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Corneal myofibroblast viability: Opposing effects of IL-1 and TGF β 1

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Abstract

The purpose of this study was to test the effect of corneal epithelial scrape on myofibroblasts associated with haze and elucidate the effect of interleukin-1 and transforming growth factor beta-1 on corneal stromal myofibroblasts viability and death *in vitro*. Corneal epithelial scrape was performed in rabbit eyes with severe haze at one month after -9 diopter photorefractive keratectomy. Corneas were processed for immunocytochemistry for myofibroblast marker α -smooth muscle actin (α -SMA) and the TUNEL assay to detect apoptosis. Rabbit corneal fibroblasts were cultured with 2 ng/ml of transforming growth factor- β 1 (TGF β 1) to induce myofibroblast differentiation confirmed by monitoring α -SMA expression. Fluorescence-based TUNEL assay was performed to analyze the apoptotic response of myofibroblasts to IL-1 α or IL-1 β in the presence or absence of TGF β 1. Dose response experiments were performed after withdrawal of TGF β 1 and exposure to 1, 5, or 10 ng/ml of IL-1 α or IL-1 β for 1 hour. Subsequent experiments were performed with myofibroblasts exposed to 5 ng/ml IL-1 α or IL-1 β in conjunction with 0, 1, 5, or 10 ng/ml TGF β 1. Corneal epithelial scrape with a scalpel blade produced myofibroblast apoptosis. Exposure to TGF β 1 *in vitro* resulted in greater than 99% transformation of corneal fibroblasts to α -SMA+ myofibroblasts. There was a statistically significant dose dependent increase in the percentage of TUNEL+ cells with either IL-1 α or IL-1 β initiated at concentrations as low as 1 ng/ml. For example, after withdrawal of TGF β 1, the % TUNEL+ cells at 1 hour after exposure to IL-1 α increased significantly with increasing concentration (0 ng/ml, 2.4 $\pm$ 0.8 [S.E.M.] %; 1 ng/ml, 15.4 $\pm$ 1.8%; 5 ng/ml, 47.4 $\pm$ 3.9%; or 10 ng/ml, 70.3 $\pm$ 3.2%). Similar results were obtained with IL-1 β . The differences between the means of apoptotic myofibroblasts for the different concentrations of cytokine for either IL-1 α or IL-1 β were significantly different (ANOVA p <0.001). When myofibroblasts were exposed to 5 ng/ml IL-1 α or IL-1 β , the % TUNEL+ cells at 1 hour were reduced in a significant dose dependent manner when TGF β 1 at a concentration of 5 ng/ml or 10 ng/ml was present in the medium (ANOVA p <0.01). IL-1 α or IL-1 β triggers the death of myofibroblasts *in vitro* and TGF β 1 reduces the IL-1 effect on cell death. TGF β 1 and IL-1 have opposing effects on myofibroblast viability and likely interact to modulate haze generation after corneal injury.

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1. Introduction

Corneal surgery, injury or infection frequently triggers the appearance of stromal myofibroblasts associated with corneal opacity or haze. The opacity develops as a result of diminished transparency of the cells themselves and the production of disordered extracellular matrix components (Masur, et al., 1996; Jester, et al., 1999a; Wilson, et al., 1999). The development of myofibroblasts has been best-characterized utilizing *in vitro* models and following photorefractive keratectomy in animal models (Masur, et al., 1996; Jester, et al., 1999a; Mohan, et al., 2003; Mohan et al., 2008). *In vitro* studies have demonstrated conclusively that the cytokine transforming growth factor (TGF) β is a key cytokine in the development of corneal myofibroblasts (Masur, et al., 1996; Jester, et al., 1999b). Recent studies have also supported an important role for platelet-derived growth factor (PDGF) in corneal myofibroblast development (Jester, et al., 2002; Kaur, et al., in press). Structural and functional defects in the epithelial basement membrane are probably requisite to penetration of epithelium-derived TGF β into the stroma at sufficient concentration to modulate development of stromal myofibroblasts from precursor cells (Masur, et al., 1996; Jester, et al., 1999a; Netto, et al., 2006a; Mohan, et al., 2008).

