

Downregulation of Matrix Metalloproteinase-2 in Corneal Fibroblasts by Interleukin-1 Receptor Antagonist Released from Corneal Epithelial Cells

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PURPOSE. The authors previously showed that the expression of various junctional proteins in corneal epithelial cells or corneal fibroblasts (CFs) is regulated by the presence of the other cell type in a coculture system. In this study, the effect of corneal epithelial cells on the expression of matrix metalloproteinases (MMPs) in CFs was studied.

METHODS. Human CFs and simian virus 40-transformed human corneal epithelial (HCE) cells were cultured on opposite sides of a collagen vitrigel membrane. Expression of MMPs in CFs was examined by reverse transcription-polymerase chain reaction and immunoblot analyses.

RESULTS. The amounts of MMP-2 mRNA and protein in CFs were decreased by the presence of HCE cells. HCE cells had no effect on the expression of MMP-1 in CFs. HCE cells released interleukin (IL)-1 receptor antagonist (IL-1RA) into the culture medium, and depletion of IL-1RA in HCE cells by RNA interference largely abolished the effect of these cells on MMP-2 expression in CFs. The downregulation of MMP-2 expression in CFs by HCE cells was blocked by an inhibitor of signaling by the mitogen-activated protein kinase (MAPK) ERK (PD98059) but was not affected by those of signaling by the MAPKs p38 (SB203580) or JNK (SP600125).

CONCLUSIONS. Corneal epithelial cells downregulated expression of MMP-2 in CFs in a manner dependent at least in part on the release of IL-1RA from the former cells. This effect might contribute to the attenuation of corneal stromal remodeling by corneal epithelial cells. (*Invest Ophthalmol Vis Sci.* 2010;51:6286–6293) DOI:10.1167/iovs.09-4753

Keratocytes, also known as corneal fibroblasts (CFs), synthesize and secrete collagen, proteoglycans, and other proteins of the extracellular matrix (ECM), and they therefore play an important role in maintenance of metabolic and physiological homeostasis of the cornea.^{1,2} During the contraction phase of wound healing, CFs transform into myofibroblasts expressing α -smooth muscle actin, which confers contractile properties on the cells.^{3,4} Myofibroblasts produce large amounts of ECM proteins and participate in ECM remodeling by releasing matrix metalloproteinases (MMPs).^{5–7} Spontaneous fibroblast activation and transformation into myofibro-

blasts during corneal wound healing result in the deposition of opaque scar tissue that can interfere with vision.⁸

The process of corneal remodeling after wounding must be tightly regulated to restore the collagen fibrillar structure of the stroma, limit scarring, and reestablish corneal transparency. The secretion of MMPs by CFs or myofibroblasts is central to this process, with the production of these enzymes being regulated at both transcriptional and posttranscriptional levels. Matrix turnover is limited in the resting cornea. Among MMPs, only the gelatinase MMP-2 is constitutively expressed by keratocytes,^{9,10} and the stromelysin MMP-7 has been localized to the epithelium of the resting cornea.¹¹ However, the collagenase MMP-1, the gelatinase MMP-9, and the stromelysin MMP-3 have also been detected in the cornea after injury.¹² Corneal epithelial cells primarily secrete MMP-9 whereas keratocytes primarily secrete MMP-2 after corneal wounding.¹³ The activity of MMP-2 declines slowly during wound healing, suggesting that such activity plays a role in long-term remodeling of the primary matrix.¹⁴ Excessive MMP activity in the cornea is implicated in the pathology of corneal ulceration.¹⁵ The deposition of scar tissue and corneal thinning in individuals with keratoconus have also been associated with increased MMP-2 activity.¹⁶

In the clinical setting, patients are often encountered in whom loss of the corneal epithelium or a delay in the resurfacing of corneal epithelial defects results in the activation of underlying fibroblasts in the stroma. These clinical observations suggest that epithelial-mesenchymal interaction is important for maintenance of the normal structure and functions of the cornea. We recently established an experimental model in which corneal epithelial cells and fibroblasts are cultured on opposite sides of a collagen vitrigel membrane.^{17–19} With this model, we showed that the presence of CFs upregulated the expression of the tight-junction proteins ZO-1, occludin, and claudin in corneal epithelial cells, and that the presence of corneal epithelial cells upregulated the expression of the gap-junction protein connexin 43 (Cx43) and the adherens-junction protein N-cadherin in CFs, which suggests that interactions between the two cell types play an important role in the maintenance of corneal homeostasis.

With regard to the mediators of such functional interactions between epithelial cells and fibroblasts in the cornea, three patterns of expression of the genes for various cytokines, growth factors, and their receptors have been identified in corneal epithelial cells and fibroblasts.²⁰ The environment of cell culture has much in common with that of healing wounds, suggesting that coculture of corneal epithelial cells and fibroblasts might also be a useful model for characterization of molecular interactions that regulate the repair of corneal injury. Cytokines have been implicated as important mediators of inflammation and tissue damage in the cornea.^{21–26} A large number of agents that regulate collagenase synthesis by cells in culture has also been identified.²⁷ Furthermore, interleukin

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(IL)-1 receptor antagonist (IL-1RA) has been shown to antagonize IL-1-stimulated collagenase synthesis.^{28,29}

We have now investigated the physiological relevance of epithelial-mesenchymal interaction in the cornea by examining the effect of corneal epithelial cells on MMP expression in CFs in our coculture system. Our results suggest that corneal epithelial cells downregulate MMP-2 production by CFs and that this effect is mediated, at least in part, through IL-1RA released from the epithelial cells.

