Downregulation of Monocyte Chemoattractant Protein 1 Expression by Prostaglandin E2 in Human Ocular Surface Epithelium

Elsewhere, we reported that in the tears and serum of patients with acute-stage Stevens-Johnson syndrome or toxic epidermal necrolysis, the levels of interleukin 6 (IL-6), IL-8, and monocyte chemoattractant protein 1 (MCP-1) were dramatically increased. We also reported that Stevens-Johnson syndrome or toxic epidermal necrolysis with severe ocular complications was associated with polymorphism of the prostaglandin E receptor 3 (EP3) gene (PTGER3). Prostanoids are a group of lipid mediators that form in response to various stimuli. They include prostaglandin D2 (PGD2), PGE2, PGF2α, PGI2, and thromboxane A2. There are 4 subtypes of the PGE receptor: EP1, EP2, EP3, and EP4. We previously reported that PGE2 suppresses polynosine–polyctydilic acid (polyI:C)-stimulated cytokine production via EP2 and/ or EP3 in human ocular surface epithelial cells. PolyI:C is a ligand of Toll-like receptor 3, which is strongly expressed in ocular surface epithelium. We found that PGE2 suppresses the production of IL-6, chemokine (C-X-C motif) ligand 10, chemokine (C-X-C motif) ligand 11, and chemokine (C-C motif) ligand 5 but not IL-8 by epithelial cells on the human ocular surface; it remains to be determined whether it also suppresses MCP-1 production. Monocyte chemoattractant protein 1 plays a significant role in the recruitment of monocytes and lymphocytes to the site of cellular immune reactions. In this study, we investigated whether PGE2 downregulates polyI:C-induced MCP-1 production.

All experiments were conducted in accordance with the principles set forth in the Declaration of Helsinki. Enzyme-linked immunosorbent assay and quantitative real-time polymerase chain reaction were performed with primary human conjunctival epithelial cells and immortalized human corneal limbal epithelial cells using previously described methods (eAppendix, http://www.archophthalmol.com).

First, we examined whether PGE2 downregulated the production and messenger RNA (mRNA) expression of MCP-1 induced by polyI:C stimulation in human conjunctival and corneal epithelial cells. We found that it significantly attenuated the production of MCP-1 (Figure A). Quantitative real-time polymerase chain reaction confirmed that the mRNA expression of MCP-1 was significantly downregulated by PGE2 (Figure, A).

Next, we examined which PGE-receptor(s) contributed to the downregulation of polyI:C-induced MCP-1. We used the EP2 agonist ONO-AE-259, the EP3 agonist ONO-AE-248, and the EP4 agonist ONO-AE-329. Enzyme-linked immunosorbent assay showed that the EP2 and EP3 agonists significantly suppressed the polyI:C-induced production of MCP-1, while the EP4 agonist did not exert suppression (Figure, B). Quantitative real-time polymerase chain reaction confirmed that the EP2 and EP3 agonists significantly downregulated the mRNA expression of MCP-1 (Figure, C). Thus, our results document that PGE2 attenuated the mRNA expression and production of MCP-1 via both EP2 and EP3.

In human macrophages, PGE2 attenuated the lipopolysaccharide-induced mRNA and protein expression of chemokines including MCP-1 through EP2. On the other hand, we demonstrated that in human ocular surface epithelial cells, PGE2 attenuated the polyI:C-induced mRNA and protein expression of MCP-1 through EP2 and EP3 but not EP4. Our findings suggest that EP2 and EP3 play important roles in the regulation of inflammation in epithelial cells, while EP2 and EP3 have important roles in immune cells such as macrophages.

In the tears and serum of patients with acute-stage Stevens-Johnson syndrome or toxic epidermal necrolysis, the levels of IL-6, IL-8, and MCP-1 were dramatically increased. Although IL-8 was not regulated by PGE2, IL-6 was regulated by PGE2 via EP3 in human ocular surface epithelial cells. Herein, we demonstrated that MCP-1 could be regulated by PGE2 via EP2 and EP3. The regulation of cytokine production by PGE2 may be associated with the pathogenesis of Stevens-Johnson syndrome or toxic epidermal necrolysis with severe ocular complications because it was associated with polymorphism of the EP3 gene (PTGER3), one of the PGE receptors (EP1, EP2, EP3, EP4). In summary, our results show that MCP-1 produced by human ocular surface epithelial cells could be downregulated by PGE2 via EP2 and EP3.

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Figure. Prostaglandin E₂ (PGE₂) attenuated the messenger RNA (mRNA) expression and production of monocyte chemoattractant protein 1 via both prostaglandin E receptor 2 (EP₂) and EP₃. A, Primary human conjunctival epithelial cells (PHCjE) and human corneal-limbal epithelial cells (HCLE) were exposed to 10 µg/mL of polyinosine–polycytidylic acid (polyI:C) and 100 µg/mL of PGE₂ for 24 hours (enzyme-linked immunosorbent assay) or 6 hours (quantitative real-time polymerase chain reaction). GAPDH indicates glyceraldehyde-3-phosphate dehydrogenase. B and C, The PHCjE and HCLE were exposed to 10 µg/mL of polyI:C and 10 µg/mL of the EP₂, EP₃, or EP₄ agonist for 24 hours (enzyme-linked immunosorbent assay) (B) or 6 hours (quantitative real-time polymerase chain reaction) (C). Data are representative of 3 separate experiments and are given as the mean (SEM) from 1 experiment carried out in 6 to 8 wells (enzyme-linked immunosorbent assay) (B) or 4 to 6 wells (quantitative real-time polymerase chain reaction) (C) per group. *P < .05; †P < .005; ‡P < .001.

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Methods. The Baron chamber used in our previous study\(^1\) was adapted to enable corneal buttons to be clamped in place and inflated (by pumping physiological saline into the posterior compartment) to restore their natural curvature. A button diameter of 8 mm or larger was deemed necessary to ensure tissue stability during this process.

The next step, obtaining fresh, full-thickness, keratoconus buttons of sufficient diameter, proved to be problematic owing to the increasing popularity of deep anterior lamellar keratoplasty. Recently, however, the opportunity arose to examine an 8-mm full-thickness (300-340 \(\mu\)m minus epithelium) keratoconus corneal button with some central scarring and a mean power greater than 51.8 diopters (Figure 1). The tissue was obtained in accordance with the tenets of the Declaration of Helsinki and with full informed consent from a 31-year-old patient at the time of penetrating keratoplasty. Using techniques detailed previously,\(^1\) the corneal button was clamped in the chamber and inflated. The central 6.3-mm region of the button was then flattened by the application of a single cut was made at a depth of 150 \(\mu\)m from the surface using an IntraLase 60-kHz femtosecond laser (Abbott Medical Optics Inc),\(^1\) thus splitting the cornea into anterior and posterior sections of roughly equal thickness. Wide-angle x-ray scattering patterns were collected at 0.25-mm intervals over each corneal section.

Figure 1. Corneal topography of the keratoconus cornea (recorded 12 years previously).\(^2\) The broken lines show the 6.3-mm region of the cornea cut with the femtosecond laser (circle) and the region of greatest corneal steepening depicted in Figure 2 (rectangle).

Figure 2. Collagen orientation in the normal (A) and keratoconus (B) posterior stroma (central 6.3 mm). The highlighted regions of the posterior (C and D) and anterior (E and F) stroma are expanded. Large vector plots showing high collagen alignment are downsized (key).