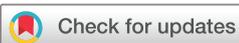


# Elevated levels of prostaglandin E<sub>2</sub> in the tears of patients with severe allergic conjunctivitis and primary cultured conjunctival cells are suppressed by ketotifen and dexamethasone

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## ABSTRACT

**Objective** We examined the production of prostaglandin E<sub>2</sub> (PGE<sub>2</sub>), which is the key prostaglandin involved in inflammatory disorders of the ocular surface. Tears and conjunctival fibroblasts were evaluated in order to assess allergic inflammation and the effect of specific drugs. **Methods and analysis** PGE<sub>2</sub> was measured in tears from both patients and normal volunteers. Primary cultures of human conjunctival fibroblasts were incubated with interleukin (IL)-4 and tumour necrosis factor (TNF)-α with or without ketotifen fumarate or dexamethasone. The culture supernatants were removed 24 hours after exposure and the concentrations of PGE<sub>2</sub> were quantified by ELISA.

**Results** Significantly higher levels of PGE<sub>2</sub> were observed in the tears of patients with severe allergic conjunctivitis than in those with post-surgical inflammation (p=0.02), and this production was reduced by eye drops. Stimulation with IL-4 and TNF-α induced the generation of PGE<sub>2</sub> in supernatants of conjunctival fibroblasts, and this production was significantly downregulated by ketotifen fumarate or steroids.

**Conclusion** PGE<sub>2</sub> may participate in the pathogenesis of severe ocular allergic disease, and both ketotifen fumarate and steroid reduce the production of PGE<sub>2</sub>.

## INTRODUCTION

Evidence suggests that ocular surface tissues such as the cornea and conjunctiva play active roles in ocular allergy; histamine receptors are expressed on conjunctival epithelial cells, and proinflammatory cytokines such as interleukin (IL)-4 and tumour necrosis factor (TNF)-α are released in tears in ocular allergic inflammation.<sup>1 2</sup> Arachidonic acid (AA), released from cell membrane phospholipids of activated cells, is metabolised to prostaglandins (PGs) by the sequential activity of cyclooxygenase (COX).<sup>3</sup> It is known that corneal and conjunctival cells also release AA in inflammatory conditions.<sup>4</sup> We have previously reported that PGE<sub>2</sub> is produced by human conjunctival and corneal cells in response to IL-4 and TNF-α stimulation.<sup>5</sup> In this

## Key messages

### What is already known about this subject?

- ▶ Prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) is produced in the tears of patients with inflammatory disorders in the ocular surface as severe allergic conjunctivitis.

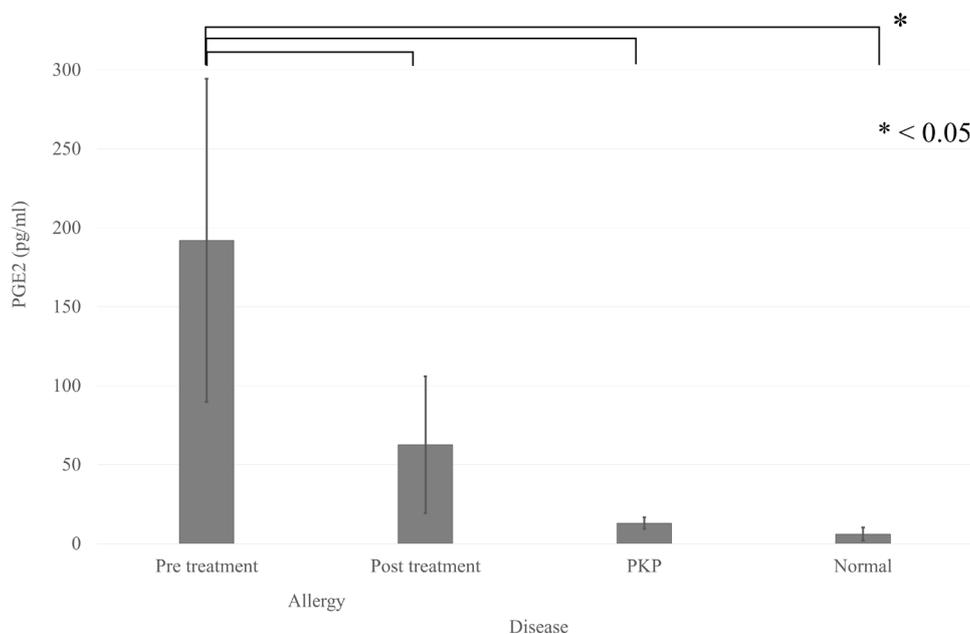
### What are the new findings?