METHODS

Antibodies and Reagents

Mouse monoclonal antibodies to MMP-1 and to MMP-2 were obtained from Daiichi Fine Chemical (Toyama, Japan), goat polyclonal antibodies to IL-1RA were from R&D Systems (Minneapolis, MN) and those to Cx43 were from Chemicon (Billerica, MA), and mouse monoclonal antibodies to α -tubulin were from Sigma (St. Louis, MO). Horseradish peroxidase-conjugated secondary antibodies were obtained from Promega (Madison, WI). A small interfering RNA (siRNA) specific for human IL-1RA mRNA and a transfection reagent were obtained from Ambion (siPORT *NeoFX*; Foster City, CA). PD98059, SB203580, and SP600125 were from Calbiochem (San Diego, CA).

Culture of Human CFs

Human CFs were prepared from the tissue remaining after corneal transplantation surgery and were cultured as described previously.³⁰ In brief, human corneas were obtained from Mid-America Transplant Service (St. Louis, MO), Northwest Lions Eye Bank (Seattle, WA), or the Eye Bank of Wisconsin (Madison, WI). The donors were white males and females ranging in age from 4 to 65 years. The cells prepared from each cornea were maintained separately in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS), and they were passaged after they had achieved ~90% confluence. Cells in the third to seventh passages were used for the experiments described in the present study. The purity of the cell cultures was assessed on the basis of both the distinctive morphology of human CFs and their reactivity with antibodies to vimentin in immunofluorescence analysis. All cells were positive for vimentin and negative for cytokeratin, indicative of the absence of contamination by epithelial cells. The human tissue was used in strict accordance with the Declaration of Helsinki.

HCE Cell Culture

Simian virus 40-transformed human corneal epithelial (HCE) cells³¹ were obtained from RIKEN Biosource Center (Tsukuba, Japan). The cells were maintained in supplemented hormonal epithelial medium, which comprises DMEM-F12 (50:50, v/v) supplemented with 15%

