Bull Yamaguchi Med Sch 60(3-4):47-55, 2013

Mechanisms of corneal damage associated with ocular surface inflammation

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(Received January 22, 2014)
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Abstract The cornea is a transparent tissue composed of epithelial cells, stromal keratocytes embedded in collagen fibers, and endothelial cells. Inflammation at the ocular surface can damage the cornea, which may become swollen or scarred, resulting in a loss of its clarity. In particular, the barrier function of the corneal epithelium can be disrupted by ocular surface inflammation, leading to adverse effects on the structure and function of the corneal stroma. Various cytokines and chemokines contribute to the inflammatory response at the ocular surface, with the proinflammatory cytokines tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) in particular playing key roles in this response. Intercellular junctions such as adherens junctions, tight junctions, and gap junctions that form between adjacent corneal epithelial cells or stromal keratocytes are essential for the maintenance of corneal homeostasis. To provide insight into the mechanisms of corneal damage induced by inflammation, we have examined the effects of TNF-α and IL-1β on tight junctions and adherens junctions in cultured monolayers of human corneal epithelial cells as well as on gap junctions in cultured stromal keratocytes. We here review the results of our recent studies and their implications for the development of new approaches to ameliorate or prevent corneal disorders associated with ocular surface inflammation.

Key words: tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), tight junction, adherens junction, gap junction

Introduction

The cornea is a transparent tissue that consists of five main layers: the epithelium, Bowman's layer, stroma, Descemet's membrane, and endothelium. The corneal epithelium, itself consisting of five or six layers of cells, functions as a barrier.[1] The corneal stroma comprises keratocytes embedded in an organized arrangement of collagen fibers, and it is largely responsible for corneal transparency, given that it accounts for up to 90% of the thickness of the entire cornea. The endothelium consists of a monolayer of cells on the inner surface of the cornea and regulates fluid exchange with the anterior chamber, thereby contributing to the control of corneal thickness and transparency.

Ocular inflammation can result in damage to the ocular surface including the cornea.[2,3] Such damage can lead to corneal swelling, scarring, or surface irregularity and thereby affect vision. Ocular surface inflammation promotes disruption of the barrier function of the corneal epithelium and thereby secondarily affects the corneal stroma. Various cytokines and chemokines are produced during such inflammation and have an impact on corneal structure and function. In particular, proinflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) play an important role in ocular

surface inflammation.[4]

Junctional structures that form between adjacent corneal epithelial cells or stromal keratocytes are required for maintenance of corneal homeostasis.[5] Intercellular junctions such as adherens junctions (AJs), tight junctions (TJs), and gap junctions (GJs) thus contribute to the formation and maintenance both of epithelial barrier function and of the keratocyte network in the stroma. Dysfunction of these junctions underlies various corneal diesases.[6-9]

The mechanisms by which ocular surface inflammation promotes disruption of the barrier function of the corneal epithelium and the network of keratocytes in the corneal stroma remain largely unknown. To provide insight into these mechanisms, we have investigated the effects of the proinflammatory cytokines TNF-α and IL-1β on the structure and function of human corneal epithelial (HCE) cells and stromal cells in culture.

I. Structure and barrier function of corneal epithelial cells

The healing of corneal epithelial wounds is important for maintenance of corneal transparency. Damage to the corneal epithelium induces migration of the remaining epithelial cells to cover the area of the defect.[10,11] We showed that corneal epithelial wound healing is promoted by fibronectin as well as by a peptide (Pro-His-Ser-Arg-Asn) derived from this extracellular matrix protein.[12] Moreover, we found that signaling pathways activated by fibronectin and mediated by the small GTPase Rac1 and the mitogen-activated protein kinase (MAPK) JNK regulate the formation of focal adhesion complexes and thereby promote the adhesion and migration of corneal epithelial cells.[13-16]

Intercellular junctions are required for the structure and barrier function of the corneal epithelium.[1,17,18] The epithelial cells possess apical-basal polarity and are connected to each other by TJs, AJs, and desmosomes.[5,19] TJs consist largely of zonula occludens (ZO), occludin, and claudin proteins and connect superficial cells of the corneal epithelium.[20,21] AJs are composed of E-cadherin, β-catenin, and α-catenin proteins and link adjacent cells in the wing and basal epithelial cell layers.[22] Desmosomes contain desmoglein and desmoplakin proteins and form between cells in middle layers of the corneal epithelium.[23] Cultured monolayers of HCE cells also form intercellular junctions, with the TJ proteins ZO-1, occludin, and claudin as well as the AJ proteins E-cadherin and β-catenin having been shown to be expressed in these cells.[24-27] We also showed that HCE cell monolayers develop barrier function, as revealed by the measurement of transepithelial electrical resistance (TER).[24-26]