Corneal epithelial and anterior stromal scrape often have a transient beneficial effect on anterior stromal opacity (haze) after photorefractive keratectomy (Kim, et al., 1999). Haze typically recurs, however, after this treatment unless topical mitomycin C is also applied to prevent myofibroblast repopulation (Netto, et al., 2006b). The mechanism of the transient decrease in the corneal haze after epithelial scrape or the modulators involved in spontaneous myofibroblast death have not been well characterized.

Interleukin (IL)-1 is an important modulator of stromal cellular functions (Wilson, et al., 1999). For example, IL-1 has been found to modulate apoptosis of keratocytes in the anterior stroma *in vivo* and *in vitro* (Wilson, et al., 1996a; Wilson, et al., 1996b). Recent studies in other organs have demonstrated that IL-1 is also an important modulator of myofibroblast functions and viability, including myofibroblast apoptosis, and that IL-1 commonly has opposing effects to TGF β in regulating myofibroblast functions (Zhang, et al., 1997; Shephard, et al., 2004; Werner, Krieg, and Smola, 2007; Bonner, et al., 1998; Wang, et al., 2000; Zhang, Gharaee-Kermani, and Phan, 1997; Zhang and Phan, 1999). The purpose of this study was to determine whether myofibroblasts undergo apoptosis in response to epithelial scrape injury and to investigate the effects of TGF β 1 and IL-1 α and IL-1 β on corneal myofibroblasts *in vitro*.

2. Materials and Methods

Induction of haze in rabbit corneas and corneal processing for TUNEL and immunocytochemistry

The Animal Control Committee at the Cleveland Clinic Foundation approved the animal studies included in this work. All animals were treated in accordance with the tenets of the ARVO statement for the Use of Animals in Ophthalmic and Vision Research. Twelve to 15 week old female New Zealand white rabbits weighing 2.5-3.0 kg each were included in this study. Haze was generated in one cornea of each rabbit using -9 diopter photorefractive keratectomy (PRK) with a Summit excimer laser (Alcon, Ft. Worth, TX), as previously described (Mohan, et al., 2003). At one month after PRK, three rabbits were euthanized and the corneas collected without additional manipulation using 0.12 forceps and sharp Westcott scissors. In four other rabbits, the animal had general anesthesia and the cornea with haze underwent epithelial scrape with a #64 Beaver blade (Fisher, Pittsburgh, PA). In two corneas, the epithelial scrape was light and injured the epithelium but did not remove it. In two corneas, heavy epithelial scrape was performed. At 1 hour after epithelial scrape, the animals were euthanized and the corneo-scleral rims were removed. The corneo-scleral rims were embedded

in liquid OCT compound (Sakura Finetek, Torrance, CA, USA) within a 24 mm × 24 mm × 5 mm mould (Fisher, Pittsburgh, PA). Specimens were centered within the mould so that the block could be bisected and transverse sections cut from the center of the cornea. Frozen tissue blocks were stored at -80° C until sectioning was performed. Central corneal sections (7 µm thick) were cut with a cryostat (HM 505M, Micron GmbH, Walldorf, Germany). Sections were placed on 25 mm × 75 mm × 1 mm microscope slides (Superfrost Plus, Fisher) and maintained frozen at -80° C until staining was performed.

Primary cell culture

Fresh rabbit corneas were obtained from Pel Freeze (Rogers, AR). The epithelial and endothelial layers were removed from corneas using 0.12 forceps and a #64 scalpel blade (BD Beaver, Franklin Lakes, NJ) under dissecting microscope using sterile technique. Small fragments of corneal stroma were then cut and placed in 60mm Primaria (BD biosciences, San Jose, CA) culture dishes with sufficient medium to cover the explants without floating them up from the bottom. Keratocytes were cultured in RSF media [MEM (JRH Bioscience, Lanexa, KS), 1% MEM vitamins 100X (Gibco, Grand Island, NY), 2% 50X Essential Amino acids (Gibco, Grand Island, NY), 1% 200mM L-Glutamine (Invitrogen Corporation, Carlsbad, CA, USA), 1% non-essential amino acids (Gibco, Grand Island, NY), 1% 100X antibiotic anti-mycotic solution (Sigma, St. Louis, MO), 1% sodium pyruvate (Gibco, Grand Island, NY), 7.5% 200 mM sodium bicarbonate (JRH Bioscience, Lanexa, KS), supplemented with 10% fetal bovine serum (FBS) (Invitrogen Corporation, Carlsbad, CA) and 5% fetal calf serum (FCS) (Gibco BRL, Grand Island, NY) at 37°C in a moist CO₂ incubator. The culture medium was changed every 48 hours until the primary cells grew to near-confluence. Primary cells were trypsinized and either stored in liquid nitrogen or immediately processed for further experiments. One to three passage corneal fibroblasts were used for all experiments.

