Inhibition by Triptolide of Chemokine, Proinflammatory Cytokine, and Adhesion Molecule Expression Induced by Lipopolysaccharide in Corneal Fibroblasts

Ying Lu,[1] *Yang Liu,*[1] *Ken Fukuda,*[1,2] *Yoshikuni Nakamura,*[1] *Naoki Kumagai,*[1] *and Teruo Nishida*[1,2]

Purpose. The production of proinflammatory cytokines and chemokines as well as the surface expression of intercellular adhesion molecule (ICAM)-1 by corneal fibroblasts contribute to corneal inflammation. The effects of triptolide on the expression of these proteins induced by lipopolysaccharide (LPS) in human corneal fibroblasts were examined in comparison with those of dexamethasone.

Methods. The release of interleukin (IL)-1β, tumor necrosis factor (TNF)-α, IL-6, granulocyte colony–stimulating factor (G-CSF), granulocyte-macrophage colony–stimulating factor (GM-CSF), monocyte chemotactic protein (MCP)-1, macrophage inflammatory protein (MIP)-1β, and IL-8 from cultured corneal fibroblasts was measured with assay kits. Surface expression of ICAM-1 on the cultured cells was measured with a whole-cell enzyme-linked immunosorbent assay.

Results. Lipopolysaccharide (LPS) induced the release of the proinflammatory cytokine IL-6 and that of the chemokines G-CSF, MCP-1, MIP-1β, and IL-8 as well as surface expression of ICAM-1 by corneal fibroblasts, whereas IL-1β, TNF-α, and GM-CSF were not detected in the culture supernatants of cells incubated with or without LPS. Triptolide and dexamethasone each inhibited in a concentration-dependent manner the LPS-induced release of IL-6, G-CSF, MCP-1, and IL-8 by corneal fibroblasts. Whereas the inhibitory effect of dexamethasone on LPS-induced IL-6 release was greater than that of triptolide, the inhibitory effect of triptolide on LPS-induced G-CSF release was more pronounced than was that of dexamethasone. Dexamethasone also inhibited LPS-induced MIP-1β release, whereas triptolide did not. Both compounds inhibited the LPS-induced surface expression of ICAM-1.

Conclusions. Triptolide inhibits the LPS-induced expression of IL-6, chemokines (G-CSF, MCP-1, IL-8), and ICAM-1 in cultured human corneal fibroblasts. This compound might thus be expected to limit the infiltration of immune cells into the cornea. (*Invest Ophthalmol Vis Sci.* 2006;47:3796–3800) DOI:10.1167/iovs.06-0319

From the Departments of [1]Biomolecular Recognition and Ophthalmology and [2]Ocular Pathophysiology, Yamaguchi University School of Medicine, Ube City, Yamaguchi, Japan.

Supported by Grants-in-Aid from the Japan Society for the Promotion of Science (JSPS; TN, NK, KF, YLu). YLu holds a JSPS postdoctoral fellowship for foreign researchers.

Submitted for publication March 23, 2006; revised May 28, 2006; accepted July 26, 2006.

Disclosure: **Y. Lu**, None; **Y. Liu**, None; **K. Fukuda**, None; **Y. Nakamura**, P; **N. Kumagai**, None; **T. Nishida**, P

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Corresponding author: Teruo Nishida, Department of Biomolecular Recognition and Ophthalmology, Yamaguchi University School of Medicine, 1-1-1 Minami-Kogushi, Ube City, Yamaguchi 755-8505, Japan; tnishida@yamaguchi-u.ac.jp.

Infectious corneal ulcer is characterized in part by infiltration into the corneal stroma of various immune cells, including neutrophils, macrophages, and lymphocytes.[1] It is also associated with the production by resident corneal fibroblasts of proinflammatory cytokines, such as interleukin (IL)-6, IL-1, and tumor necrosis factor (TNF)-α, and chemokines, including IL-8, granulocyte colony–stimulating factor (G-CSF), granulocyte-macrophage colony–stimulating factor (GM-CSF), monocyte chemotactic protein (MCP)-1, and macrophage inflammatory protein (MIP)-1β.[2–5] Together these factors induce the chemotaxis and activation of various leukocytes. We have shown that interactions between resident corneal cells, in particular corneal fibroblasts, and infiltrating immune cells play an important role in the pathogenesis of ocular inflammation.[6] Expression by corneal fibroblasts of intercellular adhesion molecule (ICAM)-1, a cell surface glycoprotein that binds leukocyte function–associated antigen 1 present on the surfaces of all leukocytes, thereby enhancing immune responses, also contributes to inflammatory corneal diseases.[7]

