than did tears from allergic patients without conjunctivitis and from patients with AKC, VKC, and SAC in descending order. Tear periostin was associated with serious comorbidities such as large papilla formation and corneal damage in AKC, although both tear IL-13 and serum periostin had little to no such abilities. Furthermore, after topical tacrolimus treatment, tear periostin tended to decrease in most patients with AKC along with their clinical improvement. **Conclusions:** Periostin produced in conjunctival tissues stimulated by IL-13 may contribute to the pathogenesis of ocular allergic diseases. Furthermore, tear periostin can be potentially applied as a biomarker to diagnose conjunctivitis in allergic patients and to evaluate disease severity as well as the efficacy of treatments in AKC. (*J Allergy Clin Immunol* 2016;■■■:■■■-■■■.)

Key words: Atopic keratoconjunctivitis, periostin, biomarker, IL-13, serum, tear

From ^athe Department of Ophthalmology, Tsurumi University School of Dental Medicine, Tsurumi, Yokohama; ^bthe Department of Allergy and Clinical Immunology, National Research Institute for Child Health and Development Laboratory, Tokyo; ^cRyogoku Eye Clinic, Tokyo; ^dthe Department of Ophthalmology, Keio University School of Medicine, Tokyo; ^ethe Department of Ophthalmology, Tokyo Dental College, Ichikawa General Hospital, Ichikawa; ^fShino-Test Corporation, Sagami-hara; ^gthe Department of Laboratory Medicine and ^hthe Division of Medical Biochemistry, Department of Biomolecular Sciences, Saga Medical School, Saga; and ⁱthe Department of Dermatology and ^jthe Department of Otorhinolaryngology, Toho University School of Medicine, Tokyo.

*These authors contributed equally to this work.

Disclosure of potential conflict of interest: H. Fujishima has received grants from Alcon Laboratories, White Medical Co, Santen, Inc, Senju Pharmaceutical Co Ltd, and Otsuka Pharmaceutical Co Ltd and has consultant arrangements with Kobayashi Pharmaceutical Co Ltd. K. Matsumoto is employed by the National Research Institute for Child Health and Development; has received grants from the National Institute for Biomedical Innovation; has received payment for lectures from MSD (Merck Sharp and Dohme) K.K., Ono Pharmaceutical Co Ltd, GlaxoSmithKline K.K., Kyorin Pharmaceutical Co Ltd, Ohtsuka Pharmaceutical K.K., Mitsubishi Tanabe Pharma, AstraZeneca K.K., Siemens Healthcare, Abbott Japan Co Ltd, Sumitomo Dainippon Pharma, Maruho Co Ltd, and Teijin Pharma Ltd; and has received payment for the development of educational presentations from Gifu Pharmaceutical University. K. Izuhara has received grants from Shino-test Co Ltd and Chugai Pharmaceutical Co Ltd; has consultant arrangements with Chugai Pharmaceutical Co Ltd; and has received payment for lectures from AstraZeneca. The rest of the authors declare that they have no relevant conflicts of interest.

This study was funded by Tsurumi University, Yokohama, Japan (to H.F.) and by the National Institute of Biomedical Innovation (grant no. ID10-43 to K.M.).

Received for publication February 29, 2015; revised October 29, 2015; accepted for publication November 18, 2015.

Corresponding author: Hiroshi Fujishima, MD, Department of Ophthalmology, Tsurumi University School of Dental Medicine, 2-1-3 Tsurumi, Tsurumi-ku, Yokohama, Kanagawa 230-8501, Japan. E-mail: fujishima117@gmail.com. Or: Kenji Izuhara, MD, PhD, Division of Medical Biochemistry, Department of Biomolecular Sciences, Saga Medical School, 5-1-1, Nabeshima, Saga, 849-8501, Japan. E-mail: kizuhara@cc.saga-u.ac.jp.

0091-6749/\$36.00

© 2016 American Academy of Allergy, Asthma & Immunology

<http://dx.doi.org/10.1016/j.jaci.2015.11.039>

Clinical courses and responses to treatments differ among ocular allergic diseases. Seasonal allergic conjunctivitis (SAC) and perennial allergic conjunctivitis, which exhibit acute clinical courses, are in most cases easily controlled by topical antiallergic drugs so that with appropriate treatment, visual disturbance does not occur.¹ In contrast, vernal keratoconjunctivitis (VKC) and atopic keratoconjunctivitis (AKC), which exhibit chronic and severe clinical courses, can be accompanied by corneal changes, tissue remodeling, and fibrosis such as corneal ulcers and the formation of giant papilla, which can lead to loss of vision.^{2,3} When patients with VKC or AKC are refractory to first-line agents such as antihistamines, prolonged use of either topical or systemic corticosteroids or immune-suppressants is required. Therefore, it is of great importance to diagnose each chronic ocular allergic disease and to estimate the severity of disease accurately to design therapeutic strategies to prevent serious comorbidities. However, accurately estimating the severity of ocular lesions is sometimes difficult, and the development of some useful biomarker for this purpose is required.

It is widely recognized that IgE-mediated hypersensitivity is dominant in SAC and perennial allergic conjunctivitis, whereas cell-mediated type 2 immune responses in addition to IgE-mediated hypersensitivity and type 1 immune responses are involved in AKC and VKC.^{3,4} However, it remains obscure how the conjunctiva and corneal changes are generated in chronic ocular allergic diseases such as AKC and VKC.

A great deal of evidence has accumulated showing that tears reflect *in vivo* inflammation in ocular allergic diseases. Inflammatory cells—neutrophils, eosinophils, and lymphocytes,

Abbreviations used

AD: Atopic dermatitis
 AKC: Atopic keratoconjunctivitis
 AR: Allergic rhinitis
 SAC: Seasonal allergic conjunctivitis
 VKC: Vernal keratoconjunctivitis

particularly T_H2 cells—exist in the tears of patients with VKC and AKC.^{4,5} Consequently, such tears contain various inflammatory factors derived from these cells. These include (1) T_H2 -type cytokines and chemokines, (2) growth factors, (3) mast cell–derived mediators, (4) metalloproteases, (5) eosinophil-derived eosinophil cationic protein, and (6) IgE.^{6–10} Although the pathological roles of the infiltrated inflammatory cells and inflammatory factors in tears remain unknown, it is possible that these cells/factors affect corneal cells or conjunctival tissues, contributing to the formation of the phenotypes seen in chronic ocular allergic diseases.

