Delayed Disruption of Barrier Function in Cultured Human Corneal Epithelial Cells Induced by Tumor Necrosis Factor-α in a Manner Dependent on NF-κB

Kazuhiro Kimura,[1] *Shinichiro Teranishi,*[2] *Ken Fukuda,*[2] *Koji Kawamoto,*[2] *and Teruo Nishida*[1,2]

Purpose. The corneal epithelium provides a barrier that is both important for corneal homeostasis and dependent on tight junctions (TJs) between adjacent epithelial cells. The authors examined the effects of tumor necrosis factor-α (TNF-α), a proinflammatory cytokine, on barrier function and the expression of TJ proteins in simian virus 40–transformed human corneal epithelial (HCE) cells.

Methods. The barrier function of cultured HCE cells was evaluated by measurement of transepithelial electrical resistance (TER). The subcellular distribution of the TJ proteins zonula occludens-1 (ZO-1) and occludin and that of the p65 subunit of nuclear factor-κB (NF-κB) were determined by immunofluorescence staining. The expression of ZO-1 and occludin and the phosphorylation and degradation of the NF-κB inhibitory protein IκB-α were examined by immunoblot analysis.

Results. TNF-α induced a decrease in the TER of HCE cells in a concentration- and time-dependent manner. It also induced the disappearance of ZO-1 from the interfaces of neighboring HCE cells without affecting the localization of occludin. The abundance of neither ZO-1 nor occludin was affected by TNF-α. TNF-α induced the phosphorylation and downregulation of IκB-α and the translocation of the p65 subunit of NF-κB to the nucleus. The NF-κB inhibitor curcumin blocked the effects of TNF-α on TER and the subcellular localization of ZO-1 at late phase.

Conclusions. TNF-α disrupted the barrier function of HCE cells, apparently by affecting the localization of ZO-1 at TJs in a manner dependent on NF-κB at late phase. This action of TNF-α may contribute to the loss of corneal epithelial barrier function associated with ocular inflammation. (*Invest Ophthalmol Vis Sci.* 2008;49:565–571) DOI:10.1167/iovs.07-0419

The corneal epithelium is the most superficial component of the cornea and serves as a barrier to prevent foreign material in the external environment, such as dust, water, and bacteria, from entering the eye.[1–4] Barrier function of the corneal epithelium can be disrupted as a result of bacterial infection or inflammation, however, resulting in various pathologic conditions of the epithelium and in stromal edema, infection, or melting.[5–7] Barrier function is thus critical for corneal homeostasis, including maintenance of corneal transparency. The epithelial barrier is formed by the apical plasma membrane and intercellular tight junctions (TJs) of the epithelial cells,[8,9] which provide a physical and a functional barrier.[10] TJs consist of multiple transmembrane, scaffolding, and signaling proteins. Transmembrane proteins of TJs include occludin, claudin, and junctional adhesion molecules (JAMs). In addition, zonula occludens (ZO-1, ZO-2, and ZO-3) proteins interact with the transmembrane proteins at the cytoplasmic face of the cell membrane and serve to anchor them to the actin cytoskeleton.[11–13] Occludin, claudin, and ZO-1 are expressed in superficial and subsuperficial cells of the corneal epithelium[14–16] and contribute to barrier function.[7,17,18]

Inflammatory response to insults such as infection and injury is mediated by tissue-resident cells and infiltrated cells, both of which secrete various cytokines and growth factors and interact with each other through autocrine and paracrine systems. Tumor necrosis factor-α (TNF-α) is a proinflammatory cytokine[19] and contributes to ocular inflammation.[20] In particular, TNF-α is thought to play an important role in pathologic conditions such as injury,[21,22] allergy,[23] infection,[24] and dry eye.[25] TNF-α also induces the production of other cytokines (including IL-6 and IL-8) and growth factors by corneal epithelial cells and stromal cells.[10,26,27] Ocular inflammation has additional effects on the structure and function of the corneal epithelium in diseases of the ocular surface.[28–30]

To provide insight into the mechanism by which inflammation can result in disruption of the barrier function of the corneal epithelium, we investigated the effects of TNF-α on such function in cultured corneal epithelial cells by measurement of transepithelial electrical resistance (TER) and distribution of ZO-1 and occludin. Given that nuclear factor-κB (NF-κB) is a key mediator of TNF-α actions,[31–33] we also examined whether the NF-κB signaling pathway might contribute to the effects of this cytokine on corneal epithelial cells.