- ▶ Significantly higher levels of PGE<sub>2</sub> were observed in the tears of patients with severe allergic conjunctivitis. Moreover, ketotifen fumarate affected PGE<sub>2</sub> production and reduced allergic inflammation.

### How might these results change the focus of research or clinical practice?

- ▶ Prostaglandins have potential importance in the management of ocular allergic inflammation and could be the pathogenesis of severe ocular allergic disease.

report, we analysed PGE<sub>2</sub> production, which has been thought to contribute to the development of inflammation, in allergic inflammation within two representative types of ocular tissues: epithelial cells and fibroblasts.<sup>5</sup> PGs have been reported to mediate various inflammatory responses in ophthalmological diseases.<sup>6</sup> COX-2 is an inducible enzyme and highly associated with ocular surface injections.<sup>7 8</sup> TNF-α is a potent inducible inflammatory cytokine and is capable of inducing PG production by cells.<sup>9–12</sup> Corneal keratocytes produce chemokines such as regulated on activation normal T cell expressed and secreted (RANTES),<sup>13</sup> monocyte chemoattractant protein (MCP)-1,<sup>14 15</sup> and eotaxin<sup>16</sup> following stimulation by IL-4 and TNF-α. These chemokines recruit eosinophils into local tissues. In our previous study, stimulation with IL-4 and TNF-α also affected the expression of many other genes in keratocytes, including those of various chemokines, such as IP-10,<sup>17</sup> the fourth most induced transcript I-TAC,<sup>17</sup> the sixth most induced transcript RANTES<sup>18</sup> and the ninth most induced



**Figure 1** PGE<sub>2</sub> production in the tears of patients, and its downregulation by ketotifen fumarate and dexamethasone. The mean production of PGE<sub>2</sub> in the tears of patients with severe allergic inflammation (n=5) was significantly higher than that in patients following 4 weeks of eye drop treatment, in patients post-surgical inflammation (n=5) and normal controls (n=5). Results are presented as means±SD. Student's t-test was performed. Allergy indicates patients with severe allergic inflammation. PKP indicates patients with post-surgical inflammation. PGE<sub>2</sub>, prostaglandin E<sub>2</sub>; PKP, penetrating keratoplasty.

transcript MCP-2. Activated conjunctival epithelial cells release biologically active compounds which play essential roles in the pathogenesis of ocular inflammation, including lipid mediators, eicosanoids, and a variety of cytokines and chemokines.

Ketotifen fumarate is non-competitive antagonist of the histamine H1 receptor and a mast cell stabiliser, which inhibits the release of inflammatory mediators from mast cells. In addition, its ophthalmic solution was safe, well tolerated, and effective in preventing the signs and symptoms of allergic conjunctivitis.<sup>19</sup>

Although previous ocular allergy studies have provided useful insights, the specific mechanisms of the regulated drug processes in severe ocular allergic diseases are not fully understood. In this study, we examined PGE<sub>2</sub> production in the tears of patients with severe ocular allergic inflammation, followed by primary cultures of the human conjunctival fibroblasts.<sup>20</sup> This study aimed to investigate the effects of ketotifen fumarate and steroids on the release of PGE<sub>2</sub> by primary cultures of human conjunctival fibroblasts.

## MATERIALS AND METHODS

Written informed consent was obtained from all subjects before participation. Patient data were anonymised before access and analysis.