Cytokines

Recombinant human interleukin-1α (IL-1α) and human interleukin-1β (IL-1β) were obtained from R & D Systems (Minneapolis, MN). Recombinant human transforming growth factor (TGF) β-1 was obtained from Sigma (St Louis, MO).

Dose-dependent effect of cytokines on corneal myofibroblasts

To determine the effect of cytokines on stromal myofibroblasts, corneal fibroblasts were first transformed to myofibroblasts using TGF β1. Corneal fibroblasts were seeded into Primaria 25cm² tissue culture flasks (Becton Dickinson, Franklin Lakes, NY) in RSF media with 10% FBS and were allowed to adhere for 24 hours. Cells were then cultured in RSF medium with 1% FBS and 2 ng/ml TGF β1. The culture medium with TGF β1 was replenished every 48 hours until the cells grew to confluence.

The dose-dependent effects of IL-1α and IL-1β were studied on the myofibroblasts. Myofibroblasts (10⁴ cells per well) were plated on eight-chamber, fibronectin-coated glass slides (BD Biosciences, Bedford, MA) and cultured in RSF media supplemented with 1% FBS for 24 to 48 hours until they were 60% to 70% confluent. The culture medium was changed to serum-free RSF medium and the cells were incubated for 24 hours on the fibronectin-coated glass slides before additional cytokines were added.

Cells were then treated with different cytokines by addition of IL-1α or IL-1β at varying concentrations from 1 to 10 ng/ml, with or without TGF β1 (varying from 1 to 10 ng/ml), in serum-free RSF. Four wells were treated with each concentration of IL-1, with or without TGF β1, for each time point. Cells with no cytokines in serum-free RSF were included as controls in each experiment.

Myofibroblast wells treated with IL-1, with or without TGF β 1, were analyzed for apoptosis using the TUNEL assay and α -SMA using immunocytochemistry. Each experiment was repeated at least three times and representative results are provided.

Immunocytochemistry

Immunofluorescent staining for α -SMA was performed using a mouse monoclonal anti-human α -smooth muscle actin clone 1A4 (Dako, Carpinteria, CA). Cell monolayers were washed with 4°C phosphate buffered saline (PBS) and fixed in chilled absolute acetone free methanol (Sigma, St. Louis, MO) for 15 minutes at -20°C. The slides or monolayers were washed twice with PBS and incubated for 90 minutes at room temperature with anti α -SMA monoclonal antibody at 1:50 dilution in 5% BSA. Slides or monolayers were then incubated at room temperature with secondary antibody, Alexa Fluor 488 (Invitrogen, Carlsbad, CA) goat anti-mouse IgG (H+L) (green) at a dilution of 1:100 in 5% BSA for 1 hour. Slides or monolayers were rinsed with PBS and coverslips were mounted with Vectashield containing DAPI (Vector Laboratories Inc., Burlingame, CA) to allow visualization of all nuclei.

Detection of apoptotic cells

Fragmented DNA characteristic of apoptosis was detected using the TUNEL assay according to a previously published method (Helena et al., 1998). Briefly, slides with corneal sections or monolayers of myofibroblasts that had been treated with the different cytokines (IL-1 α or IL-1 β , with or without TGF β 1) were first washed in PBS and fixed in Histochoice MB fixative (Electron Microscopy Science, Hatfield, PA, USA) for 30 minutes. A fluorescence-based TUNEL assay was then performed according to the manufacturer's instructions (Roche LTD, Indianapolis, IN, Cat. No. 12-156-792910).

Quantification of TUNEL-positive myofibroblasts in monolayers

For each chamber slide well, the total number of cell nuclei and the number of cells undergoing apoptosis were counted in randomly selected, noncontiguous 400X microscopic fields until 100 cells were counted. The results of four wells were averaged to obtain the result for a particular concentration of cytokine at each time point. The results were expressed as the percentage of apoptotic nuclei of myofibroblast cells/400X field.

