Inhibitory Effect of Triptolide on Chemokine Expression Induced by Proinflammatory Cytokines in Human Corneal Fibroblasts

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PURPOSE. Synthesis of various chemokines, including interleukin (IL)-8 and monocyte chemotactic protein (MCP)-1, as well as the surface expression of intercellular adhesion molecule (ICAM)-1 in corneal fibroblasts contribute to corneal inflammation. The effects of triptolide, the major constituent of extracts of the herb Tripterygium wilfordii hook f., on the expression of these proteins in human corneal fibroblasts were examined in comparison with those of dexamethasone.

METHODS. The release of IL-8 and MCP-1 from and the surface expression of ICAM-1 on cultured corneal fibroblasts were measured with enzyme-linked immunosorbent assays. The cellular abundance of the mRNAs for these proteins was determined by reverse transcription and real-time polymerase chain reaction analysis. The activities of the transcription factors NF-κB and AP-1 were assessed by cell transfection with secretory alkaline phosphatase reporter genes.

RESULTS. Both triptolide and dexamethasone inhibited in a dose-dependent manner the expression of IL-8 and MCP-1 in corneal fibroblasts induced by the proinflammatory cytokines IL-1β or tumor necrosis factor (TNF)-α. These inhibitory effects were apparent at both the mRNA and protein levels. Both compounds also had a lesser inhibitory effect on cytokine-induced ICAM-1 expression. The activation of NF-κB by IL-1β was markedly inhibited by both triptolide and dexamethasone, whereas the activity of AP-1 was not affected by either agent.

CONCLUSIONS. Like dexamethasone, triptolide inhibited IL-8 and MCP-1 expression in cultured human corneal fibroblasts exposed to proinflammatory cytokines, an action most likely mediated by inhibition of NF-κB activation. Similar effects of triptolide in vivo may be expected to limit the infiltration of neutrophils and monocytes into the cornea. (Invest Ophthalmmol Vis Sci. 2005;46:2346–2352) DOI:10.1167/iovs.05-0010

The cornea is a transparent, avascular tissue. Under normal conditions, fibroblasts are the major cellular constituent of the corneal stroma. However, the stroma of individuals with corneal ulcer also contains many infiltrated immune cells, such as neutrophils and monocytes.1 Tissue infiltration by such immune cells is triggered by chemokines, a family of low-molecular-weight cytokines that attract and activate leukocytes. Corneal fibroblasts probably contribute to the local accumulation and activation of leukocytes in the cornea through their production of chemokines such as interleukin (IL)-8 and monocyte chemotactic protein (MCP)-1, which are potent and specific chemoattractants for neutrophils and monocytes, respectively, and play an important role in infectious inflammation.2,3 The concentration of IL-8 in tear fluid is increased in individuals with corneal inflammatory diseases and associated corneal damage, and both the number of neutrophils and the concentration of IL-8 in tear fluid correlate with the severity of corneal damage.4 Expression by corneal fibroblasts of intercellular adhesion molecule (ICAM)-1, a specialized cell surface glycoprotein that binds leukocyte function-associated antigen-1 present on all leukocytes and enhances immune responses, also contributes to inflammatory corneal diseases.5 It is therefore important to characterize regulation of the expression of IL-8, MCP-1, and ICAM-1 in corneal fibroblasts to gain insight into the mechanisms that underlie the local accumulation of leukocytes in the cornea and into the pathophysiology of corneal inflammatory diseases.

Steroid administration is effective in the treatment of some cases of corneal inflammatory disease. Steroids affect the functions of both immune cells and resident corneal cells. However, their use is associated with adverse ocular effects, such as recurrence of infection, increased intraocular pressure, and the development of posterior subcapsular cataract,6 as well as with systemic complications such as osteoporosis.7 Extracts of the Chinese herb Tripterygium wilfordii hook f., the major constituent of which is the diterpene triepoxide triptolide, have been used in traditional Chinese medicine for the treatment of rheumatoid arthritis.8 Triptolide exhibits anti-inflammatory activity with immune cells such as T cells, B cells, and monocytes,8,9 it also acts on tissue-resident cells such as epithelial cells10 and fibroblasts,11 inhibiting the expression of IL-8 in bronchial epithelial cells.10 We recently showed that triptolide inhibits collagen degradation by corneal fibroblasts.12 In the present study, we examined the possible effects of triptolide, in comparison with those of dexamethasone, both on the expression of IL-8, MCP-1, and ICAM-1 and on the activities of the transcription factors nuclear factor (NF)-κB and activator protein (AP)-1 in corneal fibroblasts stimulated with the proinflammatory cytokines IL-1β or tumor necrosis factor (TNF)-α.