Periostin (*POSTN*) has recently emerged as a novel mediator in allergic inflammation.¹¹ It is produced from epithelial cells, fibroblasts, and endothelial cells by IL-4 or IL-13, the signature type 2 cytokines.^{12–14} Periostin acts on epithelial cells or fibroblasts via several α_v integrins, its receptors, on cell surfaces, causing their proliferation and nuclear factor kappa B activation.¹¹ These activities are important for the maintenance of skin homeostasis such as wound repair^{15–17}; however, overexpression of periostin as a result of type 2 immune responses plays a critical role in the onset or exacerbation of allergic inflammation in atopic dermatitis (AD) or bronchial asthma.^{18,19} Periostin has also been recognized as a novel biomarker in allergic diseases; serum periostin is elevated in patients with bronchial asthma and AD.^{18,20,21} Serum periostin reflects type 2 inflammation and fibrosis *in vivo*, which can potentially be applied to predict the efficacy of type 2 antagonists such as anti-IL-13 antibodies or anti-IgE antibodies as well as hyporesponsiveness to inhaled corticosteroids in patients with asthma.^{22–25} However, no study has yet been performed to investigate whether periostin is involved in the pathogenesis of ocular allergic diseases or whether periostin can be a biomarker for these diseases.

In this study, we examined whether conjunctival tissues of patients with ocular allergic disease produce periostin on stimulation of IL-13 and whether the produced periostin is subsequently secreted into tears. Furthermore, we investigated whether the measurement of tear periostin is useful in the diagnosis and treatment of ocular allergic diseases.

METHODS**Patients**

Patients with SAC, VKC, and AKC and healthy donors were enrolled from the Department of Ophthalmology, Tsurumi University School of Dental Medicine in Yokohama, Japan. Patients with allergic rhinitis (AR) with no conjunctivitis and patients with AD with no AKC were enrolled from Toho University School of Medicine, Tokyo, Japan. SAC, VKC, and AKC were diagnosed according to the Japanese guidelines for allergic conjunctival diseases.² SAC was diagnosed by the following symptoms and signs: ocular itching, redness, tearing or pain, filamentous or mucous discharge, chemosis, hyperemia or papillae of the palpebral conjunctiva on slit lamp examination, medical history, and positivity for serum antigen-specific IgE as indicated by SRL Inc (Tokyo, Japan) using the multiple antigen simultaneous test 33

(MAST Immunosystems, Inc, Mountain View, Calif). Samples were taken from patients with SAC who had showed some symptom in the previous year and during the cedar pollen season (February to April). VKC was diagnosed by slit lamp examination findings of typical cobblestone excrescences (giant papillary conjunctivitis of the upper tarsal conjunctiva), and severe clinical symptoms such as keratitis and positivity for serum antigen-specific IgE. VKC has no atopic pruritic eczema. AKC was diagnosed by the clinical diagnosis of AD by a dermatologist, by slit lamp examination showing the presence of keratoconjunctivitis, and by clinical symptoms indicative of AKC, which are similar to VKC. Healthy subjects had no symptoms or signs of allergic keratoconjunctivitis and tested negative for serum antigen-specific IgE antibodies. AR and AD were diagnosed on the basis of Japanese Guideline for Allergic Rhinitis 2014²⁶ and Guideline for Atopic Dermatitis 2014,²⁷ respectively. The absence of ocular lesions was confirmed by lack of symptoms and objective signs. The backgrounds of the patients and the control donors are presented in Table E1 in this article's Online Repository at www.jacionline.org.

Giant papillae were defined as papillae of 1 mm or more in diameter. In general, papillae were classified into 4 grades according to their diameters: severe (3, ≥ 0.6 mm), moderate (2, 0.3–0.5 mm), mild (1, 0.1–0.2 mm), and none (0).²

Eight patients with AKC were topically treated with 0.1% Tacrolimus hydrate suspension (Senju Pharmaceutical Co, Ltd, Osaka, Japan) for 1 month to 1 year. Tears were collected before and after the treatment.

Sample collection and all procedures were approved by the Ethics Committee of Tsurumi University School of Dental Medicine and Toho University School of Medicine in accordance with the ethical standards of the Helsinki Declaration of 1975. Informed consent was obtained from all patients and healthy volunteers.

Measurement of periostin and IL-13 by ELISA

Tears exceeding 20 μ L were collected into Nunc minisorp tubes (Nunc, Roskilde, Denmark) using a micropipette, as previously described.²⁸ IL-13 in tears was quantified by Multiplex assay kits for human IL-13 (Millipore Milliplex MAP multiplex panels; Merck Millipore, Darmstadt, Germany). Periostin in tears and serum was quantified by ELISA kits for human periostin that we established (clones nos. SS18A and SS17B).²⁹

Primary cell cultures

Human conjunctival samples were collected from normal volunteers using scissors, after informed consent was obtained from subjects. Establishment and culture of primary conjunctival epithelial cells were performed as previously described.³⁰ To culture fibroblasts, samples were incubated in Dulbecco modified Eagle medium/F12 medium (Gibco BRL, Grand Island, NY).

Immunohistochemistry of conjunctiva

Biopsy of conjunctiva from patients was performed as previously reported.³¹ Immunohistochemical staining was performed using the Vectastain ABC Kit (Vector Laboratories, Burlingame, Calif). Rabbit polyclonal antiperiostin antibodies (Abcam, Cambridge, United Kingdom) were used.

Quantitative real-time PCR

Quantitative real-time PCR was performed using a sequence detection system (ABI Prism 7500, Applied Biosystems) as previously described.¹³ Expression levels of mRNA were normalized by the median expression of glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*). Copy number was expressed as the number of transcripts per nanogram of total RNA.

Statistics

Results are presented as interquartile range in all experiments except those from *in vitro* experiments using primary epithelial cells and fibroblasts.