Lipopolysaccharide (LPS) is a component of the cell membrane of Gram-negative bacteria and is thought to contribute to bacterial keratitis. We recently showed that corneal fibroblasts are able to detect the presence of LPS and to upregulate their expression of IL-8, MCP-1, and ICAM-1 in response. This response is facilitated by serum proteins such as soluble CD14 and LPS-binding protein and is mediated in part by the transcription factor nuclear factor-κB (NF-κB).[8,9] Injection of LPS into the corneal stroma induces an acute inflammatory reaction characterized by infiltration of neutrophils and other immune cells and subsequent corneal ulceration.[10]

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.[11] Triptolide exhibits anti-inflammatory activity not only with immune cells such as T cells, B cells, and monocytes[12,13] but also with tissue resident cells such as epithelial cells[14] and fibroblasts.[15] We recently showed that triptolide inhibits collagen degradation[16] and the expression of IL-8 and MCP-1[17] by corneal fibroblasts stimulated with IL-1. We have now evaluated the effects of triptolide on the production of proinflammatory cytokines (IL-1β, TNF-α, IL-6) and chemokines (G-CSF, GM-CSF, MCP-1, MIP-1β, IL-8) and on the surface expression of ICAM-1 in corneal fibroblasts stimulated with LPS. The effects of triptolide were compared with those of dexamethasone.

Materials and Methods

Eagle minimum essential medium (MEM), fetal bovine serum (FBS), and trypsin-EDTA were obtained (Invitrogen-Gibco, Carlsbad, CA), as were 24- and 96-well culture plates (Corning-Costar, Corning, NY), collagenase, dispase, dexamethasone, and LPS derived from *Pseudomonas aeruginosa* (Sigma-Aldrich, St. Louis, MO), triptolide (Alexis Biochemicals, Carlsbad, CA), mouse monoclonal antibody to ICAM-1 (BD PharMingen, San Diego, CA), horseradish peroxidase–conjugated goat an-

Investigative Ophthalmology & Visual Science, September 2006, Vol. 47, No. 9

tibodies to mouse immunoglobulin G (Chemicon, Temecula, CA), an enzyme-linked immunosorbent assay (ELISA) kit for IL-8 (R&D Systems, Minneapolis, MN), a protein multiarray system and human cytokine assay (Bio-Plex; Bio-Rad, Hercules, CA), and a particle counter (Beckman Coulter, Fullerton, CA). All media and reagents used for cell culture were endotoxin minimized.

Isolation and Culture of Human Corneal Fibroblasts

Human corneas were obtained for corneal transplantation surgery (NorthWest Lions Eye Bank, Seattle, WA). Human tissue was used in strict accordance with the tenets of the Declaration of Helsinki. Corneal fibroblasts were prepared and cultured as described previously.[18] In brief, the endothelial layer of the rim of the cornea remaining after 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 suspension of corneal fibroblasts was obtained. Cells were cultured in MEM supplemented with 10% FBS and were used for experiments after four to seven passages under subconfluent (actively proliferating) conditions. Purity of the corneal fibroblast cultures was judged on the basis of cell morphology and reactivities with antibodies to vimentin and to cytokeratin, as previously described[18]; 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 seven passages.

Assay for IL-1β, TNF-α, IL-6, G-CSF, GM-CSF, MCP-1, MIP-1β, and IL-8

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. Cells were then incubated first for 1 hour with various concentrations of triptolide (1–30 nM) or dexamethasone (0.1–100 nM) and then for an additional 24 hours in the same medium supplemented with LPS (100 ng/mL) and 0.5% human serum (AB type). The medium was then collected and centrifuged at 120*g* for 5 minutes, and the resultant supernatant was frozen at −80°C for subsequent assay of proinflammatory cytokines and chemokines. Cells were detached from the culture plate by exposure to trypsin-EDTA, and their number was determined with a particle counter. The morphology and number of cells were not affected by incubation with LPS, triptolide, or dexamethasone for 24 hours (data not shown). Simultaneous quantification of IL-1β, TNF-α, IL-6, G-CSF, GM-CSF, MCP-1, and MIP-1β in culture supernatants was performed with a human cytokine assay system (Bio-Plex; Bio-Rad). IL-8 concentration was determined with an ELISA kit, as described previously.[8]