METHODS

Materials

Eagle’s minimum essential medium (MEM), modified Eagle’s MEM (Opti-MEM), fetal bovine serum (FBS), and trypsin-EDTA were obtained from Invitrogen-Gibco (Carlsbad, CA); cell culture dishes and 24- and 96-well culture plates from Corning-Costar (Corning, NY); collagenase,
displace, and dexamethasone from Sigma-Aldrich (St. Louis, MO); trip-
tolide from Alexis Biochemicals (Carlsbad, CA); recombinant human TNF-α and IL-1β and enzyme-linked immunosorbent assay (ELISA) kits for IL-8 and MCP-1 from R&D Systems (Minneapolis, MN); a mouse monoclonal antibody to ICAM-1 from BD-PharMingen (San Diego, CA); and horseradish peroxidase–conjugated goat antibodies to mouse IgG from Chemicon (Temecula, CA); PCR kits (RNasey Mini and Quant-iT SYBR Green) from Qiagen (Hilden, Germany); a reverse transcription system from Promega (Madison, WI); lipid transfection reagent (TransFectin) from Bio-Rad (Hercules, CA); pNF-κB-secretory alkaline phosphatase (SEAP), pAP1-SEAP vectors, and a SEAP fluorescence detection kit (BD Great EscAPE) from BD Biosciences (San Jose, CA). The pM1-CAT vector and a chlororamphenicol acetyltransferase (CAT) ELISA kit from Roche Molecular Biochemicals (Mannheim, Ger-
many). All media and reagents used for cell culture were endotoxin minim-
ized.

Isolation and Culture of Human Corneal Fibroblasts

Human corneas were obtained for corneal transplantation surgery from the NorthWest Lions Eye Bank (Seattle, WA). The human tissue was used in strict accordance with the tenets of the Declaration of Helsinki. Corneal fibroblasts were prepared and cultured as described previ-
ously. In brief, the endothelial layer of the rim of the cornea remain-
ing after corneal transplantation surgery was removed mechanically, and the tissue was then incubated with dispase (2 mg/mL in MEM) for 1 hour. After mechanical removal of the epithelial sheet, the tissue was treated with collagenase (2 mg/mL in MEM) until a single-cell suspen-
sion of corneal fibroblasts was obtained. The cells were cultured in MEM supplemented with 10% FBS and were used for experiments after four to seven passages under subconfluent (actively proliferating) con-
ditions. The purity of the corneal fibroblast cultures was judged on the basis of both cell morphology and reactivities with antibodies to vimentin and cytokeratin, as previously described. All the cells were positive for vimentin and negative for cytokeratin, suggesting that the cultures were not contaminated by epithelial cells. No changes in cell morphology or immunoreactivity were apparent after culture for up to four to seven passages. Immunofluorescence analysis with antibodies to α-smooth muscle actin also revealed that the passed cells did not express this protein, indicating that they were not transformed myo-
bronblasts.

Assay of IL-8 and MCP-1 Release

Corneal fibroblasts were cultured in 24-well plates until they achieved confluence, after which the culture medium was replaced with serum-
free MEM for 1 day. The cells were then incubated first for 1 hour with various concentrations of triptolide (0.5–30 nM) or dexamethasone (0.01–100 nM) and then for an additional 24 hours in the same medium supple-
mented with 0.1% BSA, and examined (Nighthawk system; pdi, Huntington Station, NY). The sequences of the PCR primers for ICAM-1 were 5′-TAAGGCAAGGAGGAGCA-3′ (forward) and 5′-CATCATCAAGGTTGAGGCA-3′ (reverse). Primer se-
quences for GAPDH, IL-8, and MCP-1 have been described.