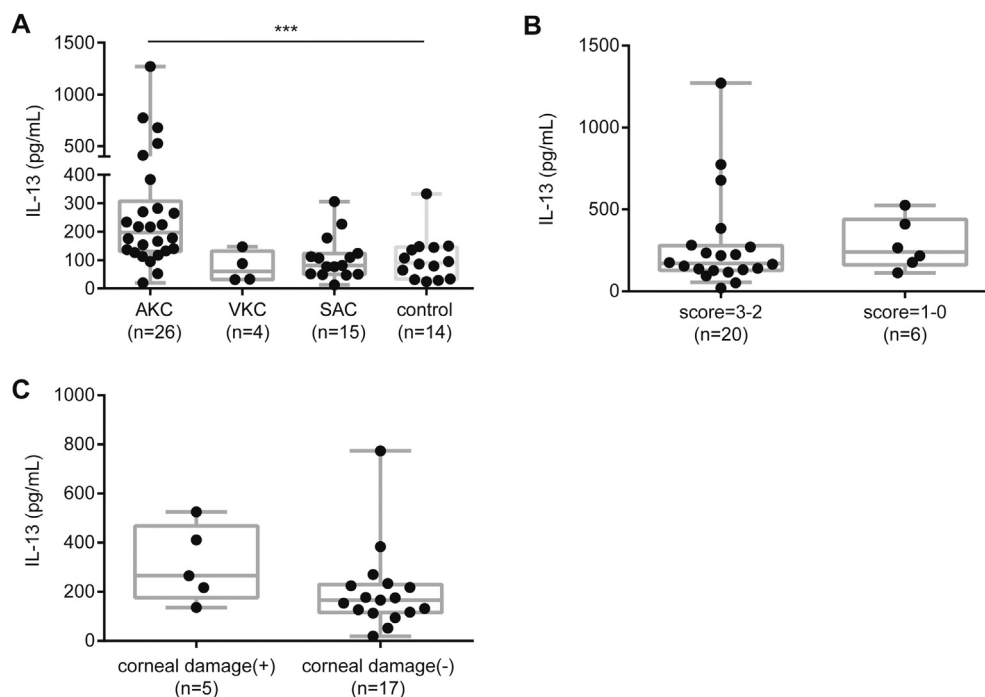


FIG 1. Elevation of IL-13 in tears of patients with AKC but not patients with VKC or SAC. **A**, Tear IL-13 in patients with AKC ($n = 26$), VKC ($n = 4$), and SAC ($n = 15$) and healthy donors ($n = 14$). **B**, Tear IL-13 in the large papilla formation group (scores 3-2, $n = 20$) and nonpapilla or less-papilla formation group (score 1-0, $n = 6$) in patients with AKC. **C**, Tear IL-13 in corneal damage-positive patients ($n = 5$) and corneal damage-negative patients ($n = 17$) in patients with AKC. *** $P < .001$ by Mann-Whitney U test.

Analysis was carried out using the Mann-Whitney U test or Spearman test. A P value of less than .05 was considered significant.

RESULTS

Elevation of IL-13 in tears of patients with AKC, but not of patients with VKC or SAC

It has been already demonstrated that various immunological mediators, particularly type 2 cytokines, are detectable in the tears of patients with ocular allergic disease.^{6-8,32} We first measured IL-13, a signature type 2 cytokine and important for fibrosis, in the tears of patients with AKC, VKC, and SAC. IL-13 in tears was elevated in patients with AKC ($n = 26$; median, 197.1 pg/mL), but not in patients with VKC ($n = 4$; median, 60.0 pg/mL) or SAC ($n = 15$; median, 80.9 pg/mL), compared with healthy donors ($n = 14$; median, 90.4 pg/mL; Fig 1, A). Elevation of IL-13 levels in patients with AKC was statistically significant against all patients with VKC ($P < .05$) and SAC ($P < .001$), and healthy donors ($P < .001$), but not patients with VKC or SAC against healthy donors.

We next examined the correlation between IL-13 levels in tears and serious comorbidities such as papilla formation or corneal damage in patients with AKC. IL-13 levels in the tears of patients with AKC were unchanged by the existence of papilla formation (score, 3-2, $n = 20$, median, 170.9 pg/mL vs score, 1-0, $n = 6$, median, 216.8 pg/mL) or corneal damage (corneal damage+, $n = 5$, median, 166.2 pg/mL vs corneal damage-, $n = 17$, median, 265.1 pg/mL; Fig 1, B and C).

Taken together, these results demonstrate that IL-13 in tears was elevated in patients with AKC, but not in patients with VKC

or SAC, and it was not associated with the severe comorbidities of AKC.

Abilities of IL-13 to produce periostin in conjunctival epithelial cells and fibroblasts

We previously found that periostin is a downstream molecule of IL-13.^{13,33} Furthermore, we and others have already demonstrated that epithelial cells, fibroblasts, and endothelial cells can produce periostin by IL-13.¹²⁻¹⁴ We next examined whether IL-13 induces periostin production in primary conjunctival epithelial cells and fibroblasts. *POSTN* mRNA was significantly expressed by IL-13 in both conjunctival epithelial cells and fibroblasts, as it is in other epithelial cells and fibroblasts (Fig 2, left panels).^{12,13,18,33} However, the expression of periostin protein in conjunctival epithelial cells was marginal; IL-13 had a tendency to upregulate its expression, although this was not statistically significant (Fig 2, right panels). In contrast, periostin protein was clearly produced in IL-13-stimulated conjunctival fibroblasts as well as in lung or skin fibroblasts.^{13,18} These results demonstrate that IL-13 clearly produces periostin in conjunctival fibroblasts and to a much smaller extent in conjunctival epithelial cells *in vitro*.

Elevation of periostin in tears of patients with ocular allergic disease

Given that IL-13 was elevated in tears of patients with AKC and that IL-13 induces periostin in primary conjunctival tissues, we explored whether periostin is produced in conjunctival tissues

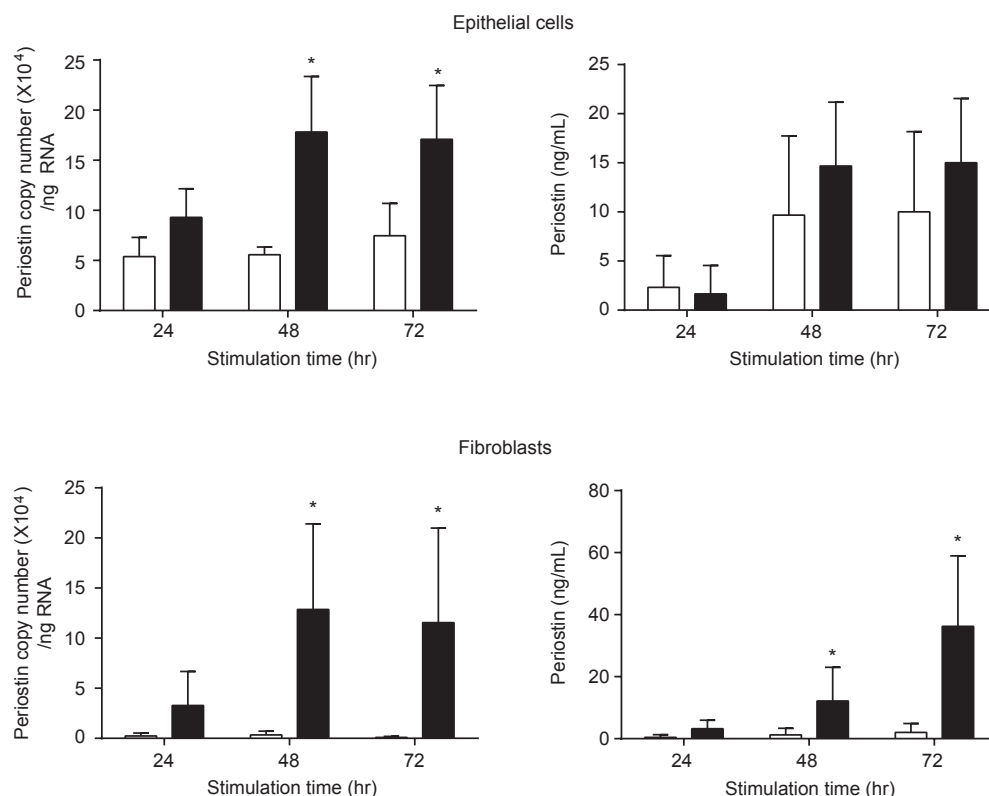


FIG 2. Periostin production from conjunctival epithelial cells and fibroblasts by IL-13. POSTN mRNA (*left panels*) and periostin protein in supernatants (*right panels*) from conjunctival epithelial cells (*upper panels*) and fibroblasts (*lower panels*) stimulated without (*white bars*) or with (*black bars*) 10 ng/mL of IL-13 for the indicated times. Data are shown as the mean $\pm$ SD from fibroblasts from 5 separate donors and from epithelial cells from 3 separate donors. * $P < .05$ by Mann-Whitney U test.