II. Effects of proinflammatory cytokines on the barrier function of corneal epithelial cells

Ocular surface inflammation associated with infection, allergy, surgery, or trauma can result in the development of corneal disorders such as superficial punctate keratitis, persistent corneal epithelial defects, corneal ulcer, and corneal opacity, potentially leading to blindness.[28,29] Both corneal resident cells and infiltrated immune cells produce various chemical mediators including cytokines and chemokines in response to ocular surface inflammation and thereby further promote the local reaction and tissue damage.[2,30-32] The proinflammatory cytokines TNF-α and IL-1β play a central role in ocular surface inflammation.[4,33] The secretion of these factors into tear fluid and tissue is thus increased during ocular surface inflammation and contributes to the pathology of associated corneal disorders.[34,35] Both TNF-α and IL-1β target corneal epithelial and stromal cells, stimulating them to produce additional cytokines and chemokines.[36,37]

We examined the effects of TNF-α and IL-1β on the barrier function of cultured monolayers of HCE cells by measurement of TER.[24-26] We found that both cytokines induce a time- and concentration-dependent decrease in TER, suggesting that they disrupt the barrier function of cultured HCE cells and that they might contribute to disruption of the corneal epithelium in individuals with ocular surface inflammation.

Both TJs and AJs are essential for cell adhesion and barrier function in various

epithelial cell types. TJs contribute to epithelial polarization and establish an apicolateral barrier to inhibit the passage of molecules through the intercellular space. TJs are positioned apical to AJs in epithelial cells.[38] We examined the effects of TNF-α and IL-1β on the expression and localization of TJ and AJ proteins in cultured monolayers of HCE cells. Immunofluorescence analysis revealed that exposure of the cells to TNF-α or IL-1β resulted in a loss of immunoreactivity for the TJ protein ZO-1 from cell borders, whereas the distribution of the AJ proteins E-cadherin or β-catenin remained unchanged. IL-1β, but not TNF-α, had a similar effect on the TJ protein occludin. These results suggest that disruption of the barrier function of the corneal epithelium by these cytokines might result from dysfunction of TJs due to the mislocalization of ZO-1. Claudin proteins have also been shown to localize to TJs in corneal epithelial cells.[27] Loss of claudin-1 and the desmosome protein desmoplakin from the surface layer of the corneal epithelium was described for patients with gelatinous drop-like corneal dystrophy.[23] Both claudins and desomoplakin are also thought to contribute to the permeability of cultured monolayers of corneal epithelial cells.[39]

We found that TNF-α and IL-1β did not down-regulate the amount of ZO-1 in cultured HCE cells, as revealed by immunoblot analysis. They also did not affect the abundance of occludin, E-cadherin, or β-catenin. The effects of TNF-α and IL-1β on the distribution of ZO-1 in these cells appear to be mediated indirectly by posttranslational modification. Both TJ and AJ proteins are associated with perijunctional actin filaments that regulate barrier function.[40,41] Rearrangement of the actin cytoskeleton thus contributes to barrier disruption,[42-44] and phosphorylation of myosin light chain (MLC) induces the reorganization of perijunctional actin and thereby disrupts TJs.[45] We have shown that TNF-α and IL-1β each induce MLC phosphorylation and rearrangement of the actin cytoskeleton in cultured monolayers of HCE cells. The disappearance of ZO-1 from cell borders induced by TNF-α and IL-1β may thus result from MLC phosphorylation and rearrangement of the actin cytoskeleton. We also found that hypoxia induces barrier disruption in association with down-regulation of ZO-1 and its loss from cell borders in HCE cell monolayers.[46-48]

These various effects of TNF-α and IL-1β on HCE cell monolayers were not associated with cytotoxicity, as revealed by measurement of the release of lactate dehydrogenase.[25,26] In contrast, TNF-α induces apoptosis in other epithelial cell types, an effect that may also contribute to barrier disruption.[49-51]