Methods

Materials

Dulbecco modified Eagle medium–nutrient mixture F12 (DMEM-F12), phosphate-buffered saline (PBS), fetal bovine serum (FBS), trypsin-EDTA, and gentamicin were obtained from Invitrogen-Gibco (Carlsbad, CA). Bovine serum albumin (BSA), bovine recombinant insulin, cholera toxin, human recombinant epidermal growth factor, and protease inhibitor cocktail were obtained from Sigma-Aldrich (St. Louis, MO). Recombinant human TNF-α was from R&D Systems (Minneapolis, MN), and curcumin was from Merck (Darmstadt, Germany). Six- or 24-well transwell plates and 24- or 96-well culture plates were obtained from Corning (Corning, NY). Rabbit polyclonal antibodies to ZO-1 (0.25 mg/mL) or to occludin (0.25 mg/mL) were obtained from Zymed-Invitrogen (Carlsbad, CA). Rabbit polyclonal antibodies to the p65

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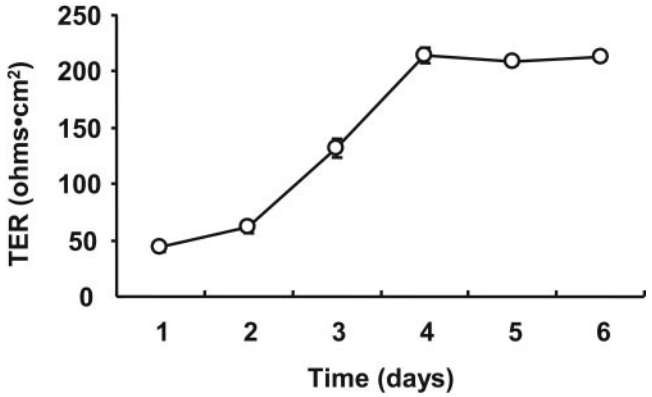

FIGURE 1. Establishment of barrier function by HCE cells. Cells were plated in a transwell apparatus and maintained in complete medium for 6 days. TER was measured daily. Data are mean ± SE from four independent experiments.

subunit of NF-κB (0.2 mg/mL), mouse monoclonal antibodies to IκB-α (0.2 mg/mL) or to phosphorylated IκB-α (0.2 mg/mL), and normal rabbit immunoglobulin G (IgG) were from Santa Cruz Biotechnology (Santa Cruz, CA). Mouse monoclonal antibodies to myosin light chain (MLC; 0.2 mg/mL) and to β-actin (27 mg/mL) were obtained from Sigma-Aldrich, and rabbit polyclonal antibodies to phosphorylated MLC (0.1 mg/mL) were from Cell Signaling (Danvers, MA). Horseradish peroxidase–conjugated goat antibodies to mouse or rabbit IgG and detection reagents (ECL Plus) were from Amersham Biosciences GE Healthcare (Little Chalfont, UK). Counterstain (TOTO-3) and fluorescent dye (AlexaFluor 488)–labeled goat antibodies to rabbit IgG were from Invitrogen (Carlsbad, CA). A cytotoxicity assay kit (CytoTox96 Non-Radioactive Cytotoxicity Assay) was obtained from Promega (Madison, WI).

Cell Culture

Simian virus 40–immortalized human corneal epithelial (HCE) cells were obtained from RIKEN Biosource Center (Tokyo, Japan). The cells were originally established and characterized by Araki-Sasaki et al.[34] They were passaged in supplemented hormonal epithelial medium (SHEM), which consists of DMEM-F12 supplemented with 15% heat-inactivated FBS, bovine insulin (5 μg/mL), cholera toxin (0.1 μg/mL), recombinant human epidermal growth factor (10 ng/mL), and gentamicin (40 μg/mL). For experiments, HCE cells were plated at a density of 1×10^5 or 5×10^5 cells per well in 24- or six-well transwell plates, respectively, or at 1×10^4 or 5×10^4 cells per well in 96- or 24-well culture plates, respectively. They were then cultured for 4 days in SHEM and for 24 hours in unsupplemented DMEM-F12 before exposure to TNF-α or curcumin in the latter medium.

Measurement of TER

HCE cells were cultured in 24-well transwell plates on filters with a pore size of 0.4 μm. Resistance was measured with the use of an EVOM volt-ohm meter (World Precision Instruments, Sarasota, FL), and TER (Ω/cm^2) was calculated by multiplying the measured resistance by the area of the transwell filter. Background resistance caused by the filter alone was subtracted from the experimental values.