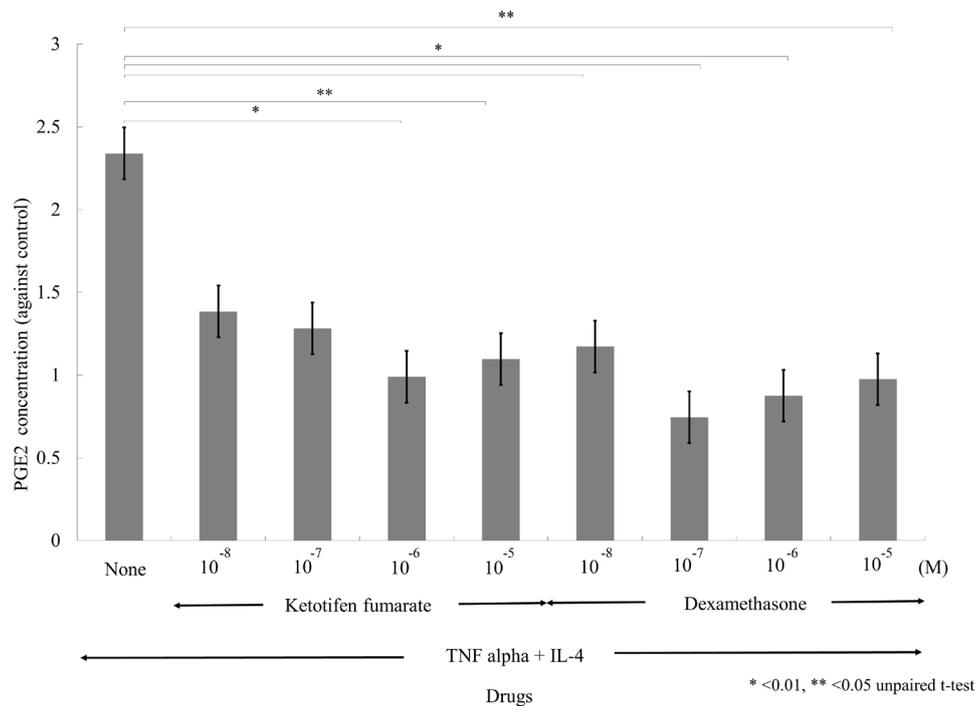
### Clinical samples

Five patients who underwent simple penetrating keratoplasty (PKP) surgery (mean age: 27.7±9.1 years) were recruited to obtain samples 1 hour post-surgical inflammation. Four patients had been performed PKP due to keratoconus, and the other had been due to bullous keratopathy. We chose

PKP case as disease control because under the epithelial defect, continuous stimulation of stromal cell may increase the production of PGE<sub>2</sub>.<sup>5</sup> In addition, five patients (mean age: 15.1±3.4 years) with atopic keratoconjunctivitis or vernal keratoconjunctivitis (VKC) with corneal erosion were recruited to obtain samples exhibiting severe allergic inflammation.<sup>21</sup> Their treatment included Zaditen (0.05% ketotifen fumarate, Santen, Osaka, Japan) and Santeson ophthalmic solution (0.1% dexamethasone, Santen, Osaka, Japan), both administered four times per day for 4 weeks. We included an additional five participants as normal controls (mean age: 31.6±4.3 years). The diagnosis of atopic dermatitis was made by a dermatology specialist based on the criteria of Hanifin *et al.*<sup>22</sup> No treatment was supplied prior to tear sample collections. In addition, samples of normal cases were collected from normal volunteers. Samples were collected before and 4 weeks after topical treatment in the control group, and a day after the operation in patients with PKP. Using a micropipette, 20 µL of tears were collected at the lateral canthus of the eyelid without anaesthetic; patients were in the supine position with their heads tilted to the side. Tear samples were centrifuged immediately at 4°C to remove cells and transferred to new tubes. Tear samples were stored at -80°C until further examination.

### Measurements

PGE<sub>2</sub> concentration was measured by ELISA (R&D, Minneapolis, Minnesota, USA). The cytokines, RANTES and IL-8 were purchased from R&D. Eotaxin was received as a gift from Dr Hiroshi Kawasaki of the Department of Clinical Immunology, The Institute of Medical Science, University of Tokyo, Tokyo, Japan.