Whole-Cell ELISA for ICAM-1

A whole-cell ELISA for ICAM-1 was performed as described previ-
ously. In brief, corneal fibroblasts (1 × 10⁶ cells per well) were grown for 3 days in 96-well plates in MEM supplemented with 10% FBS, after which the culture medium was changed to serum-free MEM for 1 day. The cells were subsequently incubated, first for 1 hour with various concentrations of triptolide (0.3–30 nM) or dexamethasone (0.01–100 nM) and then for an additional 24 hours in the same medium supplemented with IL-1β (1 ng/mL) or TNF-α (1 ng/mL). The medium was then aspirated, and the cells were washed twice with phosphate-
buffered saline (PBS), fixed for 15 minutes at room temperature with PBS containing 1% paraformaldehyde, washed with PBS, and incubated for 1 hour at 37°C with a monoclonal antibody to ICAM-1 (1:10,000 dilution) in PBS containing 1% bovine serum albumin (BSA). After they were washed three times with PBS containing 0.1% BSA, the cells were incubated for 1 hour with horseradish peroxidase–conjugated goat antibodies to mouse IgG, washed another three times with PBS con-
taining 0.1% BSA, and incubated for 20 minutes in the dark with 100 μL tetramethylbenzidine solution. The reaction was terminated by the addition of 50 μL of 1 M H₂SO₄, and the absorbance of each well was measured at 450 nm with a microplate reader. The expression of ICAM-1 was calculated as a percentage of the control value.

Quantitative RT-PCR Analysis

The abundance of IL-8, MCP-1, and ICAM-1 mRNAs was determined by reverse transcription (RT) and real-time polymerase chain reaction (PCR) analysis, as described previously. The medium of confluent corneal fibroblasts cultured in 60-mm dishes was changed to serum-
free MEM, and the cells were cultured for an additional 24 hours before incubation, first for 1 hour with 30 nM triptolide or 100 nM dexameth-
asone and then for an additional 4 hours in the same medium supple-
mented with IL-1β (1 ng/mL) or TNF-α (1 ng/mL). Total RNA was then extracted from the fibroblasts and subjected to RT, and the resultant cDNA was subjected to real-time PCR with a thermocycler (LightCycler; Roche Molecular Biochemicals). Transcripts of the constitutively expressed gene for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) served to normalize the amounts of IL-8, MCP-1, and ICAM-1 mRNAs in each sample. Real-time PCR data were analyzed with the thermocycler software (LightCycler ver. 3.1; Roche Molecular Biochemicals). To verify the specificity of amplification, we also subjected PCR products to electrophoresis on a 3% agarose gel, which was then stained with ethidium bromide (1 μg/mL) and examined (Nighthawk system). The sequences of the PCR primers for ICAM-1 were 5′-TAAGGCAAGGAGGAGCA-3′ (forward) and 5′-CATCATCAAGGTTGAGGCA-3′ (reverse). Primer se-
quences for GAPDH, IL-8, and MCP-1 have been described.

Transient Transfection

Human corneal fibroblasts (3 × 10⁴ per well) were maintained in 24-well plates for 24 hours in MEM supplemented with 10% FBS and were then transfected in modified Eagle’s MEM (Opti-MEM; Invitrogen-Gibco) with pM1-CAT and either pNF-κB-SEAP or pAP1-SEAP at a ratio of 1:4 for 4 hours with the use of transfection reagent (TransFectin; Bio-Rad). The cells were cultured for 20 hours in the preservative and then incubated in the absence or presence of IL-1β (10 ng/mL), with or without triptolide (30 nM) or dexamethasone (100 nM), for an addi-
tional 48 hours. The culture supernatants were assayed for SEAP activity, and cell lysates were assayed for CAT by ELISA, to normalize transfection efficiency.

Statistical Analysis

Data are presented as the mean ± SEM and were analyzed by the Dunnett multiple comparison test or Student’s unpaired t test. P < 0.05 was considered statistically significant.

RESULTS

Inhibition of Chemokine Expression by Triptolide

We first examined the effects of IL-1β and TNF-α on IL-8, MCP-1, and ICAM-1 expression by human corneal fibroblasts. Consistent with previous observations, incubation of the cells for 24 hours in the presence of IL-1β (1 ng/mL) or TNF-α
(1 ng/mL) resulted in 54- and 44-fold increases in the amount of IL-8 in the culture medium (Fig. 1A), 25- and 23-fold increases in the amount of MCP-1 in the culture medium (Fig. 1B), and 2.1- and 2.3-fold increases in the surface abundance of ICAM-1 (Fig. 1C), respectively.

To determine whether triptolide or dexamethasone induces a cytotoxic effect in corneal fibroblasts, we treated confluent monolayers of the cells with various concentrations of triptolide (0.3–30 nM) or dexamethasone (0.01–100 nM) for 24 hours, stained the cells with trypan blue, and assayed the culture supernatants for lactate dehydrogenase. Neither triptolide nor dexamethasone exhibited cytotoxicity at any of the concentrations examined (data not shown).

