RESEARCH LETTERS

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.1 Although IL-8 was not regulated by PGE2, IL-6 was regulated by PGE2 via EP2 in human ocular surface epithelial cells.5,6 In human macrophages, PGE2 attenuated the lipopolysaccharide-induced mRNA and protein expression of chemokines including 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.1 Although IL-8 was not regulated by PGE2, IL-6 was regulated by PGE2 via EP3 in human ocular surface epithelial cells.5 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 EP2 receptor (PTGER2) gene.2 In summary, our results show that MCP-1 produced by human ocular surface epithelial cells could be downregulated by PGE2 via EP2 and EP3.

Next, we examined which PGE2 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.

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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.

**Funding/Support:** This work was supported in part by grants-in-aid for scientific research from the Japanese Ministry of Health, Labour, and Welfare, the Japanese Ministry of Education, Culture, Sports, Science, and Technology, the Kyoto Foundation for the Promotion of Medical Science, the National Institute of Biomedical Innovation of Japan, the Intramural Research Fund of Kyoto Prefectural University of Medicine, and the
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 µm 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 cone and a single cut was made at a depth of 150 µm 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 cor-

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**Table 1.**

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<tr>
<th>Depth Profile Study of Abnormal Collagen Orientation in Keratoconus Corneas</th>
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<tr>
<td><strong>Normal</strong></td>
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<td>200-400 µm</td>
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<td>400-600 µm</td>
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**Figure 1.** Corneal topography of the keratoconus cornea (recorded 12 years previously). 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).