**Figure 2** Effects of ketotifen fumarate and dexamethasone on PGE<sub>2</sub> production by conjunctival fibroblasts. Human conjunctival fibroblasts were maintained in 96 well plates at a concentration of  $1 \times 10^5$  cells/mL. Cells were incubated with IL-4 (10 ng/mL or 100 ng/mL) and/or TNF- $\alpha$  (10 or 100 ng/mL) and harvested 24 hours later. Ketotifen fumarate and dexamethasone were added at concentrations from  $10^{-8}$  nM to  $10^{-5}$  nM. The 'None' bar indicates medium alone. The generation of PGE<sub>2</sub> was determined in cell-free supernatants by ELISA. Results are presented as mean $\pm$ SD of seven independent experiments. Significant differences at the levels of  $p < 0.05$  and  $p < 0.01$  vs time 0 are indicated by \* and \*\*, respectively (Student's t-test). IL-4, interleukin 4; PGE<sub>2</sub>, prostaglandin E<sub>2</sub>; TNF, tumour necrosis factor.

### Primary culture

Human conjunctival samples were collected using scissors from normal volunteers from whom informed consent had been obtained. Samples were incubated in Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 medium (Thermo Fisher Scientific, Waltham, Massachusetts, USA) to grow fibroblasts. Specimens were cultured in complete medium at 37°C in a humidified atmosphere supplemented with 5% CO<sub>2</sub> in air for a few weeks. The cells were grown with incubation in a flask. Fibroblasts were subcultured in 96 well plates at a concentration of  $1 \times 10^5$  cells/mL. One day later, cells were incubated with TNF- $\alpha$  (30 ng/mL) and IL-4 (30 ng/mL) in combination with ketotifen fumarate and dexamethasone:  $10^{-6}$ – $10^{-8}$  M (Sigma-Aldrich, St. Louis, Missouri, USA). Twenty-four hours later, culture supernatants were collected and PGE<sub>2</sub> levels were determined.

### Statistical analysis

The data were analysed using Prism software (V.6.04 for Mac; GraphPad Software, San Diego, California, USA). The D'Agostino-Pearson omnibus normality test was used to assess whether the data showed a normal distribution. Student's t-test was performed to assess the differences in tear and PGE<sub>2</sub> production associated with IL-4 and TNF- $\alpha$ . The same test was also used to assess differences in drug inhibition associated with ketotifen fumarate and dexamethasone. Values are expressed as mean $\pm$ SD. P

values were not adjusted for multiple comparison testing. Values below 0.05 were considered statistically significant.

### RESULTS

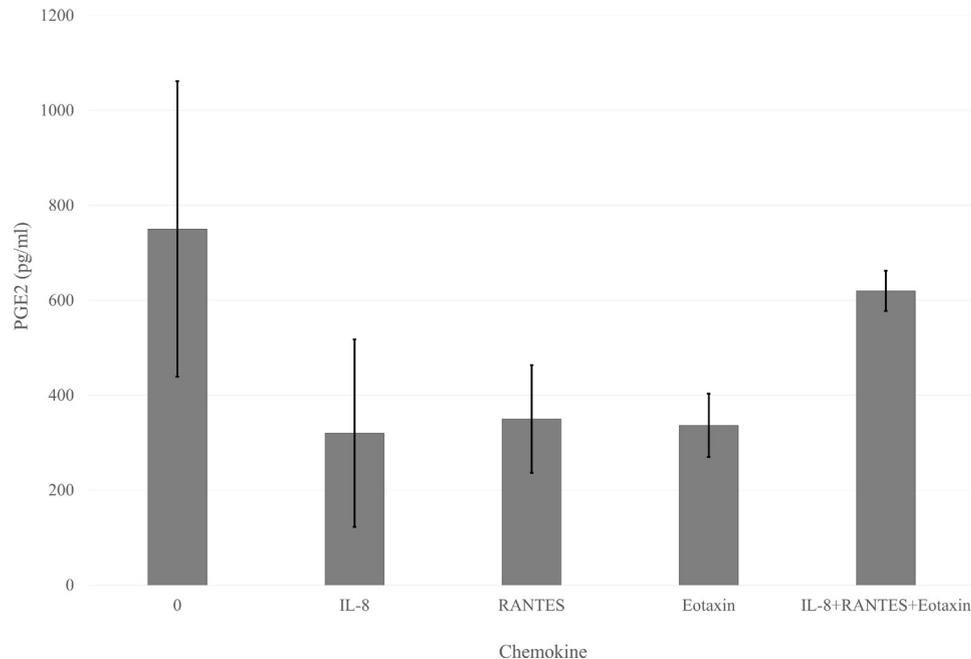
Mean tear PGE<sub>2</sub> levels are shown in figure 1; the level was  $13.0 \pm 3.6$  pg/mL in post-PKP inflammation,  $6.0 \pm 4.2$  pg/mL in normal controls and  $190 \pm 100$  pg/mL in the severe allergic inflammatory condition, which decreased significantly to  $62.6 \pm 43.3$  pg/mL after 4 weeks of treatment with ketotifen fumarate and dexamethasone ( $p = 0.04$ ).