stimulated by IL-13 and consequently can be detected in tears of patients with ocular allergic disease. Tears from all 3 patients with ocular allergic disease showed significantly high periostin levels compared with healthy donors or patients with AR or AD without conjunctivitis ($n = 18$, median, 0.2 ng/mL; $n = 9$, median, 1.6 ng/mL; $n = 16$, median, 0.8 ng/mL; Fig 3, A). Patients with AKC showed the highest levels ($n = 31$, median, 444.0 ng/mL, $P < .001$ vs healthy donors, patients with AR, or patients with AD), patients with VKC showed the second highest levels ($n = 7$, median, 67.0 ng/mL, $P < .001$ vs healthy donors or patients with AD, $P < .01$ vs patients with AR), and patients with SAC showed the third highest levels ($n = 17$, median, 12.2 ng/mL, $P < .001$ vs healthy donors or AD, $P < .05$ vs AR). Periostin and IL-13 levels in the tears of all patients with ocular allergic disease and healthy controls showed a weak but significant correlation ($n = 59$, $r = 0.3329$, $P < .05$; Fig 3, B). It is of note that periostin levels in tears of patients with AKC were approximately 1000-fold greater than those of IL-13.

We next examined the correlation between tear periostin levels and papilla formation or corneal damage in patients with AKC. Periostin levels were significantly higher in the large papilla formation group (scores 3-2, $n = 23$, median, 678.8 ng/mL) than in the nonpapilla or less-papilla formation group (score 1-0, $n = 8$, median, 23.6 ng/mL, $P < .001$; Fig 3, C). Corneal damage-positive patients ($n = 19$, median, 543.7 ng/mL) showed significantly higher periostin than did corneal damage-negative patients ($n = 8$, median, 77.0 ng/mL, $P < .01$; Fig 3, D).

The receiver-operating characteristic curve analyses for patients with AKC ($n = 31$) versus patients with AD without conjunctivitis ($n = 16$) showed that when cutoff values of tear periostin were set at 6.6 as the optimal point, the sensitivities, specificities, and areas under the curve were evaluated as 96.8, 93.8, and 0.996 (Fig 3, E), respectively. Furthermore, the receiver-operating characteristic curve analyses for corneal damage-negative ($n = 8$) versus corneal damage-positive ($n = 19$) patients with AKC showed that when the cutoff value of periostin in tears was set at 105 ng/mL as the optimal point, the sensitivities, specificities, and areas under the curve were found to be 91.3%, 75.0%, and 0.918, respectively.

These results demonstrate that periostin can be detected in tears of patients with ocular allergic disease in descending order—AKC, VKC, and SAC—which would reflect IL-13 levels in tears. Periostin in tears can discriminate patients with AKC from patients with AD without conjunctivitis. Furthermore, periostin levels in tears of patients with AKC are associated with their serious comorbidities.

Involvement of periostin in giant papilla formation

The finding that periostin levels in tears of patients with AKC were associated with the large papilla formation group suggested that periostin is involved in papilla formation in patients with ocular allergic disease. Therefore, we next examined periostin expression in giant papillae from patients with AKC. In normal conjunctiva, periostin was expressed clearly at the basement