Immunofluorescence Analysis

HCE cells were cultured in 24-well culture plates. For ZO-1 or occludin staining, the cells were fixed with 100% methanol for 20 minutes at room temperature. For staining of the p65 subunit of NF-κB, the cells were fixed with 4% paraformaldehyde in PBS, washed with PBS, and permeabilized with 100% methanol for 5 minutes at −20°C. All cells were then washed with PBS and incubated at room temperature for 1 hour with 1% BSA in PBS and then for 1 hour with antibodies to ZO-1, to occludin, or to p65 (or with normal rabbit IgG) at a 1:100 dilution in PBS containing 1% BSA. After washing with PBS, the cells were incubated at room temperature for 1 hour with fluorescent dye (AlexaFluor 488; Invitrogen)–labeled secondary antibodies at a 1:1000 dilution in PBS containing 1% BSA and then for 10 minutes with counterstain (TOTO-3; Invitrogen) for staining of nuclei. They were examined with a laser confocal microscope (LSM5; Carl Zeiss, Wexford, Germany).

Immunoblot Analysis

HCE cells were cultured in six-well transwell plates. They were lysed in 300 μL of a solution containing 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM EDTA, 5 mM NaF, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, 1 mM Na_3VO_4, and 1% protease inhibitor cocktail. Cell lysates were centrifuged at 15,000*g* for 10 minutes at 4°C, and the resultant supernatants were subjected to SDS-PAGE on a 7.5% gel. Separated proteins were transferred to a nitrocellulose membrane, which was then incubated at 4°C first for 16 hours with blocking solution (20 mM Tris-HCl [pH 7.4], 5% skim milk, 0.1% Tween 20) and then for 16 hours with antibodies to ZO-1, to occludin, to IκB-α, to phosphorylated IκB-α, to MLC, to phosphorylated MLC, or to actin at a 1:1000 dilution in blocking solution. After they were washed with a solution containing 20 mM Tris-HCl (pH 7.4) and 0.1% Tween 20, the membrane was incubated for 1 hour at room temperature with horseradish peroxidase–conjugated secondary antibodies at a 1:1000 dilution in the same solution, washed again, incubated with detection reagents (ECL Plus; Amersham Biosciences GE Healthcare) for 5 minutes, and exposed to film.

Cytotoxicity Assay

HCE cells were cultured in 96-well culture plates. Cytotoxicity of TNF-α was evaluated by determination of lactate dehydrogenase (LDH) activity released into the culture medium during 24 hours with the use of a nonradioactive assay (CytoTox96; Promega). Absorbance at 490 nm was measured with a microplate reader. Data were compared with the amount of LDH activity released from nontreated cells exposed to lysis solution and with baseline LDH release from nontreated cells exposed to culture medium only.

Statistical Analysis

Quantitative data are presented as mean ± SE. Differences were analyzed by Dunnett multiple comparison test. $P < 0.05$ was considered statistically significant.

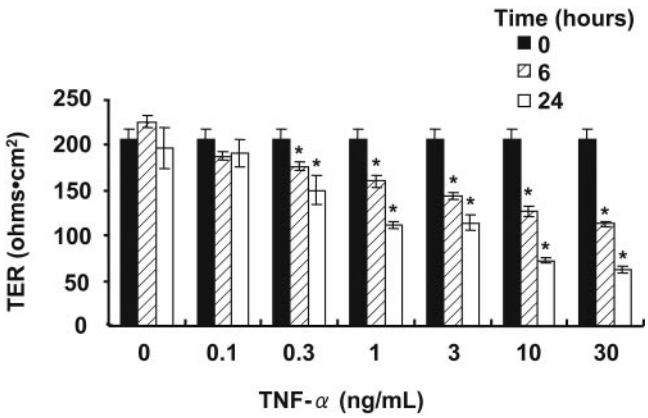

FIGURE 2. Concentration- and time-dependent effect of TNF-α on the barrier function of HCE cells. Cells were cultured in transwell plates and incubated with the indicated concentrations of TNF-α for 0, 6, or 24 hours, after which TER was determined. Data are mean ± SE from four independent experiments. *$P < 0.05$ versus value for cells incubated without TNF-α.

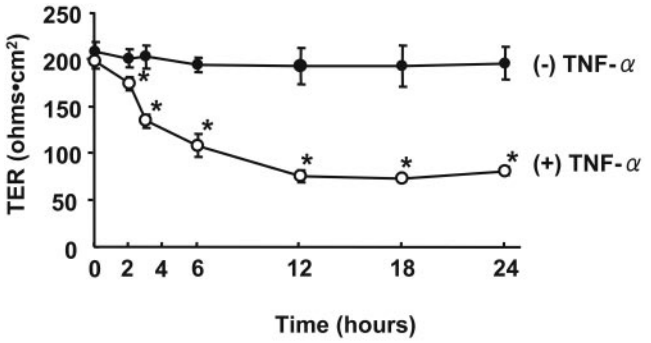

FIGURE 3. Time course of the effect of TNF-α on the barrier function of HCE cells. Cells were cultured in transwell plates and incubated for the indicated times in the absence or presence of TNF-α (10 ng/mL), after which TER was determined. Data are mean ± SE from four independent experiments. $^*P < 0.05$ versus corresponding value for cells incubated without TNF-α.

