Prostaglandin E Receptor 4 Expression in Human Conjunctival Epithelium and Its Downregulation in Devastating Ocular Surface Inflammatory Disorders

Prostanoids are a group of lipid mediators that form in response to various stimuli, including prostaglandin (PG) D$_2$, PGE$_2$, PGF$_{2\alpha}$, PGI$_2$, and thromboxane A$_2$. There are 8 types of prostanoid receptors that are conserved in mammals ranging from mice to humans: the PGD receptor, 4 subtypes of the PGE receptor (EP1, EP2, EP3, and EP4), the PGF receptor, the PGI receptor, and the thromboxane A receptor. In regard to PGE receptor subtype EP4, it was reported that EP4 messenger RNA was present in the intestinal epithelium and that EP4 maintained intestinal homeostasis and downregulated immune response. Like the intestine, the ocular surface is also one of the mucosa that are in contact with commensal bacteria. In this study, we examined the expression of EP4 in human conjunctival epithelium and compared its expression between various ocular surface diseases.

Methods. This study was approved by the Institutional Review Board of Kyoto Prefectural University of Medicine, Kyoto, Japan. For reverse transcription–polymerase chain reaction assay, we obtained human conjunctival epithelial cells from healthy volunteers by brush cytology using previously described methods. The primers were (forward) 5'-ACA ACC ATG CCT ATT TCT ACA GCC ACT ACG-3' and (reverse) 5'-AGG CTT CTA ATT ATA TTC GCA AAG TCC TCA GTG-3' for human PTGER4 and (forward) 5'-CCA TCA CCA CCT TCT AGG AG-3' and (reverse) 5'-CCT GCT TCA CCA CTT TCT-3' for human GAPDH. For immunohistochemistry, we used nearly normal bulbar conjunctival tissues obtained during surgery for conjunctivochalasis as a control, and human conjunctival tissues were also prepared from samples obtained during surgery to reconstruct the ocular surface such as treatment for various ocular surface diseases including Stevens-Johnson syndrome/toxic epidermal necrolysis (SJS/TEN), ocular cicatricial pemphigoid (OCP), and pterygium. For EP4 staining, we used the rabbit polyclonal antibody to EP4 (Cayman Chemical Co, Ann Arbor, Michigan).

Results. The presence of PTGER4 messenger RNA and EP4 protein in human conjunctival epithelium was examined by reverse transcription–polymerase chain reaction and immunohistological analysis, respectively. The PTGER4 messenger RNA was detected in normal human conjunctival epithelium (Figure, A). The sequences obtained from these polymerase chain reaction products were identical to the human PTGER4 complementary DNA sequence. The EP4 protein was also detected in the nearly normal conjunctival epithelium obtained from the patients with conjunctivochalasis (Figure, B). Next, we examined the conjunctival tissues with various ocular surface diseases. The EP4 protein was detected in conjunctival epithelium from patients with pterygium as well as in the conjunctival epithelium from...
control patients with conjunctivochalasis. However, we did not detect EP4 immunoreactivity in the conjunctival epithelium from patients with SJS/TEN or OCP (Figure, C). Our results showed that EP4 is strongly downregulated in the conjunctival epithelium of tissues with devastating ocular surface disorders such as SJS/TEN and OCP, although it is usually expressed in human conjunctival epithelium.

Comment. To our knowledge, this is the first documentation regarding downregulation of EP4 expression in human conjunctival epithelium in tissues with devastating ocular surface inflammatory disorders, although there were reports of expression of EP receptors in ocular tissues. Kabashima et al3 reported that EP4 deficiency impaired mucosal barrier function and aggregation of neutrophils and lymphocytes in the colon and that administration of an EP4-selective agonist to wild-type mice ameliorated severe colitis; they concluded that EP4 maintains intestinal homeostasis. On the other hand, Yao et al6 recently reported that PGE2 acts on its receptor EP4 on T cells and dendritic cells and promotes immune inflammation.

In human conjunctival tissues, the EP4 protein was detected in only epithelial cells but not infiltrating cells into subconjunctival tissues. Because there is mucosal inflammation on the ocular surface even in patients with chronic-phase SJS/TEN or OCP, we suspect that the downregulation of EP4 expression in conjunctival epithelium might be associated with the ocular surface inflam-
Subconjunctival Mycetoma as an Unusual Cause of Tears With Black Deposits

Ocular mycosis is a rare condition that is usually related to ocular trauma, preexisting ocular disease, or immunocompromised states. We report a case of subconjunctival mycetoma secondary to Exophiala dermatitidis in a healthy middle-aged woman with recalcitrant ocular inflammation and black deposits in her tears.

Report of a Case. A 44-year-old woman had recurrent discharge from her right eye and black deposits in her tears for 2 years. Her symptoms persisted despite the use of topical antibiotics, steroids, and antihistamine. She was otherwise healthy and was not receiving any systemic or other topical medication. She denied any history of ocular trauma or surgery. She did not use contact lenses or eye makeup.

On examination, her general condition was excellent. Her visual acuity, intraocular pressure, and fundus were all normal. There was no eyelid swelling or erythema. On exerting the right upper eyelid, some subconjunctival black deposits were noted (Figure, A). During biopsy, the conjunctiva was incised and multiple black, mulberry-like concretions extruded with mucoid discharge (Figure, B). Topical chloramphenicol, 0.5%, with dexamethasone sodium phosphate, 0.1%, eyedrops were prescribed postoperatively. Histopathological evaluation of these concretions showed large amounts of fungal hyphae (Figure, C and D) with chronic inflammation over the conjunctiva. The diagnosis was subconjunctival mycetoma. Initial culture results for fungal growth were negative, but further evaluation with 28S ribosomal RNA gene sequencing identified the causative organism as E dermatitidis. At subsequent follow-up visits, the patient had complete resolution of symptoms. Topical antifungal treatment was not given as she was asymptomatic and there was no recurrence of mycetoma at month 3 after debridement.

Comment. Tears with black deposits are extremely rare. In our case, we initially thought the black deposits were either foreign bodies or adrenochrome deposits, but they proved to be shedding from the subconjunctival mycetoma. Patients with tears with black deposits should therefore be evaluated for the presence of subconjunctival mycetoma. A similar clinical entity termed melanodacryorrhea (black tears) is caused by extraocular extension of uveal melanoma. In immunocompetent subjects, fungal infection can remain superficial and localized as illustrated in our case. Subconjunctival mycetoma has been reported after subtenon corticosteroid injection in an immunocompromised host and in an immunocompetent woman with no risk factors, similar to our patient. The Exophiala species are dematiaceous mold commonly recovered from soil, plants, water, and decaying wood materials. This strain of black yeasts has been described as the causative agent in fungal keratitis,7 but to our knowledge it has not been reported to cause subconjunctival mycetoma.7 E dermatitidis has been described as the causative agent in fungal keratitis that occurred after keratoplasty8 and laser in situ keratomileusis,9 but to our knowledge it has not been reported to cause subconjunctival mycetoma.

Treatments described for subconjunctival mycetoma are diverse, ranging from aggressive topical and systemic antifungal treatments following surgical intervention to surgical debridement alone. A study by Zeng et al10 evaluated the activity of amphotericin B, itraconazole, voriconazole, and posaconazole against E dermatitidis and reported that all 4 antifungal agents have low minimum inhibitory concentrations (range, 0.03-0.5). However, data on correlation between in vitro and in vivo susceptibility are unavailable.