Whole-Cell ELISA for ICAM-1

Whole-cell ELISA for ICAM-1 was performed as described previously.[19] In brief, corneal fibroblasts (1×10^3 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. Cells were subsequently incubated first for 1 hour with various concentrations of triptolide (1–30 nM) or dexamethasone (0.1–100 nM) and then for an additional 24 hours in the same medium supplemented with LPS (100 ng/mL) and 0.5% human serum (AB type). 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 three washes with PBS containing 0.1% BSA, the cells were incubated for 1 hour at room temperature with horseradish peroxidase–conjugated goat antibodies to mouse immunoglobulin G, washed three more times with PBS containing 0.1% BSA, and incubated for 20 minutes in the dark with 100 μL tetramethylbenzidine solution. The reaction was stopped by the addition of 50 μL of 1 M H_2SO_4, and the absorbance of each well was measured at 450 nm with a microplate reader. ICAM-1 expression was calculated as a percentage of the control value.

Statistical Analysis

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

Results

Effects of LPS on IL-1β, TNF-α, IL-6, G-CSF, GM-CSF, MCP-1, MIP-1β, and IL-8 Expression in Corneal Fibroblasts

We recently showed that LPS increases the expression of IL-8 and MCP-1 in cultured human corneal fibroblasts.[8,9] In the present study, we first examined the effects of LPS on the release of IL-1β, TNF-α, IL-6, G-CSF, GM-CSF, MCP-1, MIP-1β, and IL-8 from these cells. IL-1β, TNF-α, and GM-CSF were not detected in the culture supernatants of cells incubated in the absence or presence of LPS (100 ng/mL) for 24 hours (Table 1). G-CSF was also not detected in the culture supernatant of cells incubated in the absence of LPS, but it was present in that of cells exposed to LPS. Incubation of corneal fibroblasts with LPS also resulted in significant increases in the release of IL-6, MCP-1, MIP-1β, and IL-8.

Effects of Triptolide and Dexamethasone on IL-6, G-CSF, MCP-1, MIP-1β, and IL-8 Expression in Corneal Fibroblasts

We next investigated the effects of triptolide and dexamethasone on the expression of IL-6, G-CSF, MCP-1, MIP-1β, and IL-8 in corneal fibroblasts (Table 2). Cells were incubated with various concentrations of triptolide (1–30 nM) or dexamethasone (0.1–100 nM) for 1 hour before incubation for 24 hours in the additional absence or presence of LPS (100 ng/mL). Triptolide exhibited no effect on the basal expression of IL-6 but inhibited LPS-induced IL-6 release in a concentration-dependent manner; this latter effect was statistically significant at a concentration of 3 nM and maximal (55% inhibition) at 30 nM. Dexamethasone had a small inhibitory effect on basal IL-6 release at concentrations of 10 and 100 nM, and it inhibited LPS-induced IL-6 expression in a concentration-dependent manner; this latter effect was significant at a concentration of 1 nM and maximal (83% inhibition) at 100 nM.

Table 1. Effects of LPS on Proinflammatory Cytokine and Chemokine Release from Human Corneal Fibroblasts

Cytokine/Chemokine	None	LPS
IL-1β (pg/mL)	ND	ND
TNF-α (pg/mL)	ND	ND
IL-6 (ng/mL)	0.05 ± 0.02	14.79 ± 1.39*
GM-CSF (pg/mL)	ND	ND
G-CSF (ng/mL)	ND	2.85 ± 0.19
MCP-1 (ng/mL)	4.70 ± 0.71	11.30 ± 1.37*
MIP-1β (pg/mL)	3.00 ± 0.41	6.55 ± 0.38*
IL-8 (ng/mL)	2.58 ± 0.23	36.00 ± 1.49*

Cells were incubated in the absence or presence of LPS (100 ng/mL) for 24 hours, after which the amounts of the indicated proteins in culture supernatants were determined. Data are mean ± SEM of triplicates from an experiment that was repeated three times with similar results. ND, not detected.

* $P < 0.05$ (Student's *t* test) compared with the corresponding value for cells cultured without LPS.

