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

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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;138:459-67.)

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

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.

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

A great deal of evidence has accumulated showing that tears reflect *in vivo* inflammation in ocular allergic diseases. Inflammatory cells—neutrophils, eosinophils, and lymphocytes, 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 2014[26] 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 μL 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.