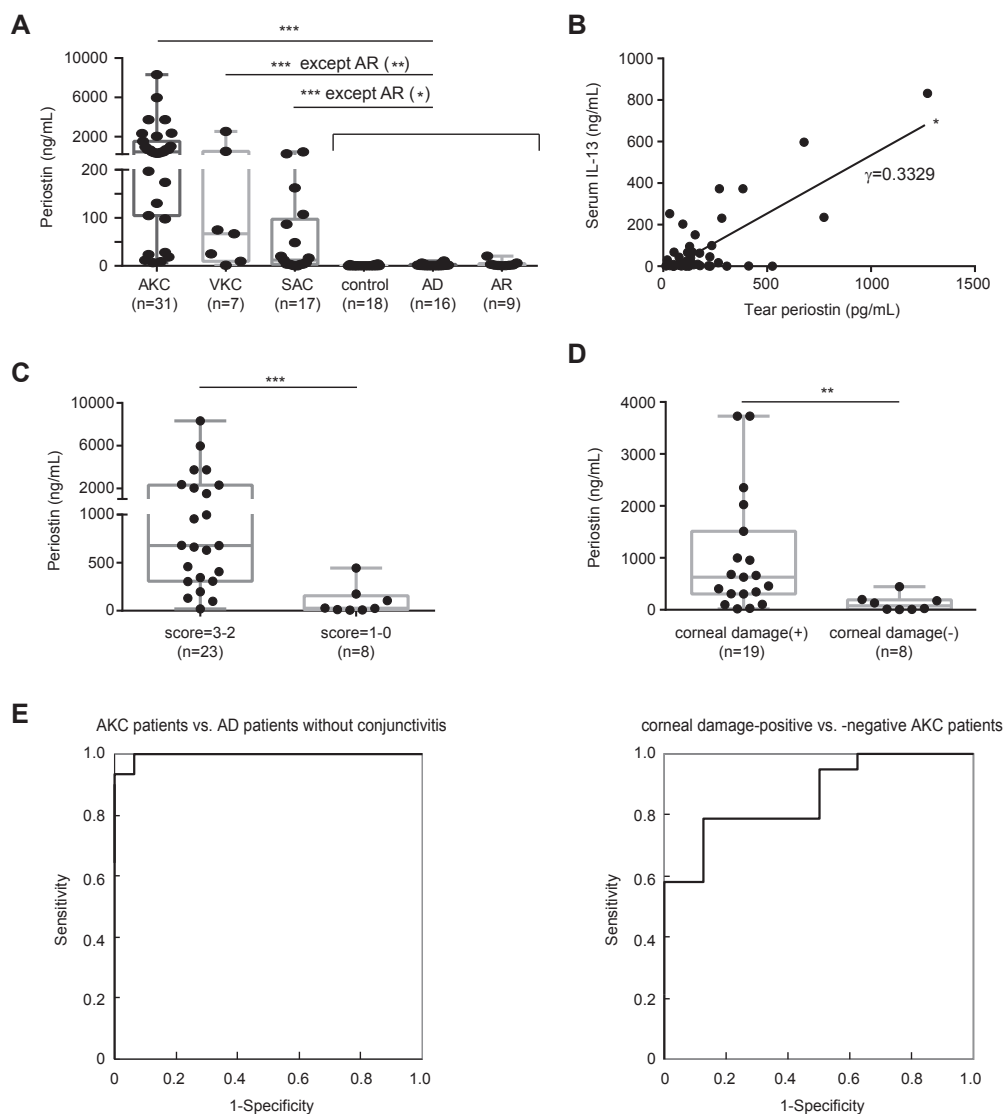


FIG 3. Elevation of periostin in tears of patients with ocular allergic disease. **A**, Tear periostin in patients with AKC (n = 31), VKC (n = 7), and SAC (n = 17), healthy donors (n = 18), and patients with AR (n = 9) or AD (n = 16) without conjunctivitis. **B**, Correlation between periostin and IL-13 in tears of patients with ocular allergic disease and healthy controls (n = 59). **C**, Tear periostin in the large papilla formation group (scores 3-2, n = 23) and nonpapilla or less-papilla formation group (score 1-0, n = 8) in patients with AKC. **D**, Tear periostin in corneal damage-positive patients (n = 19) and corneal damage-negative patients (n = 8) in patients with AKC. **E**, Left: ROC curve between patients with AKC and patients with AD without conjunctivitis for periostin in tears. ROC curve between patients with allergic ocular disease versus healthy donors for periostin in tears. Right: ROC curve between corneal damage-negative (n = 8) and corneal damage-positive (n = 19) patients with AKC for periostin in tears. ROC curve between patients with chronic ocular allergic disease versus patients with SAC for periostin in tears. ROC, Receiver-operating characteristic. * $P < .05$, ** $P < .01$, *** $P < .001$ by Mann-Whitney U test (A, C, D) or Spearman test (B).

membrane and very weakly at the epithelium, but not in subepithelial tissues (Fig 4, A, left). In contrast, giant papillae from patients with AKC showed robust periostin expression and periostin was localized mainly in the fibrotic area of the conjunctiva.

We then examined the expression of periostin and IL-13 at the mRNA level in the giant papillae from patients with AKC. Both periostin (55.5% *GAPDH*, n = 15 vs 0.6% *GAPDH*, n = 15, $P < .001$) and IL-13 (0.3% *GAPDH*, n = 14 vs 0.0% *GAPDH*,

n = 13, $P < .001$), but particularly periostin rather than IL-13, were highly expressed in giant papillae from patients with AKC compared with normal conjunctival samples (Fig 4, B and C). Expression of periostin and IL-13 mRNA in giant papillae from patients with AKC and normal conjunctival tissues showed a strong correlation (n = 27, $r = 0.8655$, $P < .001$; Fig 4, D). These results demonstrate that periostin, probably produced in fibroblasts by IL-13 stimulation, is involved in papilla formation in ocular allergic diseases.

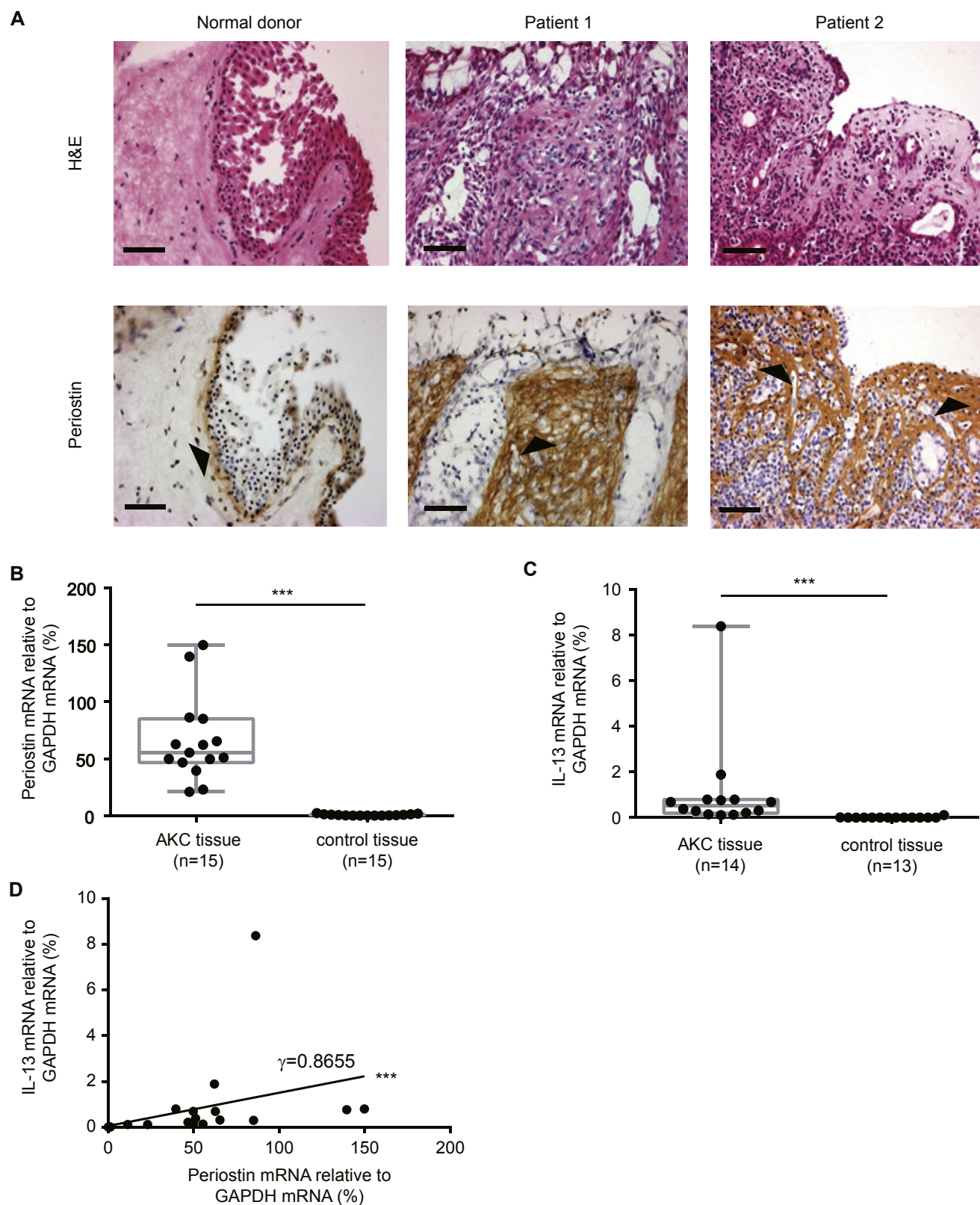


FIG 4. Expression of periostin in giant papilla formation. **A**, Staining of normal conjunctival tissues and giant papillae from patients with AKC with H&E and antiperiostin antibodies. The arrowheads indicate periostin staining. Scale bars: 50 μ m. **B** and **C**, Expression of *POSTN* (Fig 4, **B**) or *IL13* (Fig 4, **C**) at mRNA level relative to *GAPDH* mRNA in the giant papillae from patients with AKC ($n = 15$ or 14) or normal conjunctival tissues ($n = 15$ or 13). **D**, Correlation between *POSTN* and *IL13* mRNA relative to *GAPDH* mRNA in the giant papillae from patients with AKC and normal conjunctival tissues ($n = 27$). *** $P < .001$ by Mann-Whitney *U* test (Fig 4, **B** and **C**) or Spearman test (Fig 4, **D**). H&E, Hematoxylin and eosin.