TABLE 2. Effects of Triptolide and Dexamethasone on IL-6, G-CSF, MCP-1, MIP-1β, and IL-8 Release from Human Corneal Fibroblasts

	nM	IL-6 (ng/mL)	G-CSF (ng/mL)	MCP-1 (ng/mL)	MIP-1β (pg/mL)	IL-8 (ng/mL)
Trip	0	0.05 ± 0.02	ND	4.70 ± 0.71	3.00 ± 0.41	2.58 ± 0.23
	1	0.05 ± 0.03	ND	4.42 ± 0.88	3.15 ± 0.41	2.47 ± 0.24
	3	0.05 ± 0.03	ND	4.14 ± 1.13	3.33 ± 0.29	2.41 ± 0.23
	10	0.05 ± 0.02	ND	4.04 ± 0.50	3.42 ± 0.42	1.85 ± 0.22*
	30	0.04 ± 0.02	ND	2.75 ± 0.12*	3.06 ± 0.34	2.38 ± 0.19*
LPS + Trip	0	14.79 ± 1.39	2.85 ± 0.19	11.30 ± 1.37	6.55 ± 0.38	36.00 ± 1.49
	1	15.30 ± 4.26	2.11 ± 0.31*	9.31 ± 1.53	6.16 ± 0.96	25.32 ± 1.44*
	3	8.70 ± 1.13*	0.80 ± 0.09*	8.42 ± 0.47*	6.53 ± 0.47	21.84 ± 0.34*
	10	8.25 ± 0.89*	0.78 ± 0.07*	9.29 ± 0.79*	6.07 ± 0.22	21.65 ± 1.66*
	30	6.46 ± 0.69*	0.15 ± 0.01*	7.26 ± 0.29*	5.85 ± 0.59	19.31 ± 2.65*
Dex	0	0.05 ± 0.02	ND	4.70 ± 0.71	3.00 ± 0.41	2.58 ± 0.23
	0.1	0.05 ± 0.01	ND	4.97 ± 0.65	3.03 ± 0.67	2.03 ± 0.19*
	1	0.03 ± 0.01	ND	4.24 ± 0.31	2.86 ± 0.59	1.55 ± 0.13*
	10	0.01 ± 0.00*	ND	2.97 ± 0.57*	2.68 ± 0.24	1.47 ± 0.09*
	100	0.01 ± 0.00*	ND	2.54 ± 0.40*	2.89 ± 0.35	1.35 ± 0.04*
LPS + Dex	0	14.79 ± 1.39	2.85 ± 0.19	11.30 ± 1.37	6.55 ± 0.38	36.00 ± 1.49
	0.1	15.45 ± 2.58	2.49 ± 0.30	10.33 ± 1.01	5.57 ± 0.58*	26.49 ± 2.16*
	1	3.18 ± 0.34*	0.87 ± 0.11*	9.85 ± 1.40	4.80 ± 0.39*	21.61 ± 1.00*
	10	3.06 ± 0.20*	0.87 ± 0.15*	9.06 ± 0.85*	4.63 ± 0.33*	18.33 ± 1.44*
	100	2.50 ± 0.21*	0.80 ± 0.25*	7.22 ± 0.42*	4.09 ± 0.30*	17.86 ± 0.40*

Cells were incubated first with the indicated concentrations of triptolide (Trip) or dexamethasone (Dex) for 1 hour and then in the additional absence or presence of LPS (100 ng/mL) for 24 hours. The amount of each protein in culture supernatants was then determined. Data are mean ± SEM of triplicates from an experiment repeated three times with similar results. ND, not detected.

* $P < 0.05$ (Dunnett test) compared with the corresponding value for cells incubated without triptolide or dexamethasone.

Triptolide inhibited LPS-induced G-CSF release in a concentration-dependent manner; this effect was significant at a concentration of 1 nM and maximal (95% inhibition) at 30 nM. Dexamethasone similarly inhibited the stimulatory effect of LPS on G-CSF expression; this effect was significant at a concentration of 1 nM and maximal (72% inhibition) at 100 nM.

Triptolide induced a small inhibitory effect on basal MCP-1 release at a concentration of 30 nM and inhibited LPS-induced MCP-1 release in a concentration-dependent manner; this latter effect was significant at a concentration of 3 nM and maximal (36% inhibition) at 30 nM. Dexamethasone also inhibited basal and LPS-induced MCP-1 release in a concentration-dependent manner; the latter effect was significant at a concentration of 10 nM and maximal (36% inhibition) at 100 nM.

Triptolide had little or no effect on basal or LPS-induced MIP-1β release at any of the concentrations examined. In contrast, whereas dexamethasone did not significantly affect the basal expression of this chemokine, it inhibited LPS-induced MIP-1β release in a concentration-dependent manner; this effect was significant at a concentration of 0.1 nM and maximal (38% inhibition) at 100 nM.

Triptolide induced a small inhibitory effect on basal IL-8 release at concentrations of 10 and 30 nM, and it inhibited LPS-induced IL-8 release in a concentration-dependent manner; this latter effect was significant at a concentration of 1 nM and maximal (46% inhibition) at 30 nM. Dexamethasone exhibited effects similar to those of triptolide on basal and LPS-induced IL-8 release; the latter effect was significant at a concentration of 0.1 nM and maximal (50% inhibition) at 100 nM.