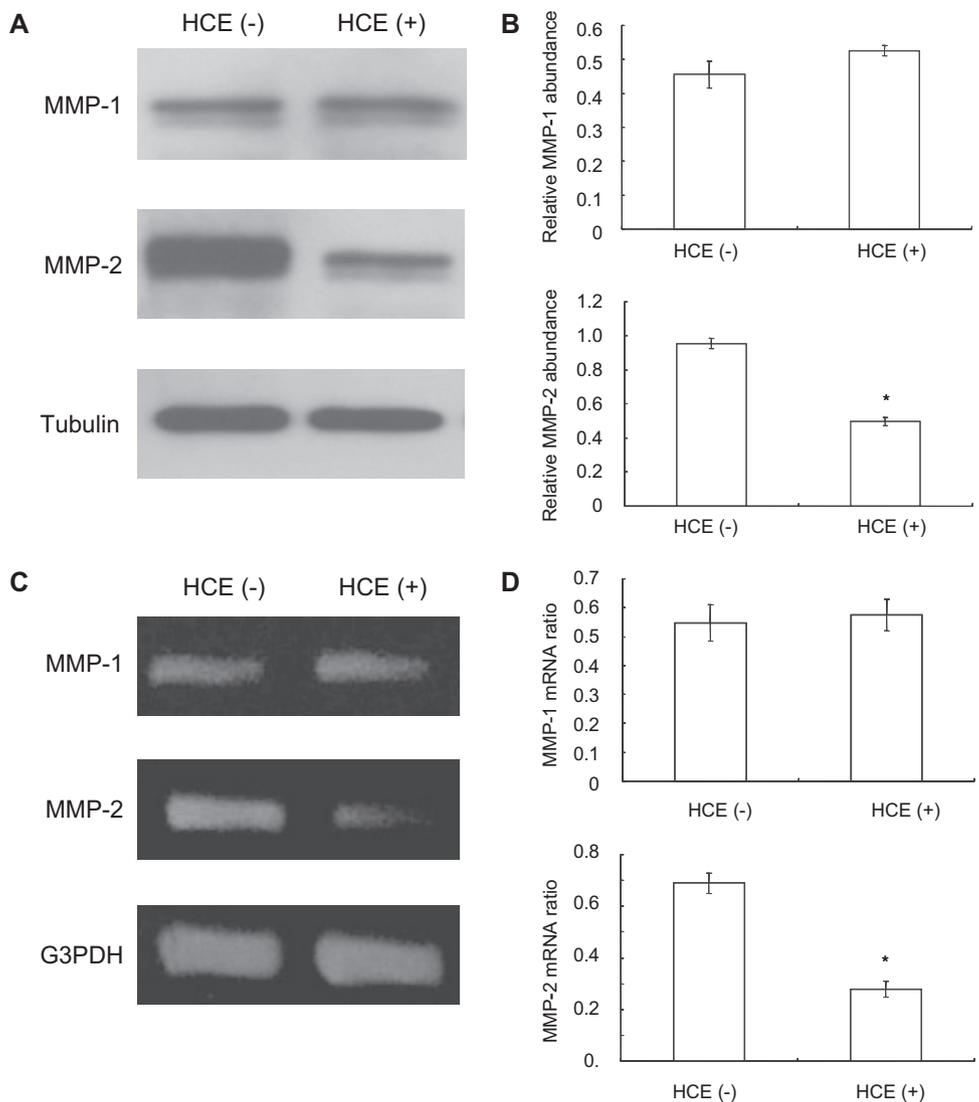


FIGURE 1. Expression of MMPs in CFs cultured with or without HCE cells. (A) Lysates of CFs cultured for 24 hours on a collagen vitrigel membrane in the absence or presence of HCE cells were subjected to immunoblot analysis with antibodies to MMP-1, MMP-2, and α -tubulin (loading control). (B) The abundance of MMP-1 and MMP-2 in CFs in experiments similar to that shown in (A) was quantified by densitometric scanning of immunoblots and normalized by the corresponding amount of α -tubulin. (C) Total RNA prepared from CFs cultured for 12 hours on a collagen vitrigel membrane in the absence or presence of HCE cells was subjected to RT-PCR analysis of MMP-1, MMP-2, and G3PDH mRNAs. (D) The abundance of MMP-1 and MMP-2 mRNAs in CFs in experiments similar to that shown in (C) was quantified by RT and real-time PCR analysis and normalized by the corresponding amount of G3PDH mRNA. Data in (B) and (D) are mean \pm SE from three separate experiments. * $P < 0.05$ versus the corresponding value for CFs incubated in the absence of HCE cells (Student's *t*-test).

heat-inactivated FBS, bovine insulin (5 $\mu\text{g}/\text{mL}$), cholera toxin (0.1 $\mu\text{g}/\text{mL}$), recombinant human epidermal growth factor (10 ng/mL), and gentamicin (40 $\mu\text{g}/\text{mL}$).

Coculture of HCE Cells and CFs on a Collagen Membrane

CFs (1×10^5 , unless indicated otherwise) were seeded on a collagen vitrigel membrane (Asahi Technoglass, Tokyo, Japan) in DMEM supplemented with 10% heat-inactivated FBS. After 24 hours, the membrane was inverted in another dish and HCE cells (2×10^5 , unless indicated otherwise), also in DMEM supplemented with 10% heat-inactivated FBS, were seeded on the empty side. After 6 to 8 hours, the membrane was again turned upside down, this time in a dish containing DMEM supplemented with 0.5% heat-inactivated FBS. The cells were then cultured at 37°C in a humidified incubator containing 5% CO_2 . As a control, CFs were seeded on a vitrigel membrane without HCE cells.

RT-PCR Analysis

Total RNA was isolated from CFs on a collagen vitrigel membrane or from HCE cells in a 30-mm dish with the use of a kit (RNeasy; Qiagen, Valencia, CA), and portions (0.5 μg) of the RNA were subjected to reverse transcription (RT) and polymerase chain reaction (PCR) analysis with a kit based on the Platinum *Taq* system (One-Step; Invitrogen, Carlsbad, CA). The PCR protocol was designed to maintain amplification in the exponential phase. The sequences of the PCR primers (sense and antisense, respectively) were 5'-AGAATTTTGACTCTCCAGAAAGC-3' and 5'-AGTTATCCCTTGCTATCCAG-3' for MMP-1, 5'-GTTCAATTGGCGGACTGTG-3' and 5'-TCACGCTCTTCAGACTTTGG-3' for MMP-2, 5'-GCCTCATGCTCTGTTCTTGG-3' and 5'-GGTTCCTGTGACGCCCTTCTG-3' for IL-1RA, and 5'-ACCAGATCCAGCCATCAC-3' and 5'-TCCACCACCCTGTTGCTGTA-3' for glyceraldehyde-3-phosphate dehydrogenase (G3PDH, internal control). The RT and PCR incubations were performed with a thermal cycler system (GeneAmp 2400-R; Perkin-Elmer, Foster City, CA). RT was performed at 50°C for 30 minutes, and PCR was performed for 25 cycles, with each cycle comprising incubations at 94°C for 2 minutes, 58°C for 30 seconds, and 72°C for 1 minute. The reaction mixture was then cooled to 4°C. The products of amplification were fractionated by electrophoresis on a 1.5% agarose gel and were stained with ethidium bromide. For RT and real-time PCR analysis, total RNA was subjected to RT with a kit (Promega), and the resultant cDNA was subjected to real-time PCR analysis by rapid cycling in glass capillaries with the use of a thermocycler (Light-Cycler; Roche Molecular Biochemicals, Indianapolis, IN).

Immunoblot Analysis

CFs on a collagen vitrigel membrane or HCE cells in a 30-mm dish were washed twice with phosphate-buffered saline and lysed in 200 μL of a solution containing 150 mM NaCl, 2% SDS, 5 mM EDTA, and 20 mM Tris-HCl (pH 7.5). Cell lysates were fractionated by SDS-polyacrylamide gel electrophoresis, and the separated proteins were transferred to a nitrocellulose membrane and exposed consecutively to primary antibodies and horseradish peroxidase-conjugated secondary antibodies. Immune complexes were detected with enhanced chemiluminescence reagents (GE Healthcare UK, Little Chalfont, UK). Band intensities in the linear range were quantitated by densitometric scanning of film with the use of an image analyzer [Multi Gauge V3 (2) software; Fuji Film, Tokyo, Japan].