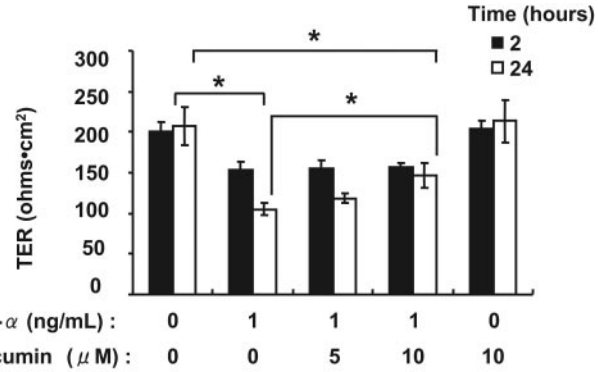

FIGURE 5. Inhibition by curcumin of the TNF-α–induced decrease in TER of HCE cells. Cells were incubated for 60 minutes with curcumin (0, 5, or 10 μM) and then for 2 or 24 hours in the additional absence or presence of TNF-α (1 ng/mL), after which TER was determined. Data are mean ± SE from four independent experiments. $^*P < 0.05$.

RESULTS

To evaluate the barrier function of HCE cells, we measured the TER of transwell cultures. TER increased in a time-dependent manner during culture for 4 days and then reached a plateau that was maintained for at least 2 days (Fig. 1), indicating that barrier function was established at this time. Exposure of HCE cells that had established barrier function to TNF-α (0–30 ng/mL) for 24 hours resulted in a concentration-dependent decrease in TER; this effect was significant at TNF-α concentrations of ≥0.3 ng/mL and was maximal at 30 ng/mL (Fig. 2). The effect of TNF-α at concentrations of ≥0.3 ng/mL was also time dependent (Fig. 2), with that of TNF-α at 10 ng/mL significant between 2 and 24 hours and maximal at 12 to 24 hours after exposure of HCE cells to this cytokine (Fig. 3).

To examine the possible role of the NF-κB signaling pathway in the effect of TNF-α on TER in HCE cells, we first investigated whether TNF-α induces the phosphorylation and degradation of the NF-κB–inhibitory protein IκB-α. Immunoblot analysis revealed that incubation of HCE cells with TNF-α (1 ng/mL) for 30 minutes resulted in the appearance of a phosphorylated form of IκB-α and a decrease in the overall abundance of this protein (Fig. 4A). Furthermore, treatment of the cells with the NF-κB inhibitor curcumin (10 μM) for 1 hour before exposure to TNF-α prevented these effects of this cytokine. We also examined whether TNF-α might affect the subcellular localization of the p65 subunit of NF-κB. Immunofluorescence microscopy revealed that p65 was largely restricted to the cytoplasm of HCE cells incubated in the absence of TNF-α (Fig. 4B). However, exposure of the cells to TNF-α (1 ng/mL) for 30 minutes induced the translocation of p65 from the cytoplasm to the nucleus. Again, this effect of TNF-α was prevented by curcumin (10 μM). These results thus showed that TNF-α activates the NF-κB signaling pathway in HCE cells.

To determine whether NF-κB activation is required for the TNF-α–induced decrease in the barrier function of HCE cells, we treated the cells with various concentrations of curcumin for 1 hour before exposure to TNF-α (1 ng/mL) for 2 or 24 hours. Curcumin inhibited in a concentration-dependent manner the effect of TNF-α on TER that was apparent at 24 hours, but it did not significantly change the effect apparent at 2 hours (Fig. 5). These results thus indicated that NF-κB activation is required for the TNF-α–induced increase in permeability of the cell monolayer apparent at 24 hours.