Inhibition by Triptolide and Dexamethasone of ICAM-1 Expression in Corneal Fibroblasts

We recently showed that LPS increases the expression of ICAM-1 in cultured human corneal fibroblasts.[8,9] Therefore, we next investigated the effects of triptolide and dexamethasone on the surface expression of ICAM-1 in corneal fibroblasts incubated in the absence or presence of LPS. Cells were incubated with various concentrations of triptolide or dexamethasone for 1 hour before incubation for 24 hours in the additional absence or presence of LPS (100 ng/mL). Although triptolide had no effect on basal ICAM-1 expression, it inhibited LPS-induced ICAM-1 expression in a concentration-dependent manner (Fig. 1A); at a concentration of 30 nM, triptolide inhibited the effect of LPS by 35%. Dexamethasone also had no effect on basal ICAM-1 expression, and it inhibited (by 27%) the effect of LPS on ICAM-1 expression only at a concentration of 100 nM (Fig. 1B).

DISCUSSION

We have shown that LPS induced the production of IL-6, G-CSF, MCP-1, MIP-1β, and IL-8 and the surface expression of ICAM-1 in human corneal fibroblasts. Furthermore, similar to the action of dexamethasone, triptolide inhibited in a concentration-dependent manner the LPS-induced release of IL-6, G-CSF, MCP-1, and IL-8 from these cells. The inhibitory effect of dexamethasone on LPS-induced IL-6 release was greater than that of triptolide, whereas the inhibitory effect of triptolide on LPS-induced G-CSF release was more pronounced than was that of dexamethasone. Dexamethasone also inhibited MIP-1β expression induced by LPS, whereas triptolide did not. Triptolide and dexamethasone exhibited similar inhibitory effects on LPS-induced surface expression of ICAM-1. Our results thus suggest that triptolide might prove effective in limiting the infiltration of immune cells into the cornea.

LPS is an important trigger for host inflammatory responses during infection of the cornea with Gram-negative bacteria.[20] Fibroblasts play a substantial role in the regulation of inflammation in the infected cornea. We have now shown that LPS stimulates proinflammatory cytokine, chemokine, and ICAM-1 expression in corneal fibroblasts, consistent with our previous observations.[8,9] We also recently showed that LPS interacts

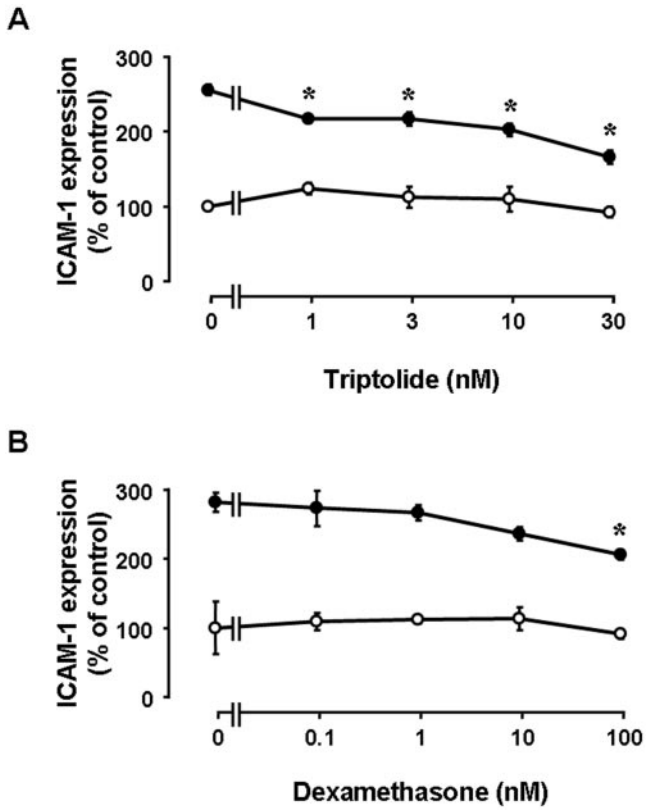

FIGURE 1. Effects of triptolide and dexamethasone on ICAM-1 expression in human corneal fibroblasts. Cells were incubated first with the indicated concentrations of triptolide (**A**) or dexamethasone (**B**) for 1 hour and then in the additional absence (*open circles*) or presence (*closed circles*) of LPS (100 ng/mL) for 24 hours. Surface expression of ICAM-1 was then determined and calculated as a percentage of the control value. Data are mean ± SEM of triplicates from an experiment that was repeated three times with similar results. $^*P < 0.05$ (Dunnett test) compared with the corresponding value for cells incubated without triptolide or dexamethasone.

with human corneal fibroblasts to induce the phosphorylation and degradation of IκBα in the cytoplasm and the consequent activation of NF-κB.[9] The activation of NF-κB by LPS may thus contribute to the LPS-induced expression of IL-6, chemokines, and ICAM-1 observed in the present study. These effects of LPS likely promote the infiltration and activation of leukocytes associated with corneal inflammation.