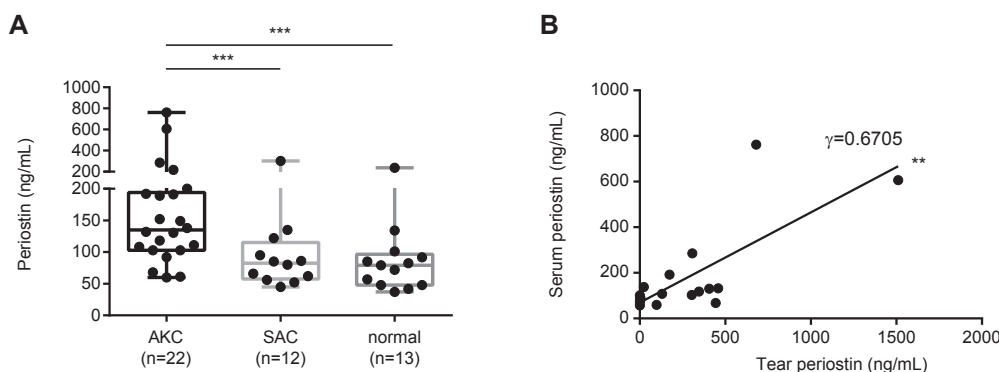


FIG 5. Correlation of serum and tear periostin in patients with ocular allergic disease. **A**, Serum periostin in patients with AKC (n = 22) and SAC (n = 12) and healthy donors (n = 13). **B**, Correlation of serum and tear periostin in patients with ocular allergic disease (n = 45). ** $P < .01$, *** $P < .001$ by Mann-Whitney U test (Fig 5, A) or Spearman test (Fig 5, B).

Correlation of periostin in serum and tears

We have already shown that serum periostin using our ELISA kit (clones nos. SS18A and SS17B) is elevated in patients with AD in association with their clinical severities.^{18,21} Therefore, we examined serum periostin in patients with ocular allergic disease and compared it with periostin in tears. Serum periostin was increased in patients with AKC (n = 22, median, 135.0 ng/mL), but not in patients with SAC (n = 12, median, 82.5 ng/mL), compared with normal donors (n = 13, median, 79.0 ng/mL, $P < .001$; Fig 5, A). Serum periostin levels were significantly correlated with tear periostin levels in patients with ocular allergic disease (n = 19, $r = .6705$, $P < .01$; Fig 5, B).

We next examined the correlation between serum periostin levels and papilla formation or corneal damage in patients with AKC, as well as the correlation between tear periostin levels and papilla formation or corneal damage in these patients. However, there was no difference of serum periostin in papilla formation (scores 3-2, n = 15, median, 132.0 ng/mL vs score 1-0, n = 7, median, 138.0 ng/mL, $P = .9869$) or corneal damage (positive, n = 16, median, 150.5 ng/mL vs negative, n = 6, median, 113.1 ng/mL, $P = .1501$).

These results demonstrate that although serum periostin is elevated in patients with AKC and associated with tear periostin in patients with overall ocular allergic disease, serum periostin is not associated with severities in patients with AKC.

Effects of treatment with tacrolimus on periostin in tears

Finally, we investigated the effects of topical treatment with tacrolimus on periostin in tears. Topical treatment with tacrolimus for patients with ocular allergic disease is approved only in Japan. Periostin in tears was measured in 8 patients with AKC before and after tacrolimus topical treatment. In all 8 patients, hyperemia and edema were improved after treatment, and proliferative signs and ulcers were improved in 2 and 3 patients, respectively, whereas others showed no change (see Table E2 in this article's Online Repository at www.jacionline.org). Periostin in tears was significantly decreased in 7 patients with AKC, whereas 1 patient showed a slight increase (Fig 6). Although the difference before and after the treatment was not statistically significant because

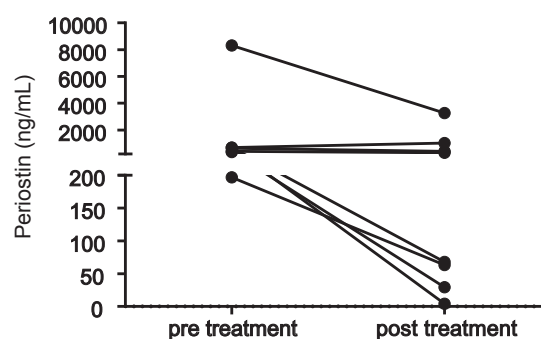


FIG 6. Effects of topical treatment with tacrolimus on tear periostin in patients with AKC before and after topical treatment with 0.1% tacrolimus hydrate suspension (n = 8).

of the small number of subjects, these results suggest that periostin in tears may reflect the effects of topical treatment with tacrolimus in patients with ocular allergic disease.

DISCUSSION

In this study, we showed that tears in patients with ocular allergic diseases, particularly AKC, contain large amounts of periostin (Fig 3, A). This is the first study showing that periostin can be detected in the tears of patients with ocular allergic disease. The overall levels were significantly higher than either tear IL-13 levels (~1000-fold) or serum periostin levels (~3-fold, Figs 1, A, 3, A, and 5, A). Furthermore, periostin in tears showed a strong correlation with IL-13 levels in the tears of patients with ocular allergic disease (Fig 3, B). We have already demonstrated that on stimulation with IL-13, lung or skin fibroblasts can produce robust amounts of periostin.^{13,18} In contrast, production of periostin in epithelial cells has a polarity; *in vitro* analyses showed that periostin can be secreted from the basal side, but not from the apical side, in bronchial epithelial cells stimulated by IL-13.¹² Bronchial epithelial cells or keratinocytes immersed in liquid culture medium did not produce even a trace amount of periostin by IL-13.^{13,18} Our present findings regarding primary conjunctival fibroblasts and epithelial cells (Fig 2) are consistent with the previous results. In accord with the *in vitro* analyses, we have confirmed that secretion of periostin into the bronchial lumens of patients with asthma does not occur or is negligible,³⁴

which is in contrast to tears. Taking these results together, it is likely that conjunctival tissues have a unique system for leaking or secreting periostin into tears.

Thus far, the pathological role of periostin in tears remains unclear. However, it has been already established that periostin acts on epithelial cells as an inflammatory mediator by inducing their proliferation and nuclear factor kappa B activation via α_v integrins.¹¹ Furthermore, in the present study, tear periostin levels were associated with corneal damage in patients with AKC (Fig 3, C and E, right panel). These results suggest that periostin in tears may act on corneal or conjunctival epithelial cells, causing complications in chronic allergic keratoconjunctivitis.