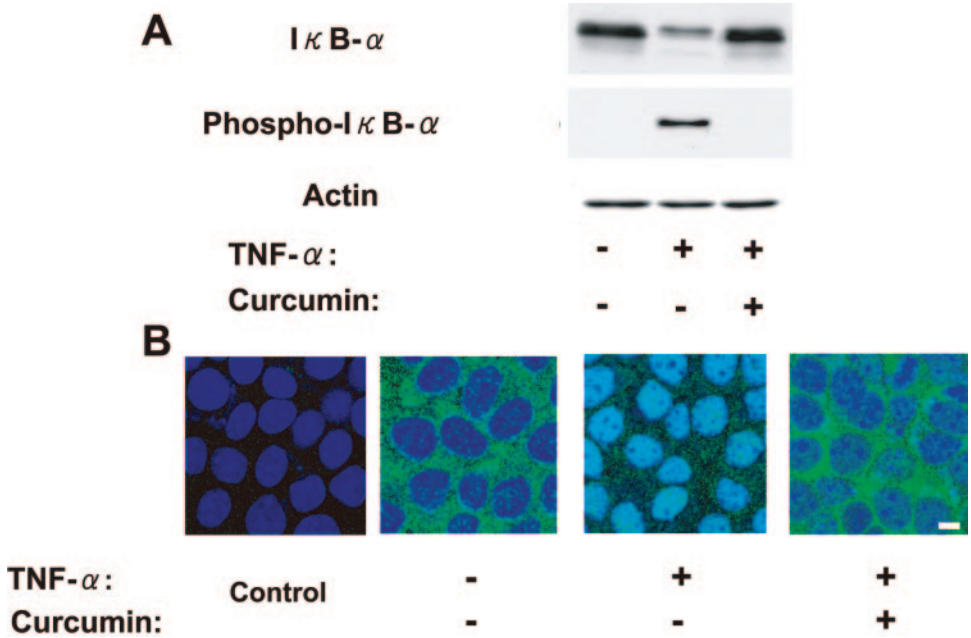

FIGURE 4. Activation of the NF-κB signaling pathway by TNF-α in HCE cells. (**A**) Cells were incubated for 1 hour in the absence or presence of curcumin (10 μM) and then for 30 minutes in the additional absence or presence of TNF-α (1 ng/mL). Cell lysates were then subjected to immunoblot analysis with antibodies to IκB-α, to phosphorylated IκB-α, or to actin (loading control). (**B**) Cells were incubated for 1 hour in the absence or presence of curcumin (10 μM) and then for 30 minutes in the additional absence or presence of TNF-α (1 ng/mL). The cells were then fixed and subjected to immunofluorescence analysis with antibodies to the p65 subunit of NF-κB (*green*). Nuclei were detected by staining with TOTO-3 (*blue*). As a control, untreated cells were stained with normal rabbit IgG in place of anti-p65. Scale bar, 10 μm. Data in (**A**) and (**B**) are representative of three independent experiments.

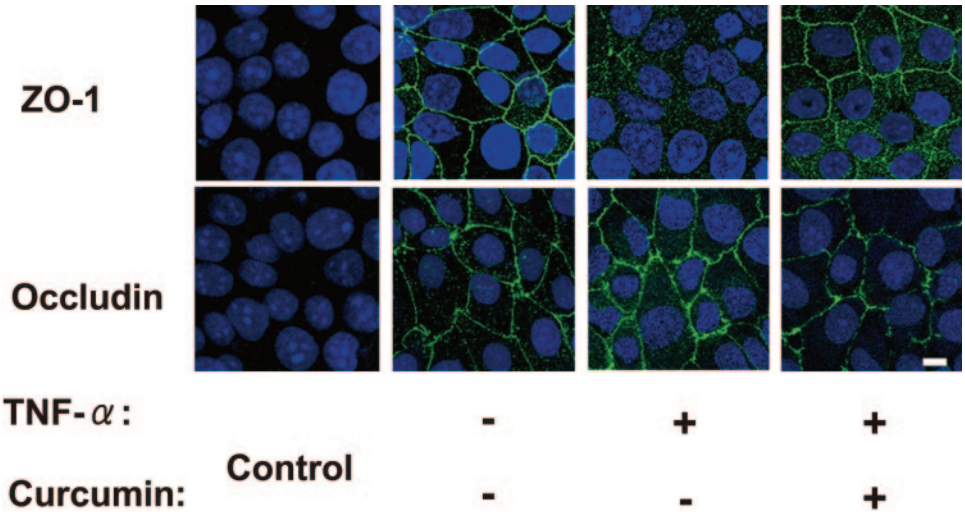

FIGURE 6. Effect of TNF-α on the distribution of ZO-1 and occludin in HCE cells. Cells were incubated in the absence or presence of curcumin (10 μM) for 1 hour and then in the additional absence or presence of TNF-α (1 ng/mL) for 24 hours. The cells were then fixed and subjected to immunofluorescence analysis with antibodies to ZO-1 or to occludin (*green*). Nuclei were stained with TOTO-3 (*blue*). As a control, untreated cells were stained with normal rabbit IgG in place of specific primary antibodies. Scale bar, 10 μm. Data are representative of three independent experiments.