Leukocytes play important roles in the pathogenesis of corneal inflammatory diseases. Migration to and infiltration into the tissue of leukocytes and the subsequent activation of these cells are regulated by chemokines and other cytokines. IL-6 promotes the differentiation of B cells,[21] whereas IL-8[22] and MCP-1[23] are potent and selective chemoattractants for neutrophils and monocytes–macrophages, respectively. We previously showed that keratocytes release IL-8 and MCP-1 and that this release might facilitate the chemotaxis of leukocytes to the corneal stroma.[24] Triptolide inhibits IL-6 expression in human bronchial epithelial cells stimulated with phorbol 12-myristate 13-acetate, TNF-α, or IL-1β,[14] LPS-stimulated mouse macrophages,[25] IL-8 expression in human bronchial epithelial cells,[13,14] TNF-α– or IL-1β–stimulated human corneal fibroblasts,[17] and MCP-1 expression in TNF-α– or IL-1β–stimulated human corneal fibroblasts.[17] G-CSF induces the proliferation, differentiation, and activation of neutrophilic granulocytes.[26] The more pronounced inhibitory effect of triptolide on LPS-induced G-CSF expression in corneal fibroblasts compared with that of dexamethasone suggests that triptolide might prove more effective than this steroid in inhibiting neutrophil-mediated corneal inflammation. MIP-1β regulates monocytes and T cells.[27] In the present study, triptolide showed little or no effect on MIP-1β expression in corneal fibroblasts, whereas dexamethasone inhibited it. Inhibition of the expression of these various factors in corneal fibroblasts might thus be expected to reduce the extent of leukocyte recruitment to the cornea and that of consequent corneal damage.

ICAM-1 is a 76- to 114-kDa cell surface glycoprotein and a member of the immunoglobulin superfamily of proteins.[28] It contributes to the local infiltration of leukocytes during immune responses by mediating the adhesion and activation of these cells.[29] ICAM-1 is thus selectively expressed in the cornea in areas of inflammation.[30] Furthermore, leukocyte infiltration into tissue is markedly reduced in ICAM-1–deficient mice compared with that in control animals.[31] We have previously shown that exposure of corneal fibroblasts to LPS increases the adherence of neutrophils to these cells, indicating that ICAM-1 expressed by corneal fibroblasts in response to LPS is biologically active.[8] Inhibition of the expression of ICAM-1 in corneal fibroblasts might thus provide a new therapeutic option in the treatment of corneal inflammatory diseases. We have now shown that, although triptolide significantly inhibited the LPS-induced increase in the surface expression of ICAM-1 in human corneal fibroblasts, the extent of this effect was limited.

Triptolide and dexamethasone each exert various effects on immune cells and on tissue resident cells that participate in inflammatory responses. The similarity in the effects of these two compounds is dependent on cell type, however. We have previously shown, for example, that dexamethasone inhibits not only the synthesis but also the activation of matrix metalloproteinases in rabbit corneal fibroblasts,[32] whereas triptolide inhibits only the synthesis of these enzymes.[16] Dexamethasone and triptolide each inhibit IL-8 and MCP-1 expression in cultured human corneal fibroblasts induced by the proinflammatory cytokines IL-1 or TNF-α.[17] Triptolide has been shown to be effective for the treatment of several autoimmune diseases such as rheumatoid arthritis,[11] lung fibrosis,[33] and uveoretinitis[34] in animal models. Furthermore, it appears to be safe and clinically beneficial in patients with rheumatoid arthritis or psoriasis vulgaris.[35–37] In the present study, dexamethasone inhibited the LPS-induced release of all the tested proteins from corneal fibroblasts, whereas the inhibitory action of triptolide was more selective. Dexamethasone was more effective at inhibiting LPS-induced IL-6 release than was triptolide, but triptolide was more effective at inhibiting LPS-induced G-CSF release than was dexamethasone. Dexamethasone also inhibited MIP-1β expression induced by LPS, but triptolide did not. Whether triptolide is a viable alternative to steroids for the treatment of corneal inflammation and whether it is devoid of adverse effects remain to be clarified.