Patients with severe allergic inflammation produced significantly higher levels of PGE<sub>2</sub> than those found after 4 weeks of topical treatment (0.05% ketotifen fumarate and 0.1% dexamethasone), and in those post-PKP inflammation, and in normal controls ( $p < 0.05$ ; figure 1).

Stimulation with 30 ng of IL-4 and TNF- $\alpha$  for 24 hours induced the production of PGE<sub>2</sub> by fibroblasts, and this production was significantly downregulated by ketotifen fumarate at concentrations of  $10^{-6}$  M ( $p < 0.01$ ) and  $10^{-5}$  M ( $p < 0.05$ ). Dexamethasone reduced the PGE<sub>2</sub> by fibroblasts at concentrations of  $10^{-8}$  M,  $10^{-7}$  M and  $10^{-6}$  M (all  $p < 0.01$ ), as well as  $10^{-5}$  M ( $p < 0.05$ ; figure 2). Chemokines such as IL-8, RANTES and eotaxin did not induce the production of PGE<sub>2</sub> by fibroblasts (figure 3).

### DISCUSSION

This study identified the production of PGE<sub>2</sub> in tears of patients with severe allergic conjunctivitis, and that this



**Figure 3** PGE<sub>2</sub> production by conjunctival fibroblasts stimulated with chemokines. Human conjunctival fibroblasts were maintained in 96 well plates at a concentration of  $1 \times 10^5$  cells/mL. Cells were incubated with IL-8 (30 ng/mL), RANTES (30 ng/mL) and eotaxin (30 ng/mL), and harvested 24 hours later. The left bar '0' indicates medium alone. The generation of PGE<sub>2</sub> was determined in cell-free supernatants by ELISA. Results are presented as means $\pm$ SD of at least three independent experiments. No significant differences were observed between the chemokine combinations (Student's t-test). IL-8, interleukin 8; PGE<sub>2</sub>, prostaglandin E<sub>2</sub>; RANTES, regulated on activation normal T cell expressed and secreted.

production was decreased by the administration of 0.05% ketotifen fumarate and 0.1% dexamethasone. PGE<sub>2</sub> was also produced by primary cultured conjunctival fibroblasts from normal volunteers following stimulation with IL-4 and TNF- $\alpha$ , and this production was also decreased by ketotifen fumarate or dexamethasone. PGs induce vasodilation, which is an important feature of local inflammation.<sup>23</sup> AA, which is released from the cell membrane phospholipids of activated cells, is metabolised to PGs by the sequential activity of COX-1 and COX-2.<sup>24</sup>