To investigate the effect of TNF-α on TJ structure in HCE cells, we examined the distribution of the TJ proteins ZO-1 and occludin by immunofluorescence microscopy. ZO-1 and occludin were localized at the interfaces of adjacent HCE cells in the absence of TNF-α (Fig. 6). However, exposure of the cells to TNF-α (1 ng/mL) for 24 hours resulted in a loss of ZO-1 immunoreactivity from the cellular borders, and this effect was blocked by curcumin (10 μM). In contrast, the distribution of occludin at the cell surface was not affected by TNF-α in the absence or presence of curcumin. We investigated the time course of the effect of TNF-α on ZO-1 distribution. Exposure of the cells to TNF-α (1 ng/mL) for 2 or 4 hours did not affect the localization of ZO-1 at the cell surface, whereas exposure to the cytokine for 12 or 24 hours induced a loss of ZO-1 immunoreactivity from the cellular borders in a manner sensitive to curcumin (Fig. 7). We also examined the expression of ZO-1 and occludin in HCE cells by immunoblot analysis. Exposure of the cells to TNF-α (1 ng/mL) for 24 hours had no effect on the abundance of either TJ protein (Fig. 8).

We next investigated the effect of TNF-α on MLC phosphorylation (Fig. 9). Immunoblot analysis revealed that incubation of HCE cells with TNF-α (1 ng/mL) for 24 hours resulted in the appearance of the phosphorylated form of MLC and that this MLC phosphorylation was inhibited by curcumin (10 μM). The overall abundance of MLC was not affected by TNF-α in the absence or presence of curcumin.

Finally, we investigated whether TNF-α or curcumin might exert a cytotoxic effect in HCE cells by measuring the release of LDH. Treatment of the cells for 24 hours with TNF-α (1 or 10 ng/mL) or curcumin (5 or 10 μM) did not significantly increase the release of this cytosolic enzyme (Fig. 10).

DISCUSSION

We have shown that TNF-α, a proinflammatory cytokine, reduced the barrier function of HCE cells, as revealed by the measurement of TER. Moreover, immunofluorescence analysis revealed that TNF-α induced the disappearance of the TJ protein ZO-1 from the interfaces of adjacent HCE cells. Immunoblot analysis showed that this effect was not accompanied by the downregulation of ZO-1 expression. Neither the distribution nor the expression of occludin was affected by TNF-α. We also found that TNF-α activated the NF-κB signaling pathway in HCE cells and that the NF-κB inhibitor curcumin blocked the effects of TNF-α on both TER and the localization of ZO-1. Neither TNF-α nor curcumin had a cytotoxic effect on HCE cells. Our results thus suggest that TNF-α disrupts the barrier function of HCE cells by inducing the loss of ZO-1 from cell borders in a manner dependent on the NF-κB signaling pathway.

Although we found that TNF-α activated the NF-κB signaling pathway and reduced barrier function in HCE cells, the NF-κB

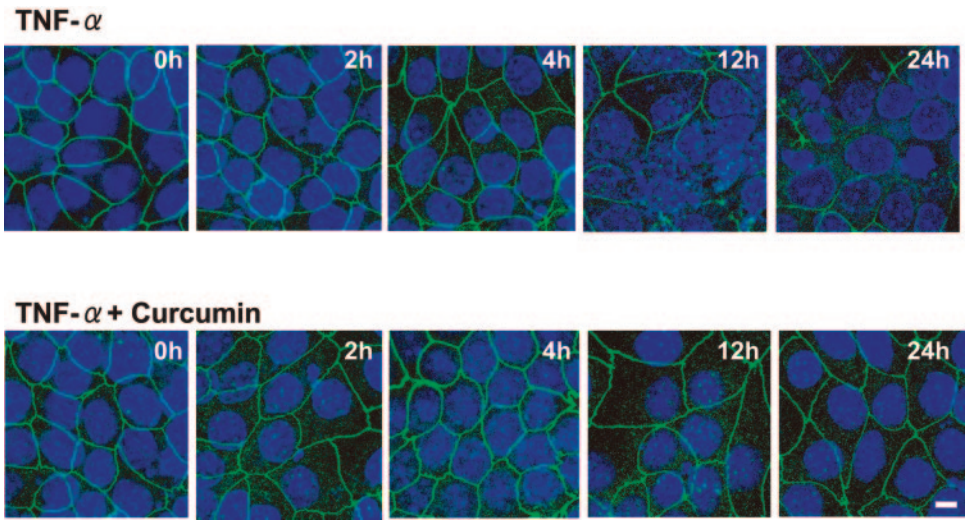

FIGURE 7. Time course of the effect of TNF-α on the distribution of ZO-1 in HCE cells. Cells were incubated in the absence or presence of curcumin (10 μM) for 1 hour and then in the additional presence of TNF-α (1 ng/mL) for 0, 2, 4, 12, or 24 hours. The cells were then fixed and subjected to immunofluorescence analysis with antibodies to ZO-1 (*green*). Nuclei were stained with TOTO-3 (*blue*). Scale bar, 10 μm. Data are representative of three independent experiments.