Microscopy

Alpha SMA stained and TUNEL stained slides or monolayers of myofibroblasts were viewed and photographed with a Leica DM5000 microscope equipped with Q-Imaging Retiga 4000RV (Surrey, BC, Canada) camera and ImagePro software. Corneal sections were also viewed and photographed with the Leica DM5000 microscope.

Statistical analysis

Statistical comparisons between the groups were performed using analysis of variance (ANOVA) with Student-Newman-Keuls method test where applicable (Sigma Stat software 3.5). p values less than 0.01 were considered statistically significant.

3. Results

Effect of corneal epithelial scrape on corneal myofibroblasts

Light epithelial scrape of a rabbit cornea with haze and associated myofibroblasts triggered myofibroblast apoptosis detected with the TUNEL assay (Fig. 1). Heavy scrape of the epithelium resulted in complete removal of all detectable myofibroblasts in the anterior stroma of rabbit corneas with PRK-induced haze (not shown).

Effect of TGF β 1 on cultured stromal fibroblasts

Stromal fibroblasts cultured in presence of 2 ng/ml TGF β 1 and 1% FBS differentiated into myofibroblasts. After 4 to 5 days of culture with TGF β 1 greater than 99% of cells expressed the α -smooth muscle actin marker for mature myofibroblasts (Fig. 2).

Dose dependent effect of IL-1 α and IL-1 β on myofibroblast viability

A pilot study was performed in which myofibroblast monolayers were exposed to variable concentrations of IL-1 α or IL-1 β in serum free medium for 30 minutes, 1 hour or 3 hours. Monolayers were then fixed and stained with the TUNEL assay. These pilot studies indicated that concentrations of IL-1 α or IL-1 β from 1 to 10 ng/ml for 1 hour were ideal for quantitative studies of the effects of the cytokines on corneal myofibroblast apoptosis.

Quantitative dose response studies were then performed by exposing myofibroblast monolayers to 1, 5 or 10 ng/ml of IL-1 α or IL-1 β for 1 hour prior to staining with the TUNEL assay or immunocytochemistry α -SMA on adjacent slides of a culture plate (Fig. 3). The percentage myofibroblasts that underwent apoptosis in cells exposed to 0 ng/ml, 1 ng/ml, 5 ng/ml or 10 ng/ml of IL-1 α for one hour was 2.4 ± 0.8 (standard error of the mean), 15.4 ± 1.8 , 47.4 ± 3.9 , 70.3 ± 3.2 , respectively (Fig. 4a). The differences between the different concentrations of IL-1 α were statistically significant ($p < 0.01$, ANOVA). The percentage of cells that were undergoing apoptosis in cells exposed to 0 ng/ml, 1 ng/ml, 5 ng/ml or 10 ng/ml of IL-1 β for one hour was 2.4 ± 0.8 , 12.8 ± 0.7 , 53.5 ± 4.1 , 84.6 ± 3.7 , respectively (Fig. 4b). The differences between the different concentrations of IL-1 β were statistically significant ($p < 0.01$, ANOVA). Thus, there was a dose response increase in percentage of apoptotic myofibroblasts with increasing dosage of either of IL-1 α or IL-1 β .

Dose-dependent effect of TGF β 1 on IL-1 α - or IL-1 β -induced corneal myofibroblast apoptosis

Monolayers of corneal myofibroblasts were exposed to 5 ng/ml concentration of IL-1 α or IL-1 β and concurrent addition of 1, 5, or 10 ng/ml of TGF β 1 for one hour. TUNEL assays were then performed on the fixed cells. TGF β 1 at 0 ng/ml, 1 ng/ml, 5 ng/ml or 10 ng/ml resulted in 47.4 ± 3.9 , 47.3 ± 2.0 , 36.1 ± 3.4 , and 21.4 ± 2.3 percent apoptotic myofibroblasts, respectively, when added to cells concurrent to IL-1 α (Fig. 5a). The difference in the mean values for the 5 ng/ml and 10 ng/ml TGF β 1 concentrations with IL-1 α present were significantly different from the other TGF β 1 concentrations and from each other (ANOVA, $p < 0.01$). TGF β 1 at 0 ng/ml, 1 ng/ml, 5 ng/ml or 10 ng/ml resulted in 53.5 ± 4.1 , 52.6 ± 6.0 , 38.9 ± 4.8 , and 20.6 ± 2.1 percent apoptotic myofibroblasts, respectively, when added to cells concurrent to IL-1 β (Fig. 5b). The difference in the mean values for the 5 ng/ml and 10 ng/ml TGF β 1 concentrations with IL-1 β present were significantly different from the other TGF β 1 concentrations and from each other (ANOVA, $p < 0.01$). Thus, the percentage of myofibroblasts undergoing apoptosis triggered by either IL-1 α or IL-1 β was reduced in a dose response manner by exposure to TGF β 1.