It has been reported previously that a conjunctival allergic reaction can be induced with antigen in sensitised guinea pigs, and that in the acute phase of allergic conjunctivitis, the PGE<sub>2</sub> levels can be determined in lavage fluid; PGE<sub>2</sub> was produced simultaneously in the conjunctiva and exhibited identical profiles of synthesis in response to antigen provocation.<sup>25</sup> In this study, PGE<sub>2</sub> was identified in the tears of patients with severe allergic conjunctivitis, who were thought to be in the late phase of this condition. Levels of PGE<sub>2</sub> were higher in the tears of patients with severe allergy than in tears 4 weeks after eye drop treatment, and in post-surgical patients with PKP. The PKP tear samples, used as inflammatory controls, likely involve quite different inflammatory mechanisms and are not chronic. It is therefore not surprising that PGE<sub>2</sub> levels in patients with PKP were close to baseline. While our PKP surgical procedure usually concludes with a subconjunctival steroid injection and coverage with a medical use contact lens, PGE<sub>2</sub> production was significantly higher in the tears from patients with a severe

allergy. This may be due to the corneal ulcers in patients with severe allergic disease.<sup>5</sup> PGE<sub>2</sub> was produced from the conjunctival fibroblasts by the action of IL-4 or TNF- $\alpha$  in tears,<sup>5</sup> as discussed below.

In vitro concentrations of procollagens and inflammatory cytokines in fibroblasts from patients with VKC, including TNF- $\alpha$  and other cytokines, have been shown to be increased.<sup>26</sup> TNF- $\alpha$  levels have been shown to be increased in the tears of patients with atopy after conjunctival allergen challenge.<sup>11</sup> We have also reported increased levels of IL-4 in tears from patients with VKC.<sup>27</sup> We have stimulated ocular surface cells by IL-4 and TNF- $\alpha$  through allergic inflammation in vitro. Cooperation between proinflammatory cytokines is well documented in the literature. Production of PGE<sub>2</sub> was induced in primary cultured corneal keratocytes, conjunctival epithelial cells, and fibroblasts by IL-4 and TNF- $\alpha$ . Kinetic studies have demonstrated that PGE<sub>2</sub> is significantly increased in a time-dependent manner.<sup>5</sup> In this paper, corneal epithelial cells produced almost no PGE<sub>2</sub>. The reason for this is still unknown, but we hypothesise that corneal epithelial cells did not release PGE<sub>2</sub> and may be the target cell of this inflammatory response. Thus, we are now investigating how PGE<sub>2</sub> affects corneal epithelial cells. Repeated induction of PGE<sub>2</sub> production by conjunctival cells may cause corneal epithelial injection, and once the epithelial barrier is broken, keratocytes could produce more PGE<sub>2</sub> and epithelial erosions or ulcers can occur in severe allergic conjunctivitis. The effects of PGE<sub>2</sub> on corneal epithelial cells are

still unclear. We are studying these effects because we believe the corneal epithelium is a target of PGE<sub>2</sub>. Any event which induces the production of PGE<sub>2</sub> may play an important role in allergic inflammation and result in severe clinical manifestations.

The effect of steroids on COX enzyme inhibition is now widely accepted. The anti-inflammatory effects of ketotifen fumarate are therefore likely to be due to the inhibition of COX. The identification of selective inhibitors of COX-2 will therefore lead to advances in therapy.<sup>24</sup> In this study, the production of PGE<sub>2</sub> by conjunctival fibroblasts was decreased by ketotifen fumarate, which is an antihistamine drug with an incompletely understood efficacy. Ketotifen fumarate has a strong inhibitory effect of platelet-activating factor (PAF), which enhances AA metabolism in addition to its role in both the acute and delayed phases of allergy. There is one speculation that under the circumstances an environment without mast cells, PGE<sub>2</sub> production was suppressed by its PAF inhibitory effect. Although this agent has distinct antihistamine molecular mechanisms of action and exhibits different immunoregulatory profiles, the direct effects on antigen presentation processes remain unknown. This is why we have studied the effects of this drug on primary cultured ocular surface cells. The pathogenesis of topical therapeutic antihistamine drugs in the inhibition of allergic inflammation may be due to other reasons.