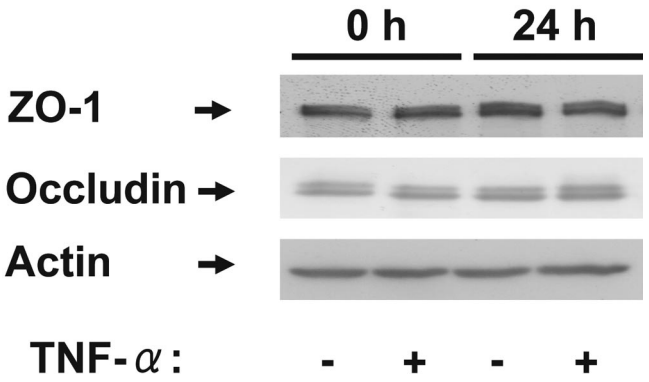

FIGURE 8. Lack of effect of TNF-α on the expression of ZO-1 and occludin in HCE cells. Cells were incubated for 0 or 24 hours in the absence or presence of TNF-α (1 ng/mL), after which cell lysates were subjected to immunoblot analysis with antibodies to ZO-1, to occludin, or to actin (loading control). Data are representative of three independent experiments.

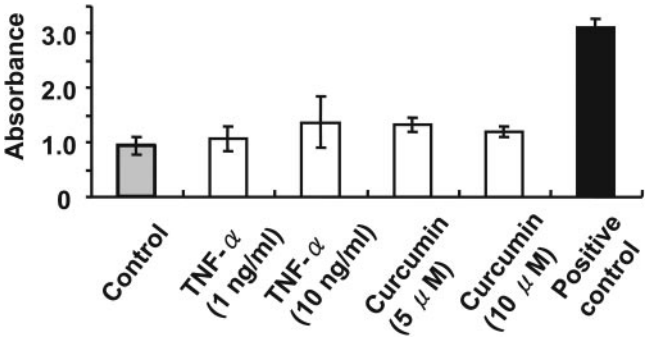

FIGURE 10. Lack of a cytotoxic effect of TNF-α or curcumin on HCE cells. Cells were incubated in the absence or presence of TNF-α (1 or 10 ng/mL) or curcumin (5 or 10 μM) for 24 hours, after which culture supernatants were assayed for LDH with a colorimetric assay and measurement of absorbance at 490 nm. The amount of LDH released from cells by cell lysis solution was determined as a positive control. Data are mean ± SE from four independent experiments.

inhibitor curcumin blocked the effect of TNF-α on TER apparent at 24 hours but not that apparent at 2 hours. We also showed that TNF-α did not affect the distribution of ZO-1 at the cell surface at 2 or 4 hours, with the TNF-α–induced loss of ZO-1 immunoreactivity from the cellular borders apparent at 12 to 24 hours blocked by curcumin. TNF-α has been shown to activate several additional signaling pathways, including those mediated by various isoforms of mitogen-activated protein kinase (ERK, p38, or JNK) or by JAK-STAT, in other cell types.[35–37] Our results suggest that the initial phase of the TNF-α–induced change in TER in HCE cells is not directly related to the dissociation of ZO-1 from TJs or to the NF-κB signaling pathway.

TNF-α has been detected in the corneas of patients or model animals with keratitis.[10,38] Moreover, the amount of TNF-α in tear fluid was found to be markedly increased in humans with ocular allergy or keratitis.[39,40] We have previously shown that TNF-α downregulates intercellular communication mediated by gap junctions in human corneal fibroblasts.[41] These observations thus suggest that TNF-α is an important contributor to inflammation in the cornea. We have now shown that TNF-α disrupts the barrier function of cultured HCE cells. TNF-α has also been shown to disrupt barrier function in other types of epithelial cell, including retinal pigment epithelial cells, intestinal epithelial cells, and airway epithelial cells.[42–44]

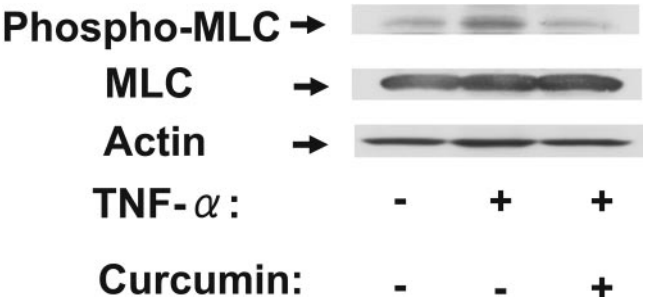

FIGURE 9. Effect of TNF-α on the phosphorylation of MLC in HCE cells. Cells were incubated in the absence or presence of curcumin (10 μM) for 1 hour and then in the additional absence or presence of TNF-α (1 ng/mL) for 24 hours, after which cell lysates were subjected to immunoblot analysis with antibodies to phosphorylated MLC, to total MLC, or to actin (loading control). Data are representative of three independent experiments.