4. Discussion

Previous studies have demonstrated that corneal myofibroblasts associated with haze undergo spontaneous apoptosis at low levels (Wilson, Chaurasia, and Medeiros, 2007). It was hypothesized that spontaneous myofibroblast apoptosis associated with the disappearance of stromal opacity is triggered when levels of TGF β levels fall to critical concentrations needed to support myofibroblast viability following repair of defective epithelial basement membrane (Netto, et al., 2006a). The present study demonstrates that epithelial scrape injury triggers extensive apoptosis of myofibroblasts associated with haze, similar to anterior keratocytes in the anterior stroma (Wilson, et al., 1996a). In order to detect myofibroblast apoptosis, the corneal epithelium must be scraped gently because vigorous scraping results in complete

removal of myofibroblasts and associated extracellular matrix. Even after vigorous scraping and removal of myofibroblasts, however, more myofibroblasts and haze recur (Kim, et al., 1999), although the response can be blunted by treatment with topical mitomycin C (Netto, et al., 2006b).

IL-1 has been shown to have a role in modulating keratocyte apoptosis that occurs in the anterior stroma in response to epithelial injury (Wilson, et al., 1996a; Wilson, et al., 1996b; Mohan, et al., 1997). In other organs, such as skin and lung, it has been shown that 1) IL-1 modulates myofibroblast apoptosis and 2) the effect of IL-1 on myofibroblasts is counteracted by TGF β (Zhang, Gharaee-Kermani, and Phan, 1997; Zhang and Phan, 1999; Shephard, et al., 2004; Werner, Krieg and Smola, 2007). The *in vitro* experiments in this study were performed to analyze the effects of IL-1 and TGF β on corneal myofibroblast apoptosis. It was found that both IL-1 α and IL-1 β triggered myofibroblast apoptosis in a dose response manner *in vitro*. It was also noted that, similar to results with myofibroblasts from other organs, TGF β counteracted this IL-1 effect. Thus, it appears that IL-1 and TGF β have opposing effects on corneal myofibroblast viability and likely the balance of these cytokines in the corneal stroma is important in modulating myofibroblast differentiation, viability and programmed cell death.

Based on these experiments and previous studies demonstrating the importance of TGF β in myofibroblast development (Masur, et al., 1996; Jester, et al., 1999a), it seems likely that myofibroblast differentiation from precursor cells is triggered by penetration of TGF β into the stroma at sufficiently high levels to activate the maturation of myofibroblast precursors. PDGF released from epithelium, produced by other stromal cells or produced in autocrine fashion by myofibroblasts likely has a role in modulating myofibroblast differentiation (Jester, et al., 2002; Kaur, et al., in press). Once myofibroblasts develop in the anterior corneal stroma, persistent levels of TGF β are likely necessary to maintain myofibroblast viability. Once TGF β levels fall, for example, when basement membrane barrier function is restored (Netto, et al., 2006a), myofibroblasts undergo apoptosis. The present study suggests that IL-1 could modulate this effect *in vivo*.

If IL-1 does modulate myofibroblast death *in vivo*, it is important to determine the source of this IL-1. Even though the corneal epithelium constitutively produces IL-1 α (Wilson, et al., 1994) and IL-1 β (Weng, et al., 1997), it seems unlikely that epithelium could be the source for stromal IL-1 months to years after the epithelium recovers from injury since IL-1 lacks a signal sequence for exocytosis and the cell must be injured or die for the cytokine to be released (Wilson, Liu and Mohan, 1999). More likely the IL-1 that modulates myofibroblasts *in vivo* would be derived from stromal cells. Thus, months to years after haze is generated by an injury such as PRK, the keratocytes that repopulate the anterior stroma or the myofibroblasts themselves would seem to be the most likely sources for IL-1 to modulate myofibroblast apoptosis. Quiescent keratocytes in unwounded human cornea do not produce IL-1 α or IL-1 β detectable by immunocytochemistry (Wilson, et al., 1994; Weng, et al., 1997). However, West-Mays and coworkers (1997) demonstrated that corneal fibroblasts derived from keratocytes during corneal wound healing are activated to produce autocrine IL-1 α . Recently, it was demonstrated that, at one month after PRK, stromal cells in the anterior stroma produce IL-1 α (Wilson and Esposito, in press). It's as yet unknown whether these IL-1 α expressing-cells are corneal fibroblasts, myofibroblasts, keratocytes or other cells, or how long this stromal IL-1 production persists following injury, but if expression is maintained in the stroma for many months or years it could provide a source for myofibroblast regulation *in vivo*. Alternatively, myofibroblasts themselves could produce autocrine IL-1 α and/or IL-1 β that fail to trigger myofibroblast apoptosis when the cells are simultaneously stimulated by TGF β (Fig. 6). The latter possibility is under investigation.