We recently showed that neutrophils stimulate collagen degradation by corneal fibroblasts[6] and that triptolide inhibits IL-1–induced collagen degradation by these cells.[16] Interactions of inflammatory cells with resident corneal fibroblasts, therefore, likely play an important role in corneal inflammation. The downregulation by triptolide of the production of chemokines and other cytokines and of the surface expression of ICAM-1 by LPS-stimulated corneal fibroblasts suggests that this compound may be of therapeutic benefit in diseases characterized by corneal stromal inflammation.

References

1. Prause JU, Jensen OA. PAS-positive polymorphonuclear leucocytes in corneal ulcers. *Acta Ophthalmol (Copenh).* 1980;58:556–566.

2. Thakur A, Willcox MD. Cytokine and lipid inflammatory mediator profile of human tears during contact lens associated inflammatory diseases. *Exp Eye Res*. 1998;67:9–19.
3. Spandau UH, Toksoy A, Verhaart S, Gillitzer R, Kruse FE. High expression of chemokines Gro-alpha (CXCL-1), IL-8 (CXCL-8), and MCP-1 (CCL-2) in inflamed human corneas in vivo. *Arch Ophthalmol*. 2003;121:825–831.
4. Hong JW, Liu JJ, Lee JS, et al. Proinflammatory chemokine induction in keratocytes and inflammatory cell infiltration into the cornea. *Invest Ophthalmol Vis Sci*. 2001;42:2795–2803.
5. Cubitt CL, Lausch RN, Oakes JE. Differential regulation of granulocyte-macrophage colony-stimulating factor gene expression in human corneal cells by pro-inflammatory cytokines. *J Immunol*. 1994;153:232–240.
6. Li Q, Fukuda K, Lu Y, et al. Enhancement by neutrophils of collagen degradation by corneal fibroblasts. *J Leukoc Biol*. 2003; 74:412–419.
7. Whitcup SM, Nussenblatt RB, Price FW Jr, Chan CC. Expression of cell adhesion molecules in corneal graft failure. *Cornea*. 1993;12: 475–480.
8. Kumagai N, Fukuda K, Fujitsu Y, et al. Lipopolysaccharide-induced expression of intercellular adhesion molecule-1 and chemokines in cultured human corneal fibroblasts. *Invest Ophthalmol Vis Sci*. 2005;46:114–120.
9. Fukuda K, Kumagai N, Yamamoto K, et al. Potentiation of lipopolysaccharide-induced chemokine and adhesion molecule expression in corneal fibroblasts by soluble CD14 or LPS-binding protein. *Invest Ophthalmol Vis Sci*. 2005;46:3095–3101.
10. Schultz CL, Morck DW, McKay SG, Olson ME, Buret A. Lipopolysaccharide induced acute red eye and corneal ulcers. *Exp Eye Res*. 1997;64:3–9.
11. Gu WZ, Brandwein SR. Inhibition of type II collagen-induced arthritis in rats by triptolide. *Int J Immunopharmacol*. 1998;20: 389–400.
12. Chang DM, Chang WY, Kuo SY, Chang ML. The effects of traditional antirheumatic herbal medicines on immune response cells. *J Rheumatol*. 1997;24:436–441.
13. Qiu D, Zhao G, Aoki Y, et al. Immunosuppressant PG490 (triptolide) inhibits T-cell interleukin-2 expression at the level of purine-box/nuclear factor of activated T-cells and NF-κB transcriptional activation. *J Biol Chem*. 1999;274:13443–13450.
14. Zhao G, Vaszar LT, Qiu D, Shi L, Kao PN. Anti-inflammatory effects of triptolide in human bronchial epithelial cells. *Am J Physiol Lung Cell Mol Physiol*. 2000;279:L958–L966.
15. Tao X, Schulze-Koops H, Ma L, et al. Effects of *Tripterygium wilfordii* hook F extracts on induction of cyclooxygenase 2 activity and prostaglandin E2 production. *Arthritis Rheum*. 1998;41: 130–138.
16. Lu Y, Fukuda K, Seki K, et al. Inhibition by triptolide of IL-1-induced collagen degradation by corneal fibroblasts. *Invest Ophthalmol Vis Sci*. 2003;44:5082–5088.
17. Lu Y, Fukuda K, Nakamura Y, et al. Inhibitory effect of triptolide on chemokine expression induced by proinflammatory cytokines in human corneal fibroblasts. *Invest Ophthalmol Vis Sci*. 2005;46: 2346–2352.