TNF-α was found to induce the disappearance of ZO-1 from the interfaces of neighboring HCE cells. The distribution of ZO-1 at apical cell-cell junctions is thought to reflect the formation of a tight barrier in corneal epithelial cells.[15,45] Indeed, the translocation of ZO-1 to the apical surface is an important step in barrier formation.[9,46] These observations suggest that the redistribution of ZO-1 induced by TNF-α in HCE cells contributes to the disruption of barrier function induced by this cytokine. The loss of ZO-1 from the borders of HCE cells revealed by immunofluorescence analysis was not accompanied by a decrease in the total amount of ZO-1 revealed by immunoblot analysis. The lack of a correlation between the total cellular expression of TJ-associated proteins and their junctional localization has been demonstrated.[47–49] In contrast, TNF-α was shown to downregulate transcription of the occludin gene.[50] Our findings suggest that TNF-α may affect the structure and function of TJs in HCE cells by modulating the junctional localization of ZO-1 rather than its expression.

TNF-α regulates the proteolysis of various proteins.[51,52] It upregulates the expression of metalloproteinase 9 (MMP9) and MMP13 in certain cell types, including corneal epithelial cells.[53–55] An increase in MMP9 activity at the ocular surface in response to dryness has been shown to induce disruption of corneal epithelial barrier function as a result of the loss of TJs from superficial corneal epithelial cells.[56] Dry eye is associated with an increase in the concentration of TNF-α in tear fluid.[25] MMP9 knockout mice were shown to be resistant to the disruption of corneal epithelial barrier function associated with experimental dry eye.[56] TNF-α also regulates the ubiquitin system, which targets proteins for degradation, in a manner dependent on NF-κB activity.[57,58] We have now shown that the activation of NF-κB was required for the disruption of barrier function in HCE cells and the associated redistribution of ZO-1 induced by TNF-α. Activation of the ubiquitin system alters the distribution of ZO-1, resulting in loss of the integrity of TJs, in kidney epithelial cells.[59] Even though an overall decrease in ZO-1 abundance was not detected by immunoblot analysis, it is thus possible that the activation of MMPs or the ubiquitin system by TNF-α contributes to the disruption of the TJ structure by the proteolysis of ZO-1 at the borders of HCE cells.

TJ proteins are structurally and functionally associated with perijunctional actin filaments that regulate barrier function.[60,61] Moreover, MLC phosphorylation induces the reorganization of perijunctional actin and disrupts TJs.[62,63] TNF-α induces MLC kinase expression and MLC phosphorylation and thereby increases TJ permeability in intestinal epithelial cells.[62,64,65] We also found that TNF-α induced MLC phosphor-

ylation in HCE cells, suggesting that remodeling of perijunctional actin associated with this effect may contribute to the barrier disruption elicited by TNF-α in these cells.

TNF-α did not exert a cytotoxic effect in HCE cells, as revealed by the measurement of LDH release. The barrier disruption induced by TNF-α in intestinal epithelial cells was also found not to correlate with apoptosis.[66] In contrast, TNF-α induces apoptosis in other epithelial cell types, and this effect contributes to barrier disruption.[67,68] Although our results suggest that the barrier disruption induced by TNF-α in HCE cells is not attributable to cell damage or cell death, we are unable to exclude completely a contribution of apoptosis to this effect. We also found that, under our experimental conditions, TER reached a relatively stable plateau of $214 \pm 3.2\ \Omega/cm^2$ after culture of HCE cells for 4 days. Steady state TER values for intestinal or kidney epithelial cells have previously been determined as 327 ± 9 and $121 \pm 4\ \Omega/cm^2$, respectively,[66,69] similar to the value for HCE cells.

Ocular inflammation is associated with the production of various cytokines and growth factors and the disruption of TJs in the corneal epithelium, resulting in an increase in paracellular permeability. Disruption of corneal epithelial barrier function by TNF-α may thus play an important role in corneal epithelial disorders associated with ocular inflammation. Maintenance of corneal epithelial TJs is thus a potential goal in the development of new drug therapies for corneal epithelial disorders.

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