We next investigated the effects of triptolide and dexamethasone on IL-8, MCP-1, and ICAM-1 expression in corneal fibroblasts treated with IL-1β or TNF-α. The cells were incubated with various concentrations of triptolide or dexamethasone for 1 hour before incubation for 24 hours in the absence or presence of IL-1β (1 ng/mL) or TNF-α (1 ng/mL). Triptolide induced a small inhibitory effect on IL-8 release at a concentration of 10 nM, but otherwise did not significantly modify the basal expression of this chemokine (Fig. 2A). In contrast, triptolide significantly inhibited IL-1β- or TNF-α-induced IL-8 release in a dose-dependent manner. At a concentration of 30 nM, triptolide inhibited this effect of IL-1β by 52% and that of TNF-α by 43%. Dexamethasone exhibited effects similar to those of triptolide on basal as well as IL-1β- or TNF-α-induced IL-8 release (Fig. 2B). At 100 nM, it inhibited this effect of IL-1β by 60% and that of TNF-α by 44%.

Triptolide inhibited basal MCP-1 release at all concentrations examined (Fig. 3A). It also inhibited IL-1β- or TNF-α-induced MCP-1 release in a dose-dependent manner. At a concentration of 50 nM, triptolide inhibited the effect of IL-1β by 75% and that of TNF-α by 66%. Dexamethasone induced a small inhibitory effect on basal MCP-1 release at a concentration of 10 nM. It also inhibited in a dose-dependent manner the MCP-1 expression induced by IL-1β or TNF-α (Fig. 3B). At 100 nM, dexamethasone inhibited the effect of IL-1β by 64% and that of TNF-α by 57%.

Triptolide (30 nM) significantly inhibited ICAM-1 expression induced by IL-1β or TNF-α by 37% and 30%, respectively, but it had no significant effect on ICAM-1 expression at other concentrations examined (Fig. 4A). Dexamethasone (100 nM) inhibited the effect of IL-1β on ICAM-1 expression by 29% but otherwise had no significant effect on ICAM-1 expression (Fig. 4B).

Quantitative RT-PCR analysis revealed that culture of corneal fibroblasts with IL-1β (1 ng/mL) or TNF-α (1 ng/mL) for 4 hours resulted in 930- and 610-fold increases, respectively, in
the amount of IL-8 mRNA, and that triptolide (30 nM) inhibited these effects by 72% and 85%, respectively, whereas dexamethasone (100 nM) inhibited them by 50% and 60%, respectively (Fig. 5A). IL-1 \( \beta \) or TNF-\( \alpha \) induced 146- and 56-fold increases, respectively, in the amount of MCP-1 mRNA in corneal fibroblasts, and triptolide (30 nM) inhibited these effects by 88% and 76%, respectively, whereas dexamethasone (100 nM) inhibited them by 81% and 65%, respectively (Fig. 5B). IL-1 \( \beta \) or TNF-\( \alpha \) induced 91- and 130-fold increases, respectively, in the amount of ICAM-1 mRNA in corneal fibroblasts, and triptolide (30 nM) inhibited each of these effects by 32%, whereas dexamethasone (100 nM) inhibited the effect of IL-1 \( \beta \) by 49% and that of TNF-\( \alpha \) by 51% (Fig. 5C).

**Discussion**

We have shown that not only dexamethasone but also triptolide, the major component of extracts of the Chinese herb *T. wilfordii* hook f, inhibited, in a dose-dependent manner, the release of IL-8 and MCP-1 from human corneal fibroblasts induced by the proinflammatory cytokines TNF-\( \alpha \) or IL-1\( \beta \). Both triptolide and dexamethasone inhibited the cytokine-induced expression of ICAM-1 on the surface of these cells only at the highest concentration examined, however. Inhibition of NF-\( \kappa \)B activity, but not of AP-1 activity, accompanied and may contribute to the effects of triptolide and dexamethasone on chemokine production. Our results thus suggest that triptolide may downregulate IL-8 and MCP-1 expression in corneal fibroblasts in vivo and thereby limit the local accumulation of neutrophils and monocytes in the corneal stroma.