Cell Transfection

HCE cells (2×10^5) were seeded in 30-mm dishes and cultured for 24 hours to 50% to 60% confluence. An siRNA specific for human IL-1RA mRNA (1 μM) was mixed with 5 μL of transfection reagent (siPORT *NeoFX*) and then diluted with 200 μL of multi-purpose medium (OPTI-MEM; Invitrogen). After incubation for 10 minutes at room temperature, the mixture was added to the culture dishes, and the cells were

cultured for various times. Given that the depletion of IL-1RA was most pronounced after transfection for 24 hours, the siRNA-treated HCE cells were cocultured with CFs after this time.

IL-1RA Assay

Culture supernatants were assayed for IL-1RA with the use of a bead-based assay (Bio-Plex system; Bio-Rad, Hercules, CA).

Statistical Analysis

Quantitative data are presented as mean \pm SE from three independent experiments and were analyzed by Student's *t*-test, the Tukey-Kramer test, or Dunnett's test. A *P* value of <0.05 was considered statistically significant.

RESULTS

Effect of HCE Cells on the Expression of MMPs in CFs

We first investigated the effect of the presence of HCE cells on the expression of MMPs in CFs. Immunoblot analysis revealed that the amount of MMP-2 in CFs cultured for 24 hours in the presence of HCE cells was markedly reduced compared with that in those cultured without HCE cells (Figs. 1A, 1B). RT-PCR as well as RT and real-time PCR analyses showed that the abundance of MMP-2 mRNA in CFs cultured for 12 hours in the presence of HCE cells was similarly decreased compared with that in those cultured alone (Figs. 1C, 1D). In contrast, the presence of HCE cells had no effect on the expression of MMP-1 in CFs at the mRNA or protein level (Fig. 1). Examination of the time course of the effect of HCE cells on the expression of MMP-2 in CFs revealed that the decrease in the abundance of MMP-2 protein was similarly apparent at 24 and 48 hours (Fig. 2A), whereas that in the amount of MMP-2 mRNA was apparent at 12 to 24 hours (Fig. 2B).

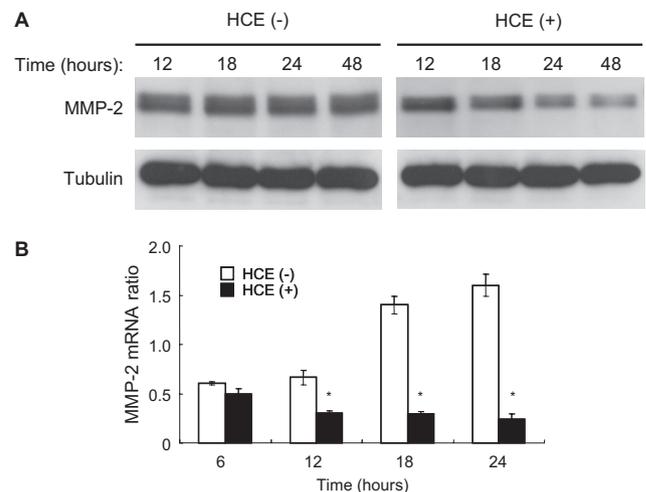


FIGURE 2. Time course of the effect of HCE cells on the expression of MMP-2 in CFs. (A) Lysates of CFs cultured for 12 to 48 hours on a collagen vitrigel membrane in the absence or presence of HCE cells were subjected to immunoblot analysis with antibodies to MMP-2 and to α -tubulin. Data are representative of three independent experiments. (B) Total RNA prepared from CFs cultured for 6 to 24 hours on a collagen vitrigel membrane in the absence or presence of HCE cells was subjected to RT and real-time PCR analysis of MMP-2 mRNA. Data were normalized by the corresponding amount of G3PDH mRNA and are mean \pm SE from three separate experiments. **P* < 0.05 versus the corresponding value for CFs incubated in the absence of HCE cells (Student's *t*-test).