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Fig. 1.

Myofibroblast apoptosis following epithelial scrape in corneas with haze. Sections underwent double TUNEL assay (red) and immunocytochemistry for α -smooth muscle actin (green). Coverslips were mounted with DAPI to stain cell nuclei (blue). A. At one month after -9 diopter PRK without epithelial scrape, myofibroblasts stained for α smooth muscle actin were detected in the anterior stroma (arrows). TUNEL-positive cells are noted in the epithelium (e), but not in the myofibroblasts in this section. When many sections from unscraped corneas with haze were examined, rare myofibroblasts were noted to be TUNEL+, but the majority were TUNEL-. The asterisks indicate an artifactual separation between the epithelium and stroma that occurred during preparation of this section. B. At one month after -9 diopter PRK, the epithelium was scraped gently with a scalpel blade and 1 hour later the cornea was processed for immunocytochemistry. Myofibroblast cells (arrows) in the anterior stroma double stained for the myofibroblast marker α -smooth muscle actin (green) and the TUNEL assay for apoptosis. Heavy scraping of the epithelium with a scalpel completely removed the myofibroblasts and associated extracellular matrix material (not shown). Magnification 500X.

Fig. 2. Alpha smooth muscle actin (green) staining in myofibroblasts cultured in RSF with 1% FBS and 2 ng/ml TGF β 1. Blue is DAPI stained nuclei. Magnification 200X.

Fig. 3. Effect of IL-1 α and IL-1 β on apoptosis of corneal myofibroblasts. Representative TUNEL and immunocytochemistry for alpha smooth muscle actin staining of myofibroblasts in consecutive slides exposed to vehicle (A, D), 5 ng/ml IL-1 α (B, E) or 5 ng/ml IL-1 β (C, F). Red is TUNEL assay staining. Green is α -smooth muscle actin staining. Blue is DAPI staining of nuclei. Magnification 400X.

Fig. 4.

Dose response study showing % apoptosis in myofibroblasts *in vitro* in response to one hour of exposure to 1, 5, or 10 ng/ml of IL-1 α (a) or IL-1 β (b). The error bars represent the mean $\pm$ the standard error of the mean. *, **, and *** indicate that the means are different from one another (ANOVA, $p < 0.01$).

Fig. 5. Percent apoptosis in treated with 5 ng/ml IL-1 α (A) or IL-1 β (B), along with increasing dosages of TGF β 1 from 0 to 10 ng/ml. The error bars represent the means $\pm$ standard error of the mean. *, **, and *** indicate that the means are significantly different from MEM alone and are significantly different compared to each other (ANOVA $p < 0.01$)

Fig. 6.

Schematic diagram of hypothesized interactions between epithelial TGF β and PDGF and stromal IL-1 in modulating myofibroblast viability and death. **A.** After corneal injuries that lead to structural and/or functional defects in the epithelial basement membrane (BM), TGF β and PDGF from the epithelium gain access to the stroma at sufficient concentrations to stimulate differentiation of myofibroblast precursors. **B.** The myofibroblast developmental sequence includes the development of a pro-apoptotic autocrine IL-1 cycle that, however, is blocked by TGF β . Alternatively, paracrine IL-1 could be released by other stromal cells such as keratocytes (not shown). **C.** Eventually, repair of the epithelial basement membrane (BM) leads to a fall in epithelium-derived TGF β and PDGF in the corneal stroma and the IL-1 effect

on myofibroblasts is unchecked. **D.** IL-1 unopposed by TGF β triggers apoptosis of myofibroblasts.