The regulation of corneal fibroblasts by cytokines is likely to be an important determinant of neutrophil and monocyte recruitment to ocular lesions. Various cytokines released into tear fluid affect corneal fibroblast function either individually in the transactivation activity of NF-\( \kappa \)B and that triptolide or dexamethasone inhibited this effect by 39% and 17%, respectively (Fig. 6). In contrast, neither IL-1\( \beta \), triptolide, nor dexamethasone affected the transactivation activity of AP-1 (Fig. 7).

**Effects of Triptolide and Dexamethasone on NF-\( \kappa \)B and AP-1 Activity in Corneal Fibroblasts**

To determine whether the transactivation activities of NF-\( \kappa \)B or AP-1 are inhibited by triptolide or dexamethasone, we transfected corneal fibroblasts with pNF-\( \kappa \)B-SEAP or pAP-1-SEAP plasmids together with pM1-CAT. After incubation of the transfected cells in the absence or presence of IL-1\( \beta \) (1 ng/mL), 30 nM triptolide, or 100 nM dexamethasone for 24 hours, the amount of MCP-1 mRNA in culture supernatants was then determined by ELISA. Data are the mean ± SEM of triplicates from an experiment that was repeated three times with similar results. *P < 0.05, **P < 0.01 (Dunnett test) versus the corresponding value for cells incubated without triptolide or dexamethasone.

**Figure 3.** Dose-dependent inhibition by triptolide and dexamethasone of MCP-1 release from corneal fibroblasts. Cells were incubated first with the indicated concentrations of triptolide (A) or dexamethasone (B) for 1 hour and then in the additional absence (○) or presence of IL-1\( \beta \) (1 ng/mL; ◆) or TNF-\( \alpha \) (1 ng/mL; □) for 24 hours. The amount of MCP-1 in culture supernatants was then determined by ELISA. Data are the mean ± SEM of triplicates from an experiment that was repeated three times with similar results. *P < 0.05, **P < 0.01 (Dunnett test) versus the corresponding value for cells incubated without triptolide or dexamethasone.

**Figure 4.** Effects of triptolide and dexamethasone on ICAM-1 expression in corneal fibroblasts. Cells were incubated first with the indicated concentrations of triptolide (A) or dexamethasone (B) for 1 hour and then in the additional absence (○) or presence of IL-1\( \beta \) (1 ng/mL; ◆) or TNF-\( \alpha \) (1 ng/mL; □) for 24 hours. The surface expression of ICAM-1 was then examined by ELISA and was calculated as a percentage of the control value. Data are the mean ± SEM of triplicates from an experiment that was repeated three times with similar results. *P < 0.01 (Dunnett test) versus the corresponding value for cells incubated without triptolide or dexamethasone.
detected in individuals with corneal inflammatory diseases. Increased local expression of IL-8 and MCP-1 has been identified as chemoattractants for neutrophils and monocytes-macrophages, respectively. IL-8 and MCP-1 are potent and selective chemoattractants for leukocytes as well as the subsequent activation of these cells through interaction with the β2 integrin chain expressed on their surface. ICAM-1 is selectively expressed in the cornea in areas of inflammation. Furthermore, leukocyte infiltration into tissues is markedly reduced in ICAM-1-deficient mice compared with that in control animals. We have previously shown that exposure of corneal fibroblasts to TNF-α increases the adherence of neutrophils to these cells, indicating that ICAM-1 expressed by corneal fibroblasts in response to TNF-α is biologically active. Inhibition or modulation of the expression of ICAM-1 in corneal fibroblasts may thus provide a new therapeutic option in the treatment of corneal inflammatory diseases. Our data indicate that, although triptolide significantly inhibited the increase in the amount of ICAM-1 mRNA in human corneal fibroblasts induced by TNF-α, its effect on the expression of ICAM-1 on the cell surface was limited.

Leukocytes play important roles in the pathogenesis of corneal inflammatory diseases. We recently showed that keratoconjunctivitis and correlates with the severity of disease. We have now shown that, in human corneal fibroblasts, triptolide inhibited, in a dose-dependent manner, the expression of ICAM-1, at both the protein and mRNA levels, induced by proinflammatory cytokines.

Leukocytes release chemokines that may facilitate the chemotaxis of leukocytes to the corneal stroma. Various mediators have been identified as chemokine receptors for leukocytes. Migration and infiltration into tissues by leukocytes as well as the subsequent activation of these cells are regulated by chemokines. IL-8 and MCP-1 are potent and selective chemoattractants for neutrophils and monocytes-macrophages, respectively. Increased local expression of IL-8 and MCP-1 has been detected in individuals with corneal inflammatory diseases.