Giant papillary formation is a major characteristic of chronic allergic keratoconjunctivitis, including AKC and VKC, and is thought to be one factor causing corneal damage.³⁵ A giant papilla is composed of conjunctival epithelial cells and activated fibroblasts with infiltration of excess amounts of inflammatory cells, although the mechanism of its formation is unclear.^{36,37} The histological analyses in this study showed that periostin was highly deposited in the fibrotic area of papillae and that periostin expression was associated with that of IL-13 (Fig 4). These results suggest that giant papilla formation is a consequence of type 2 immune responses and that periostin is involved in the formation of giant papilla downstream of IL-13 signals.

The present finding, that tear periostin was upregulated in patients with ocular allergic disease, suggests that tear periostin can be a novel and useful biomarker for these diseases. The utility of tear periostin as a biomarker has been summarized as (1) discrimination of overall ocular allergic diseases from the normal condition, (2) discrimination of AKC from AD without conjunctivitis, and (3) evaluation of the severities of AKC such as papilla formation and corneal damage. Particularly, diagnosis of conjunctival lesions in patients with AD or estimation of the clinical severity in patients with AKC is of great importance to design treatment strategies. The abilities of tear periostin to estimate the clinical severity of AKC are superior to those of tear IL-13 or serum periostin (Fig 1, C, and Fig 3, D). The superiority to tear IL-13 is explained by relatively abundant amounts of tear periostin compared with tear IL-13 (~1000-fold), whereas the superiority to serum periostin is explained by detection of lesion-specific periostin.

Another potential of tear periostin as a biomarker was demonstrated by the finding that it tended to be decreased by topical treatment with tacrolimus along with improved clinical features in most patients with AKC (Fig 6). Topical or systemic treatment of immune-suppressants is widely used for patients with chronic allergic keratoconjunctivitis, and topical tacrolimus treatment is approved for use in patients with chronic allergic keratoconjunctivitis only in Japan.^{2,38} However, no biomarker to evaluate the efficacy of these immune-suppressants is thus far available. Oray and Toker⁸ reported that several inflammatory cytokines and chemokines in tears are decreased after topical treatment with cyclosporine A in patients with VKC. However, again tear periostin has the advantage of occurring in larger amounts than these cytokines/chemokines. Further studies aiming to explore this potential are needed.

We thank Dr Dovie R. Wylie for the critical review of this manuscript.

Key messages

- Periostin, a novel mediator and biomarker in allergic inflammation, produced in conjunctival tissues stimulated by IL-13, may contribute to the pathogenesis of ocular allergic diseases.
- Furthermore, tear periostin can be potentially applied as a biomarker to diagnose conjunctivitis in allergic patients and to evaluate disease severity as well as efficacy of treatments in AKC.

REFERENCES

- Leonardi A, Bonini S. Is visual function affected in severe ocular allergies? *Curr Opin Allergy Clin Immunol* 2013;13:558-62.
- Takamura E, Uchio E, Ebihara N, Ohno S, Ohashi Y, Okamoto S, et al. Japanese guideline for allergic conjunctival diseases. *Allergol Int* 2011;60:191-203.
- Sy H, Bielory L. Atopic keratoconjunctivitis. *Allergy Asthma Proc* 2013;34:33-41.
- Saban DR, Calder V, Kuo CH, Reyes NJ, Dartt DA, Ono SJ, et al. New twists to an old story: novel concepts in the pathogenesis of allergic eye disease. *Curr Eye Res* 2013;38:317-30.
- Leonardi A, DeFranchis G, Zancanaro F, Crivellari G, De Paoli M, Plebani M, et al. Identification of local Th2 and Th0 lymphocytes in vernal conjunctivitis by cytokine flow cytometry. *Invest Ophthalmol Vis Sci* 1999;40:3036-40.
- Leonardi A, Curnow SJ, Zhan H, Calder VL. Multiple cytokines in human tear specimens in seasonal and chronic allergic eye disease and in conjunctival fibroblast cultures. *Clin Exp Allergy* 2006;36:777-84.
- Leonardi A, Sathe S, Bortolotti M, Beaton A, Sack R. Cytokines, matrix metalloproteinases, angiogenic and growth factors in tears of normal subjects and vernal keratoconjunctivitis patients. *Allergy* 2009;64:710-7.
- Oray M, Toker E. Tear cytokine levels in vernal keratoconjunctivitis: the effect of topical 0.05% cyclosporine A therapy. *Cornea* 2013;32:1149-54.
- Martinez R, Acera A, Soria J, Gonzalez N, Suarez T. Allergic mediators in tear from children with seasonal and perennial allergic conjunctivitis. *Arch Soc Esp Ophthalmol* 2011;86:187-92.
- Wakamatsu TH, Satake Y, Igarashi A, Dogru M, Ibrahim OM, Okada N, et al. IgE and eosinophil cationic protein (ECP) as markers of severity in the diagnosis of atopic keratoconjunctivitis. *Br J Ophthalmol* 2012;96:581-6.
- Izuhara K, Arima K, Ohta S, Suzuki S, Inamitsu M, Yamamoto K. Periostin in allergic inflammation. *Allergology Int* 2014;63:143-51.
- Sidhu SS, Yuan S, Innes AL, Kerr S, Woodruff PG, Hou L, et al. Roles of epithelial cell-derived periostin in TGF- β activation, collagen production, and collagen gel elasticity in asthma. *Proc Natl Acad Sci U S A* 2010;107:14170-5.
- Takayama G, Arima K, Kanaji T, Toda S, Tanaka H, Shoji S, et al. Periostin: a novel component of subepithelial fibrosis of bronchial asthma downstream of IL-4 and IL-13 signals. *J Allergy Clin Immunol* 2006;118:98-104.
- Shoda T, Futamura K, Kobayashi F, Saito H, Matsumoto K, Matsuda A. Cell type-dependent effects of corticosteroid on periostin production by primary human tissue cells. *Allergy* 2013;68:1467-70.
- Nishiyama T, Kii I, Kashima TG, Kikuchi Y, Ohazama A, Shimazaki M, et al. Delayed re-epithelialization in periostin-deficient mice during cutaneous wound healing. *PLoS One* 2011;6:e18410.
- Ontsuka K, Kotobuki Y, Shiraishi H, Serada S, Ohta S, Tanemura A, et al. Periostin, a matricellular protein, accelerates cutaneous wound repair by activating dermal fibroblasts. *Exp Dermatol* 2012;21:331-6.
- Elliott CG, Wang J, Guo X, Xu SW, Eastwood M, Guan J, et al. Periostin modulates myofibroblast differentiation during full-thickness cutaneous wound repair. *J Cell Sci* 2012;125:121-32.
- Masuoka M, Shiraishi H, Ohta S, Suzuki S, Arima K, Aoki S, et al. Periostin promotes chronic allergic inflammation in response to Th2 cytokines. *J Clin Invest* 2012;122:2590-600.
- Bentley JK, Chen Q, Hong JY, Popova AP, Lei J, Moore BB, et al. Periostin is required for maximal airways inflammation and hyperresponsiveness in mice. *J Allergy Clin Immunol* 2014;134:1433-42.
- Matsumoto H. Serum periostin: a novel biomarker for asthma management. *Allergol Int* 2014;63:153-60.