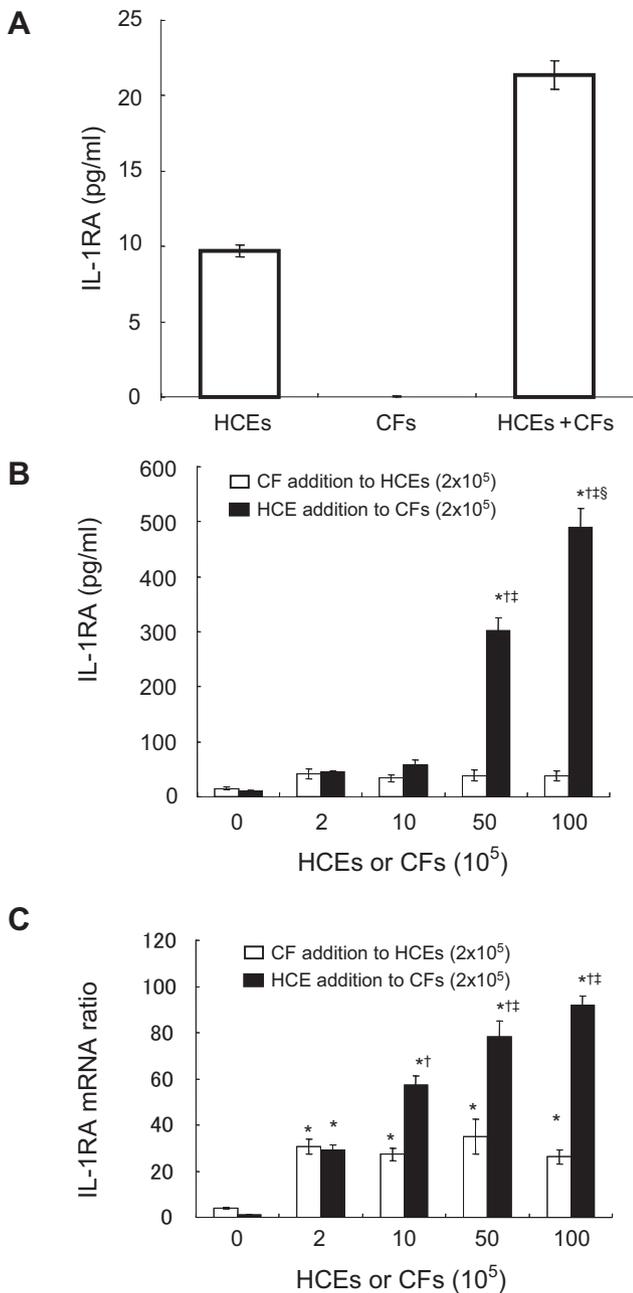


FIGURE 3. Release of IL-1RA in cultures of HCE cells and CFs. (A) HCE cells (2×10^5) or CFs (1×10^5) were cultured alone or together on a collagen vitrigel membrane for 24 hours, after which the concentration of IL-1RA in culture supernatants was assayed with the use of the Bio-Plex system. (B) HCE cells (2×10^5) or CFs (2×10^5) were cultured together with the indicated number of cells of the other cell type on a collagen vitrigel membrane for 24 hours, after which the concentration of IL-1RA in culture supernatants was determined. (C) HCE cells or CFs were cultured as in (B) for 12 hours, after which total RNA was isolated from all cells and subjected to RT and real-time PCR analysis of IL-1RA mRNA; data were normalized by the corresponding amount of G3PDH mRNA. Data in (B) and (C) are mean \pm SE from three separate experiments. * $P < 0.05$ versus the corresponding value for HCEs or CFs cultured alone; † $P < 0.05$ versus the corresponding value for the addition of 2×10^5 cells; ‡ $P < 0.05$ versus the corresponding value for the addition of 10×10^5 cells; § $P < 0.05$ versus the corresponding value for the addition of 50×10^5 cells (Tukey-Kramer test).

Role of IL-1RA in the Downregulation of MMP-2 Expression in CFs by HCE Cells

IL-1RA has been shown to inhibit collagenase synthesis in human monocytes.^{28,29} To examine whether IL-1RA might be responsible for the downregulation of MMP-2 expression in CFs by HCE cells, we first assayed the culture supernatants of HCE cells and CFs incubated alone or together on a collagen vitrigel membrane for IL-1RA. IL-1RA was detected in the culture supernatants of HCE cells incubated alone but not in those of CFs. Moreover, the amount of IL-1RA released by cocultures of the two cell types was greater than that released by HCE cells cultured alone (Fig. 3A). Further investigation revealed, however, that the amount of IL-1RA released by cocultures of HCE cells and CFs was highly dependent on HCE cell number but was largely independent of the number of CFs (Fig. 3B). RT and real-time PCR analysis also showed that the abundance of IL-1RA mRNA in cocultures was dependent on HCE cell number to a much greater extent than on the number of CFs (Fig.