Inhibition of IL-8 or MCP-1 synthesis by corneal fibroblasts may thus reduce the extent of neutrophil or monocyte recruitment to the cornea as well as that of consequent corneal damage.

Our results suggest that triptolide has therapeutic potential as an alternative to steroids for suppressing leukocyte infiltration into the cornea.

ICAM-1 is a cell surface glycoprotein of 76 to 114 kDa and a member of the immunoglobulin superfamily of proteins. It contributes to the local infiltration of leukocytes during immune responses by mediating the adhesion and activation of these cells through interaction with the β2 integrin chain expressed on their surface. ICAM-1 is selectively expressed in the cornea in areas of inflammation. Furthermore, leukocyte infiltration into tissues is markedly reduced in ICAM-1-deficient mice compared with that in control animals. We have previously shown that exposure of corneal fibroblasts to TNF-α increases the adherence of neutrophils to these cells, indicating that ICAM-1 expressed by corneal fibroblasts in response to TNF-α is biologically active. Inhibition or modulation of the expression of ICAM-1 in corneal fibroblasts may thus provide a new therapeutic option in the treatment of corneal inflammatory diseases. Our data indicate that, although triptolide significantly inhibited the increase in the amount of ICAM-1 mRNA in human corneal fibroblasts induced by TNF-α, its effect on the expression of ICAM-1 on the cell surface was limited.

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Triptolide and dexamethasone exert various effects on immune cells as well as on tissue-resident cells that participate in inflammatory responses. The similarity in the effects of these two compounds is dependent on cell type, however. Both dexamethasone and triptolide inhibit cytokine synthesis in monocytes. T cells, and macrophages and induce apoptosis in T cells. Both agents inhibit phagocytosis by neutrophils whereas dexamethasone also inhibits apoptosis in these cells. Triptolide inhibits IgG secretion from B cells, whereas dexamethasone has no such effect. We have shown that dexamethasone inhibits not only the synthesis but also the activation of matrix metalloproteinases in rabbit corneal fibroblasts whereas triptolide inhibits only the synthesis of these enzymes. Steroids have been administered widely for their anti-inflammatory activity in human diseases, but they are not free of adverse effects. Such adverse reactions may be avoided if triptolide proves effective for the treatment of corneal inflammation. Triptolide has been shown to be effective in the treatment of several autoimmune diseases such as rheumatoid arthritis, lung fibrosis, and uveoretinitis in animal models. Furthermore, clinical studies of triptolide in patients with rheumatoid arthritis have shown that the drug is safe and clinically beneficial. Whether triptolide is a viable alternative to steroids for treatment of corneal inflammation and is devoid of adverse effects remain to be clarified.

The transcription factor NF-κB plays an important role in intracellular signaling induced by IL-1 or TNF-α and is therefore a potential target for new therapeutic approaches to inflammatory diseases. In the current study, triptolide inhibited the activation of NF-κB by IL-1β in human corneal fibroblasts, suggesting that this effect of triptolide contributes to its inhibition of chemokine expression stimulated by proinflammatory cytokines. Triptolide has been shown to inhibit cytokine production in T cells and bronchial epithelial cells as well as to sensitize lung cancer cells to apoptosis by inhibition of NF-κB activity. The mechanism by which triptolide inhibits the activation of NF-κB remains to be determined.

AP-1 also contributes to IL-1 and TNF-α signaling pathways in many cell types. In the present study, however, the transcription activity of AP-1 in corneal fibroblasts was not increased by IL-1β. Although triptolide has been shown to inhibit AP-1 activity in articular chondrocytes and gastric cancer cells, it did not affect AP-1 activity in corneal fibroblasts. We recently showed that neutrophils stimulate collagen degradation by corneal fibroblasts and that triptolide inhibits IL-1-induced collagen degradation by corneal fibroblasts. Interactions of inflammatory cells with resident corneal fibroblasts therefore very likely play an important role in corneal inflammation. The inhibition by triptolide of IL-8 and MCP-1 synthesis is not specific to corneal fibroblasts, given that this agent has been shown to exert a similar action in cultured bronchial epithelial cells. Nevertheless, our present results suggest that triptolide may be of therapeutic benefit in diseases characterized by corneal stromal inflammation.

References