21. Kou K, Okawa T, Yamaguchi Y, Ono J, Inoue Y, Kohno M, et al. Periostin levels correlate disease severity and chronicity in patients with atopic dermatitis. *Br J Dermatol* 2014;171:283-91.
22. Corren J, Lemanske RF, Hanania NA, Korenblat PE, Parsey MV, Arron JR, et al. Lebrikizumab treatment in adults with asthma. *N Engl J Med* 2011;365:1088-98.
23. Hanania NA, Wenzel S, Rosen K, Hsieh HJ, Mosesova S, Choy DF, et al. Exploring the effects of omalizumab in allergic asthma: an analysis of biomarkers in the EXTRA study. *Am J Respir Crit Care Med* 2013;187:804-11.
24. Kanemitsu Y, Matsumoto H, Izuhara K, Tohda Y, Kita H, Horiguchi T, et al. Increased periostin associates with greater airflow limitation in patients receiving inhaled corticosteroids. *J Allergy Clin Immunol* 2013;132:305-12.
25. Nagasaki T, Matsumoto H, Kanemitsu Y, Izuhara K, Tohda Y, Kita H, et al. Integrating longitudinal on pulmonary function and inflammation using asthma phenotypes. *J Allergy Clin Immunol* 2014;133:1474-7.
26. Okubo K, Kurono Y, Fujieda S, Ogino S, Uchio E, Odajima H, et al. Japanese guideline for allergic rhinitis 2014. *Allergol Int* 2014;63:357-75.
27. Katayama I, Kohno Y, Akiyama K, Aihara M, Kondo N, Saeki H, et al. Japanese guideline for atopic dermatitis 2014. *Allergol Int* 2014;63:377-98.
28. Fujishima H, Takeuchi T, Shinozaki N, Saito I, Tsubota K. Measurement of IL-4 in tears of patients with seasonal allergic conjunctivitis and vernal keratoconjunctivitis. *Clin Exp Immunol* 1995;102:395-8.
29. Okamoto M, Hoshino T, Kitasato Y, Sakazaki Y, Kawayama T, Fujimoto K, et al. Periostin, a matrix protein, is a novel biomarker for idiopathic interstitial pneumonias. *Eur Respir J* 2011;37:1119-27.
30. Fukagawa K, Okada N, Fujishima H, Nakajima T, Takano Y, Tanaka M, et al. Corneal and conjunctival fibroblasts are major sources of eosinophil-recruiting chemokines. *Allergol Int* 2009;58:499-508.
31. Fujishima H, Fukagawa K, Satake Y, Saito I, Shimazaki J, Takano Y, et al. Combined medical and surgical treatment of severe vernal keratoconjunctivitis. *Jpn J Ophthalmol* 2000;44:511-5.
32. Nivenius E, Montan PG, Chrysanthou E, Jung K, van Hage-Hamsten M, van der Ploeg I. No apparent association between periocular and ocular microcolonization and the degree of inflammation in patients with atopic keratoconjunctivitis. *Clin Exp Allergy* 2004;34:725-30.
33. Yuyama N, Davies DE, Akaiwa M, Matsui K, Hamasaki Y, Suminami Y, et al. Analysis of novel disease-related genes in bronchial asthma. *Cytokine* 2002;19:287-96.
34. Nakamura Y, Nagashima H, Ohta S, Ono J, Yamauchi K, Izuhara K. Periostin in the bronchial lavage fluid of asthma patients. *Allergol Int* 2015;64:209-10.
35. Asano-Kato N, Fukagawa K, Okada N, Kawakita T, Takano Y, Dogru M, et al. TGF- β 1, IL-1 β , and Th2 cytokines stimulate vascular endothelial growth factor production from conjunctival fibroblasts. *Exp Eye Res* 2005;80:555-60.
36. Hu Y, Adan ES, Matsumoto Y, Dogru M, Fukagawa K, Takano Y, et al. Conjunctival in vivo confocal scanning laser microscopy in patients with atopic keratoconjunctivitis. *Mol Vis* 2007;13:1379-89.
37. Hu Y, Matsumoto Y, Adan ES, Dogru M, Fukagawa K, Tsubota K, et al. Corneal in vivo confocal scanning laser microscopy in patients with atopic keratoconjunctivitis. *Ophthalmology* 2008;115:2004-12.
38. Fukushima A, Ohashi Y, Ebihara N, Uchio E, Okamoto S, Kumagai N, et al. Therapeutic effects of 0.1% tacrolimus eye drops for refractory allergic ocular diseases with proliferative lesion or corneal involvement. *Br J Ophthalmol* 2014;98:1023-7.

TABLE E1. Backgrounds of the subjects used in this study

Characteristic	AKC	VKC	SAC	Control	AD control	AR control	Total
N	41	7	27	26	16	9	126
Sex: female (%)	22.0	28.6	63.0	65.4	50.0	36.4	46.0
Age (y)	24.6 ± 14.8	24.6 ± 20.8	43.0 ± 20.8	34.2 ± 12.9	35.4 ± 10.6	44.5 ± 13.9	33.5 ± 17.4
Hyperemia score	2.1 ± 1.0	2.6 ± 0.8	1.5 ± 0.6	0.2 ± 0.4	0.0 ± 0.0	0.0 ± 0.0	1.5 ± 1.1
Edema score	2.0 ± 1.0	2.6 ± 0.8	1.1 ± 0.7	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.3 ± 1.2
Proliferation score	2.0 ± 1.0	2.6 ± 0.8	1.0 ± 0.5	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.2 ± 1.2
Giant papillae (%)	44.4	57.1	0	0	0	0	16.5
Corneal ulcer (%)	34.5	71.4	0	0	0	0	13.2

TABLE E2. Clinical signs and tear periostin concentrations in pre-/post-topical treatment with FK506

No.	Age (y)	Sex	Administration term (m)	Tear periostin (ng/mL)		Hyperemia score		Edema score		Proliferation score		Giant papillae		Corneal ulcer		Corneal damage score	
				Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
1	13	F	12	8322.5	3260.6	3	1	3	1	3	3	+	+	—	—	A3D3	A1D1
2	13	M	3	662.3	4.1	3	0	3	0	3	3	+	+	—	—	A3D3	—
3	27	M	2	629.3	394.3	3	1	3	1	3	3	+	+	+	—	A1D1	—
4	13	M	4	405.7	303.7	3	2	3	2	3	3	+	+	+	—	A3D3	A3D3
5	12	M	6	345.9	29.4	3	0	3	0	3	0	+	—	+	—	A3D2	—
6	13	M	8	196.9	63.3	2	0	2	0	3	1	+	—	—	—	—	—
7	8	F	1	678.8	1025.4	3	1	3	1	3	3	+	+	—	—	—	—
8	41	M	1	678.0	68.0	3	2	2	1	3	3	+	+	+	+	A3D3	A2D3

F, Female; M, male.