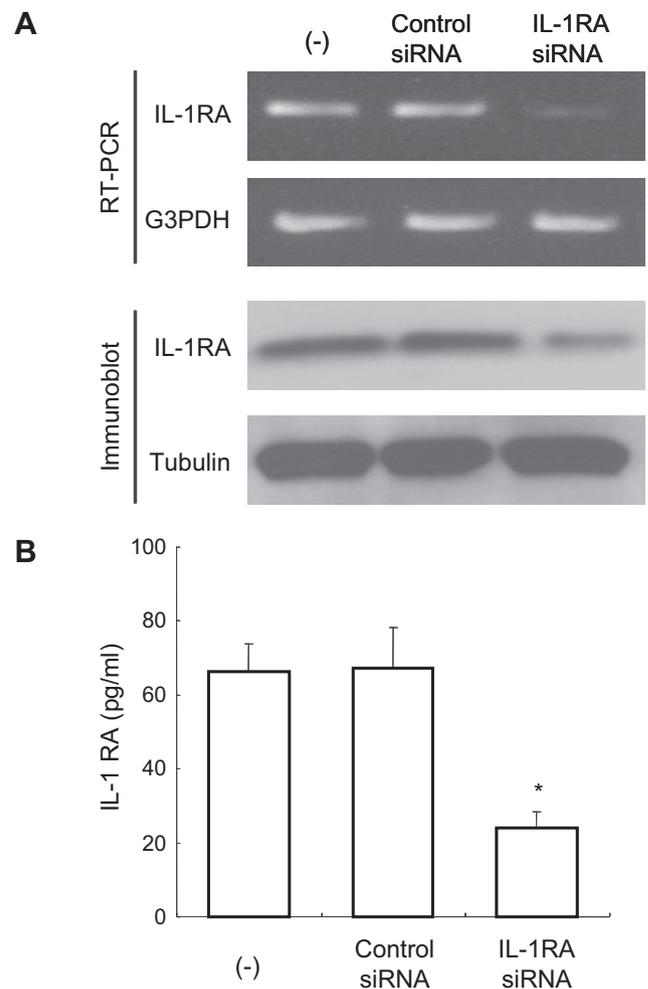


FIGURE 4. Depletion of IL-1RA in HCE cells by RNAi. (A) HCE cells were transfected [or not (-)] with an siRNA specific for IL-1RA mRNA or with a corresponding scrambled (control) siRNA for 24 hours. The cells were then subjected to RT-PCR analysis of IL-1RA and G3PDH mRNAs (top) or to immunoblot analysis with antibodies to IL-1RA and to α -tubulin (bottom). (B) HCE cells (2×10^5) in a 30-mm dish were transfected for 24 hours as in (A), after which the concentration of IL-1RA in the culture medium was determined with the Bio-Plex system. Data are mean \pm SE from three separate experiments. * $P < 0.05$ versus the value for nontransfected cells or for cells transfected with the control siRNA (Dunnett's test).

3C). These results thus suggested that IL-1RA released by cocultures of HCE cells and CFs was largely derived from the HCE cells.

We next determined whether depletion of IL-1RA in HCE cells by RNA interference (RNAi) might influence the effect of these cells on MMP-2 expression in CFs. Examination of the time course of IL-1RA depletion in HCE cells revealed that the effect of the IL-1RA siRNA was most pronounced after transfection for 24 hours (data not shown). RT-PCR and immunoblot analyses of the transfected cells at this time revealed that the amounts of IL-1RA mRNA and protein were reduced by 60 to 70% (Fig. 4A). A similar reduction was apparent in the amount of IL-1RA released from the transfected cells into the culture medium (Fig. 4B). Transfection of HCE cells

with the IL-1RA siRNA for 24 hours before coculture with CFs largely abolished the inhibitory effect of the HCE cells on the expression of MMP-2 in CFs at both the mRNA and protein levels (Fig. 5). The expression of MMP-1 by CFs was not affected by HCE cells transfected with the IL-1RA siRNA. These results thus indicated that IL-1RA derived from HCE cells mediates the inhibitory effect of these cells on MMP-2 expression in CFs.

Role of MAPK Signaling in HCE Cell-Induced Downregulation of MMP-2 in CFs

Finally, we investigated the signaling pathways responsible for the downregulation of MMP-2 expression in CFs by HCE cells. CFs were cultured for 12 hours on a collagen vitrigel mem-