CC chemokines, such as eotaxin and RANTES, are important in recruiting eosinophils into tissues affected by allergy.<sup>28</sup> RANTES has been found in the tears of patients with allergic conjunctivitis,<sup>29</sup> and is produced by conjunctival and corneal cells. We have previously reported that eotaxin is present in the tears of patients with allergy and severe corneal injury, collating with the number of eosinophils in the tears.<sup>30</sup> We have also found that IL-4 induces eotaxin production by human corneal keratocytes.<sup>16</sup> In this study, we investigated the induction of PGE<sub>2</sub> production by fibroblasts associated with IL-8, RANTES and eotaxin. No effects on fibroblasts were observed (figure 3). Chemokines have no direct effects on keratocytes during the induction of PGE<sub>2</sub> production. This result implies that PGE<sub>2</sub> production by fibroblasts is not promoted by chemokines via autocrine or paracrine stimulations, but instead by the direct stimulation of IL-4 and TNF-α.

In summary, we found that PGE<sub>2</sub> was produced in the tears of patients with severe allergic conjunctivitis, and produced by cultured conjunctival fibroblast cells in a sequential manner following stimulation with IL-4 and TNF-α. Ketotifen fumarate affected this PGE<sub>2</sub> production and reduced allergic inflammation with unknown antihistamine efficacy. PGs have an important role in the study of allergic inflammation and have potential importance in managing ocular allergic inflammation.

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**Competing interests** None declared.

**Patient consent for publication** Not required.

**Ethics approval** This study was performed in accordance with the Declaration of Helsinki and was approved by the institutional ethics review board of Tsurumi University School of Dental Medicine (Kanagawa, Japan; IRB number: 1111).

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**Data availability statement** Data may be obtained from a third party and are not publicly available.

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#### REFERENCES

- 1 Church MK, McGill JI. Human ocular mast cells. *Curr Opin Allergy Clin Immunol* 2002;2:419–22.
- 2 Takano Y, Fukagawa K, Shimmura S, et al. IL-4 regulates chemokine production induced by TNF-alpha in keratocytes and corneal epithelial cells. *Br J Ophthalmol* 1999;83:1074–6.
- 3 Fujishima H, Sanchez Mejia RO, Bingham CO, et al. Cytosolic phospholipase A2 is essential for both the immediate and the delayed phases of eicosanoid generation in mouse bone marrow-derived mast cells. *Proc Natl Acad Sci U S A* 1999;96:4803–7.
- 4 Tripathi T, Alizadeh H. Significance of arachidonic acid in ocular infections and inflammation. *Inflamm Cell Signal* 2014;1.
- 5 Shimizu E, Yazu H, Satake Y, et al. The effect of cytokine-stimulation and pharmacologic intervention on PGE2 production in primary human conjunctival and corneal cells. *Ocul Immunol Inflamm* 2020;28:384–90.
- 6 Ueta M. Regulation of ocular surface inflammation by prostaglandin E receptor subtype EP3. *Cornea* 2010;29:S57–61.
- 7 Szerenyi KD, Campos M, McDonnell PJ. Prostaglandin E2 production after lamellar keratectomy and photorefractive keratectomy. *J Refract Corneal Surg* 1994;10:413–6.
- 8 Venza I, Giordano L, Piraino G, et al. Prostaglandin E2 signalling pathway in human T lymphocytes from healthy and conjunctiva basal cell carcinoma-bearing subjects. *Immunol Cell Biol* 2001;79:482–9.
- 9 Cunliffe IA, Richardson PS, Rees RC, et al. Effect of TNF, IL-1, and IL-6 on the proliferation of human tenon's capsule fibroblasts in tissue culture. *Br J Ophthalmol* 1995;79:590–5.
- 10 Cook EB, Stahl JL, Barney NP, et al. Olopatadine inhibits TNFalpha release from human conjunctival mast cells. *Ann Allergy Asthma Immunol* 2000;84:504–8.
- 11 Vesaluoma M, Rosenberg ME, Teppo A, et al. Tumour necrosis factor alpha (TNFalpha) in tears of atopic patients after conjunctival allergen challenge. *Clin Exp Allergy* 1999;29:537–42.
- 12 Abu el-Asrar AM, Geboes K, Tabbara KF, et al. Immunopathogenesis of conjunctival scarring in trachoma. *Eye* 1998;12 (Pt 3a):453–60.
- 13 Yamagami S, Miyazaki D, Ono SJ, et al. Differential chemokine gene expression in corneal transplant rejection. *Invest Ophthalmol Vis Sci* 1999;40:2892–7.
- 14 Bian ZM, Elnor VM, Lukacs NW, et al. Glycated human serum albumin induces IL-8 and MCP-1 gene expression in human corneal keratocytes. *Curr Eye Res* 1998;17:65–72.
- 15 Hong JW, Liu JJ, Lee JS, et al. Proinflammatory chemokine induction in keratocytes and inflammatory cell infiltration into the cornea. *Invest Ophthalmol Vis Sci* 2001;42:2795–803.
- 16 Fukagawa K, Nakajima T, Saito H, et al. IL-4 induces eotaxin production in corneal keratocytes but not in epithelial cells. *Int Arch Allergy Immunol* 2000;121:144–50.
- 17 Fukagawa K, Okada N, Fujishima H, et al. Corneal and conjunctival fibroblasts are major sources of eosinophil-recruiting chemokines. *Allergol Int* 2009;58:499–508.