18. Kumagai N, Fukuda K, Nishida T. Synergistic effect of TNF-α and IL-4 on the expression of thymus- and activation-regulated chemokine in human corneal fibroblasts. *Biochem Biophys Res Commun*. 2000;279:1–5.
19. Kumagai N, Fukuda K, Fujitsu Y, Nishida T. Expression of functional ICAM-1 on cultured human keratocytes induced by tumor necrosis factor-α. *Jpn J Ophthalmol*. 2003;47:134–141.
20. Schultz CL, Buret AG, Olson ME, et al. Lipopolysaccharide entry in the damaged cornea and specific uptake by polymorphonuclear neutrophils. *Infect Immun*. 2000;68:1731–1734.
21. Kishimoto T, Akira S, Narazaki M, Taga T. Interleukin-6 family of cytokines and gp130. *Blood*. 1995;86:1243–1254.
22. Yoshimura T, Matsushima K, Tanaka S, et al. Purification of a human monocyte-derived neutrophil chemotactic factor that has peptide sequence similarity to other host defense cytokines. *Proc Natl Acad Sci USA*. 1987;84:9233–9237.
23. Matsushima K, Larsen CG, DuBois GC, Oppenheim JJ. Purification and characterization of a novel monocyte chemotactic and activating factor produced by a human myelomonocytic cell line. *J Exp Med*. 1989;169:1485–1490.
24. Kumagai N, Fukuda K, Ishimura Y, Nishida T. Synergistic induction of eotaxin expression in human keratocytes by TNF-α and IL-4 or IL-13. *Invest Ophthalmol Vis Sci*. 2000;41:1448–1453.
25. Lin N, Sato T, Ito A. Triptolide, a novel diterpenoid triepoxide from *Tripterygium wilfordii* Hook. f., suppresses the production and gene expression of pro-matrix metalloproteinases 1 and 3 and augments those of tissue inhibitors of metalloproteinases 1 and 2 in human synovial fibroblasts. *Arthritis Rheum*. 2001;44:2193–2200.
26. Pitrak DL. Effects of granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor on the bactericidal functions of neutrophils. *Curr Opin Hematol*. 1997;4:183–190.
27. Maurer M, von Stebut E. Macrophage inflammatory protein-1. *Int J Biochem Cell Biol*. 2004;36:1882–1886.
28. Pardi R, Inverardi L, Bender JR. Regulatory mechanisms in leukocyte adhesion: flexible receptors for sophisticated travelers. *Immunol Today*. 1992;13:224–230.
29. Rothenberg ME. Eosinophilia. *N Engl J Med*. 1998;338:1592–1600.
30. Goldberg MF, Ferguson TA, Pepose JS. Detection of cellular adhesion molecules in inflamed human corneas. *Ophthalmology*. 1994; 101:161–168.
31. Kelly KJ, Williams WW Jr, Colvin RB, et al. Intercellular adhesion molecule-1-deficient mice are protected against ischemic renal injury. *J Clin Invest*. 1996;97:1056–1063.
32. Lu Y, Fukuda K, Liu Y, Kumagai N, Nishida T. Dexamethasone inhibition of IL-1-induced collagen degradation by corneal fibroblasts in three-dimensional culture. *Invest Ophthalmol Vis Sci*. 2004;45:2998–3004.
33. Krishna G, Liu K, Shigemitsu H, et al. PG490–88, a derivative of triptolide, blocks bleomycin-induced lung fibrosis. *Am J Pathol*. 2001;158:997–1004.
34. Wu Y, Wang Y, Zhong C, et al. The suppressive effect of triptolide on experimental autoimmune uveoretinitis by down-regulating Th1-type response. *Int Immunopharmacol*. 2003;3:1457–1465.
35. Tao X, Cush JJ, Garret M, Lipsky PE. A phase I study of ethyl acetate extract of the Chinese antirheumatic herb *Tripterygium wilfordii* hook F in rheumatoid arthritis. *J Rheumatol*. 2001; 28:2160–2167.
36. Tao X, Younger J, Fan FZ, Wang B, Lipsky PE. Benefit of an extract of *Tripterygium wilfordii* Hook f in patients with rheumatoid arthritis: a double-blind, placebo-controlled study. *Arthritis Rheum*. 2002;46:1735–1743.
37. Wu SX, Guo NR. Clinical observation on effect of triptolide tablet in treating patients with psoriasis vulgaris. *Chin J Integr Med*. 2005;11:147–148.