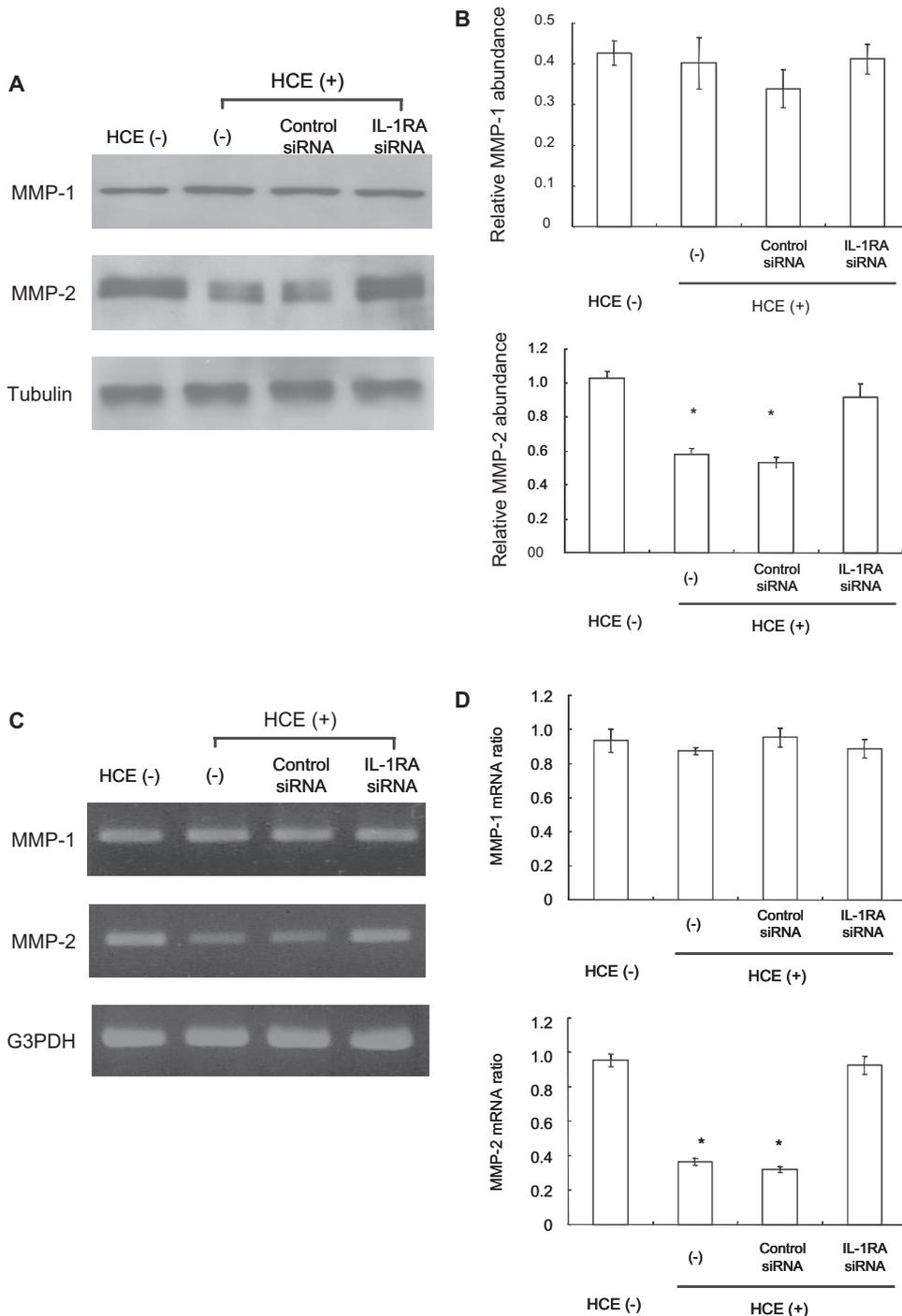


FIGURE 5. Attenuation of the inhibitory effect of HCE cells on MMP-2 expression in CFs by RNAi-mediated depletion of IL-1RA. (A) CFs were incubated on a vitrigel membrane for 24 hours in the absence or presence of HCE cells that had been transfected (or not) for 24 hours with either an siRNA specific for IL-1RA mRNA or a corresponding scrambled siRNA. Lysates of the CFs were then subjected to immunoblot analysis with antibodies MMP-1, MMP-2, and α -tubulin. (B) The abundance of MMP-1 and MMP-2 in CFs in experiments similar to that shown in (A) was quantified by densitometric scanning of immunoblots and normalized by the corresponding amount of α -tubulin. (C) CFs cultured for 12 hours as in (A) were subjected to RT-PCR analysis of MMP-1, MMP-2, and G3PDH mRNAs. (D) The abundance of MMP-1 and MMP-2 mRNAs in CFs in experiments similar to that shown in (C) was quantified by RT and real-time PCR analysis and normalized by the corresponding amount of G3PDH mRNA. Data in (B) and (D) are mean \pm SE from three separate experiments. * $P < 0.05$ versus the corresponding value for CFs incubated in the presence of HCE cells transfected with the IL-1RA siRNA (Dunnett's test).

brane in the absence or presence of HCE cells and were then exposed for 12 hours to 10 μ M PD98059, 10 μ M SB203580, or 10 μ M SP600125, which are inhibitors of signaling by the mitogen-activated protein kinases (MAPKs) ERK (extracellular signal-regulated kinase), p38, and JNK (c-Jun NH₂-terminal kinase), respectively. The inhibitor concentrations were selected on the basis of results obtained in a previous study with this coculture system¹⁸ and in preliminary experiments. Immunoblot analysis revealed that the HCE cell-induced decrease in the amount of MMP-2 in CFs was blocked by PD98059 but not by SB203580 or SP600125 (Fig. 6). As a control, we also found that the upregulation of Cx43 expression in CFs by HCE cells was blocked by PD98059 and SB203580, consistent with our previous observations.¹⁸

DISCUSSION

With the use of a coculture system based on a collagen vitrigel membrane, we have shown that the presence of HCE cells resulted in downregulation of MMP-2 expression at both the protein and mRNA levels in human CFs. Depletion of IL-1RA in HCE cells by RNAi largely abolished the inhibitory effect of these cells on MMP-2 expression in CFs. These results indicate that IL-1RA released from HCE cells is responsible, at least in part, for the downregulation of MMP-2 expression in CFs. Furthermore, the inhibitory effect of HCE cells on MMP-2 expression in CFs was blocked by PD98059, an inhibitor of signaling by the MAPK ERK, but it was not affected by SB203580 or SP600125, inhibitors of signaling by the MAPKs p38 and JNK, respectively.

The downregulation of MMP-2 expression in CFs by HCE cells was not accompanied by a similar effect on the expression of MMP-1, suggesting that the effect of HCE cells on MMP-2 expression is specific. MMP-2 preferentially catalyzes the degradation of collagen type IV, a component of the corneal epithelial basement membrane, rather than that of collagen type I, the principal ECM component of the corneal stroma. Interaction of the intact corneal epithelium with underlying CFs might thus play an important role in corneal homeostasis.