- 18 Yazu H, Fukagawa K, Okada N, *et al.* Effects of docosahexaenoic acid on chemokine expression in human conjunctival fibroblasts. *Curr Eye Res* 2020;45:81–6.
- 19 Greiner JV, Mundorf T, Dubiner H, *et al.* Efficacy and safety of ketotifen fumarate 0.025% in the conjunctival antigen challenge model of ocular allergic conjunctivitis. *Am J Ophthalmol* 2003;136:1097–105.
- 20 Fukagawa K, Tsubota K, Simmura S, *et al.* Chemokine production in conjunctival epithelial cells. *Adv Exp Med Biol* 1998;438:471–8.
- 21 Fujishima H, Okada N, Matsumoto K, *et al.* The usefulness of measuring tear periostin for the diagnosis and management of ocular allergic diseases. *J Allergy Clin Immunol* 2016;138:459–67.
- 22 Hanifin JM, Ling MR, Langley R, *et al.* Tacrolimus ointment for the treatment of atopic dermatitis in adult patients: Part I, efficacy. *J Am Acad Dermatol* 2001;44:S28–38.
- 23 Murrant CL, Dodd JD, Foster AJ, *et al.* Prostaglandins induce vasodilatation of the microvasculature during muscle contraction and induce vasodilatation independent of adenosine. *J Physiol* 2014;592:1267–81.
- 24 Vane JR, Bakhle YS, Botting RM. Cyclooxygenases 1 and 2. *Annu Rev Pharmacol Toxicol* 1998;38:97–120.
- 25 Meijer F, Tak C, van Haeringen NJ, *et al.* Interaction between nitric oxide and prostaglandin synthesis in the acute phase of allergic conjunctivitis. *Prostaglandins* 1996;52:431–46.
- 26 Leonardi A, Borghesan F, DePaoli M, *et al.* Procollagens and inflammatory cytokine concentrations in tarsal and limbal vernal keratoconjunctivitis. *Exp Eye Res* 1998;67:105–12.
- 27 Fujishima H, Takeuchi T, Shinozaki N, *et al.* Measurement of IL-4 in tears of patients with seasonal allergic conjunctivitis and vernal keratoconjunctivitis. *Clin Exp Immunol* 1995;102:395–8.
- 28 Ying S, Robinson DS, Meng Q, *et al.* C-C chemokines in allergen-induced late-phase cutaneous responses in atopic subjects: association of eotaxin with early 6-hour eosinophils, and of eotaxin-2 and monocyte chemoattractant protein-4 with the later 24-hour tissue eosinophilia, and relationship to basophils and other C-C chemokines (monocyte chemoattractant protein-3 and RANTES). *J Immunol* 1999;163:3976–84.
- 29 Chihara J, Yamada H, Takamura E, *et al.* Possible presence of RANTES in tears of patients with allergic conjunctivitis. *Int Arch Allergy Immunol* 1995;106:428.
- 30 Fukagawa K, Nakajima T, Tsubota K, *et al.* Presence of eotaxin in tears of patients with atopic keratoconjunctivitis with severe corneal damage. *J Allergy Clin Immunol* 1999;103:1220–1.