The collagen vitrigel system for cell culture is based on a membrane produced from collagen type I.³² We applied this system to the coculture of HCE cells and CFs, with the two

cell types being cultured on opposite sides of the membrane. Given that the epithelial cells and fibroblasts do not come into direct contact with each other in this system, it is likely that a factor (or factors) released by the epithelial cells is responsible for the downregulation of MMP-2 in the fibroblasts. We found that HCE cells secreted IL-1RA and that RNAi-mediated depletion of IL-1RA in HCE cells largely prevented the inhibitory effect of these cells on MMP-2 expression in CFs. These results thus implicate IL-1RA released by HCE cells as a mediator of the downregulation by these cells of MMP-2 expression in CFs.

In the normal cornea, MMP-2 is present at only low levels and in the form of the proenzyme.^{9,33,34} After injury or in response to the release of cytokines, the expression or activity of several MMPs is upregulated in the cornea.³⁵ MMP-9 may also be released from the secretory granules of neutrophils recruited in response to inflammation.³⁶ MMP-1, -2, -3, and -9 participate in corneal epithelial repair and stromal remodeling.^{33,37} Although their mechanisms of action are uncertain, MMP-1 is required for the migration of corneal epithelial cells over a matrix of collagen type I *in vitro*.³⁸ The subepithelial expression of MMP-9 also parallels degradation of the epithelial basement membrane in the cornea,^{39,40} whereas MMP-2 and MMP-3 produced by stromal fibroblasts may contribute to long-term stromal remodeling and basement membrane synthesis.⁹

We have shown previously that the presence of HCE cells upregulated the expression of the gap-junction protein Cx43¹⁸ as well as that of the adherens-junction protein N-cadherin¹⁹ in CFs, which suggests that HCE cells play an important role in the maintenance of stromal function. These effects of HCE cells on Cx43 and N-cadherin expression were found to be mediated in part by insulin-like growth factor-1 released from the epithelial cells, whereas the downregulation of MMP-2 expression in CFs observed in the present study appears to be attributable, at least in part, to IL-1RA released by the HCE cells.

We found that inhibition of signaling by the MAPK ERK, but not that of signaling by p38 or JNK, blocked the downregulation of MMP-2 in CFs by HCE cells. We previously found that both ERK and p38 participate in the HCE cell-induced upregulation of Cx43 in CFs.¹⁸

IL-1RA has been shown to protect limbal epithelial cells from apoptosis.⁴¹ Upregulation of IL-1RA is also thought to

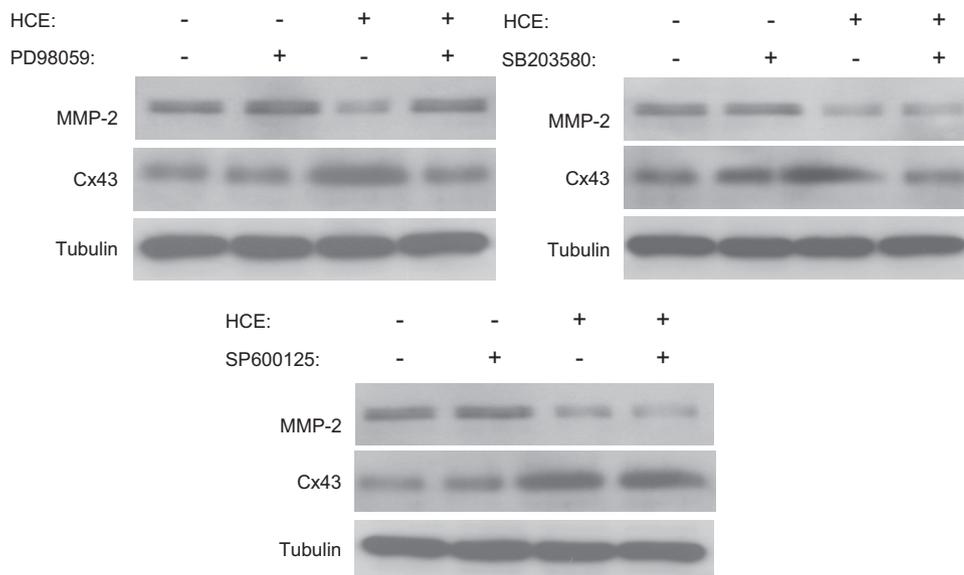


FIGURE 6. Effects of MAPK inhibitors on the HCE cell-induced downregulation of MMP-2 expression in CFs. CFs were cultured on a collagen vitrigel membrane in the absence or presence of HCE cells for 12 hours before exposure to either 10 μ M PD98059, 10 μ M SB203580, or 10 μ M SP600125 for 12 hours, as indicated. Lysates of the CFs were then subjected to immunoblot analysis with antibodies to MMP-2, Cx43, and α -tubulin.

attenuate inflammation in part by blocking the action of IL- β .^{42,43} Our results now indicate that IL-1RA-induced down-regulation of MMP-2 expression in CFs might contribute to inhibition of corneal inflammation or attenuation of stromal remodeling during wound healing. Reestablishment of a mature corneal epithelium may serve as a signal for termination of corneal wound healing, including cessation of the production of MMPs by CFs. Clinical evidence that the persistence of corneal epithelial defects can lead to corneal ulceration as the stromal remodeling process continues in an uncontrolled manner is consistent with this notion. Our demonstration that IL-1RA derived from corneal epithelial cells inhibits the production of MMP-2 by CFs also suggests that IL-1RA or a related molecule may prove effective for the treatment of corneal ulceration.

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