III. Role of the NF-κB signaling pathway in barrier disruption induced by proinflammatory cytokines

Proinflammatory cytokines activate several signaling pathways including those mediated by the transcription factor NF-κB and the MAPKs ERK, p38, and JNK.[52] Given that the NF-κB signaling pathway had been implicated in the regulation of barrier function in epithelial cells[53] and that it is a key mediator of TNF-α and IL-1β actions,[54-56] we examined whether this pathway might also contribute to the effects of these cytokines in cultured HCE cells. We found that TNF-α and IL-1β indeed activated NF-κB signaling in HCE cell monolayers, as evidenced by the phosphorylation and degradation of the endogenous NF-κB inhibitor IκB-α and the consequent translocation of the p65 subunit of NF-κB to the nucleus.[24-26] We examined the possible role of the NF-κB signaling pathway in disruption of the barrier function of HCE cells by proinflammatory cytokines with the use of the pathway inhibitor curcumin.[52] Curcumin blocked the disappearance of ZO-1 from cell borders and attenuated the loss of TER induced by TNF-α in these cells.[52] Curcumin inhibited in a concentration-dependent manner the effect of TNF-α on TER that was apparent at 24 h (late phase), but it did not significantly affect that apparent at 2 h (early phase), consistent with the notion that NF-κB activation is required only for the late phase of the TNF-α- or IL-1β-induced increase in the permeability of HCE cell monolayers. This agent thus warrants further investigation as a potential drug to protect against disruption of corneal epithelial barrier function associated with ocular surface inflammation. We and others also recently showed

that rebamipide, an analog of 2(1*H*)-quinolinone,[58] suppressed both the TNF-α-induced disappearance of ZO-1 from the borders of adjacent HCE cells as well as the associated increase in monolayer permeability.[24,59] Moreover, rebamipide ameliorated corneal epithelial damage as well as inhibited the loss of ZO-1 from the borders of corneal epithelial cells in a rat model of dry eye.[24] Rebamipide thus also appears to protect the corneal epithelium from disruption of barrier function associated with ocular surface inflammation.

IV. Effects of proinflammatory cytokines on keratocytes

The corneal stroma is composed of keratocytes embedded in an extracellular matrix consisting predominantly of collagen type I. Keratocytes are connected to each other by GJs in a three-dimensional network structure.[60-62] GJs mediate the diffusion of ions and small molecules including metabolites and second messengers such as cyclic AMP between neighboring cells, thereby allowing the synchronization of tissue differentiation and function and maintenance of homeostasis.[63] These junctions are composed of connexins, a diverse family of proteins that are expressed in a cell type-specific manner, with connexin43 (Cx43) being one of the most abundant members of this family.[64] Functional GJs have been detected in both the normal and wounded rabbit corneal stroma as well as in the normal human corneal stroma.[65,66] Keratocytes have also been found both to express Cx43 and to form functional GJs in culture.[60,67,68] Inflammation of the corneal stroma is associated with the infiltration of leukocytes and changes in tissue structure.[37,69] We found that TNF-α down-regulated the expression of Cx43 and inhibited gap-junctional intercellular communication (GJIC) in cultured human corneal keratocytes.[60,61] In contrast, IL-1β did not exhibit such effects. Thus, whereas TNF-α and IL-1β have similar effects on the barrier function of cultured HCE cells, they have opposite effects on the expression of Cx43 and GJIC in cultured keratocytes.

Connexins have a high turnover rate in various tissues that is dependent on protein degradation by both lysosomal and proteasomal systems.[70] We showed that the ubiquitin-proteasome pathway contributes to the TNF-α-induced down-regulation of Cx43 expression and inhibition of GJIC in cultured human keratocytes.[61,68] Moreover, we also found that the JNK signaling pathway plays a role in these effects of TNF-α. Cross talk between these pathways may thus regulate the disruption of GJIC among keratocytes in the corneal stroma during ocular inflammation. Given that GJs play an important role in maintenance of both tissue structure and function,[63,71] the degradation of Cx43 in response to inflammation in the cornea may affect not only GJIC among stromal keratocytes but also the architecture of the stroma.

V. Conclusion

In this review, we have addressed the mechanisms of corneal disorders associated with ocular surface inflammation. In particular, we have focused on the effects of the proinflammatory cytokines TNF-α and IL-1β on corneal epithelial barrier function and GJIC among keratocytes in the corneal stroma. Inflammation at the ocular surface is characterized by complex interactions among tissue resident cells, infiltrated immune cells, and, in the case of infection, pathogenic microorganisms as well as by the production of various cytokines and chemokines. Access of these molecules to the corneal stroma is facilitated by disruption of the barrier function of the corneal epithelium, resulting in the activation of keratocytes and the further promotion of inflammation. Maintenance of the corneal epithelial barrier is thus considered key to dampening the inflammatory response and preventing damage to the corneal stroma. Further studies of the mechanisms by which inflammation disrupts both the barrier function of the corneal epithelium and GJIC among stromal keratocytes may provide a basis for the development of new treatments to prevent or ameliorate corneal disorders associated with ocular surface inflammation.

Acknowledgments

I thank Yukari Mizuno and Shizuka Murata as well as other members of the Department of Ophthalmology at Yamaguchi University for assistance with the studies described in this review.

Conflict of Interest Statement

The author states no conflict of interest.

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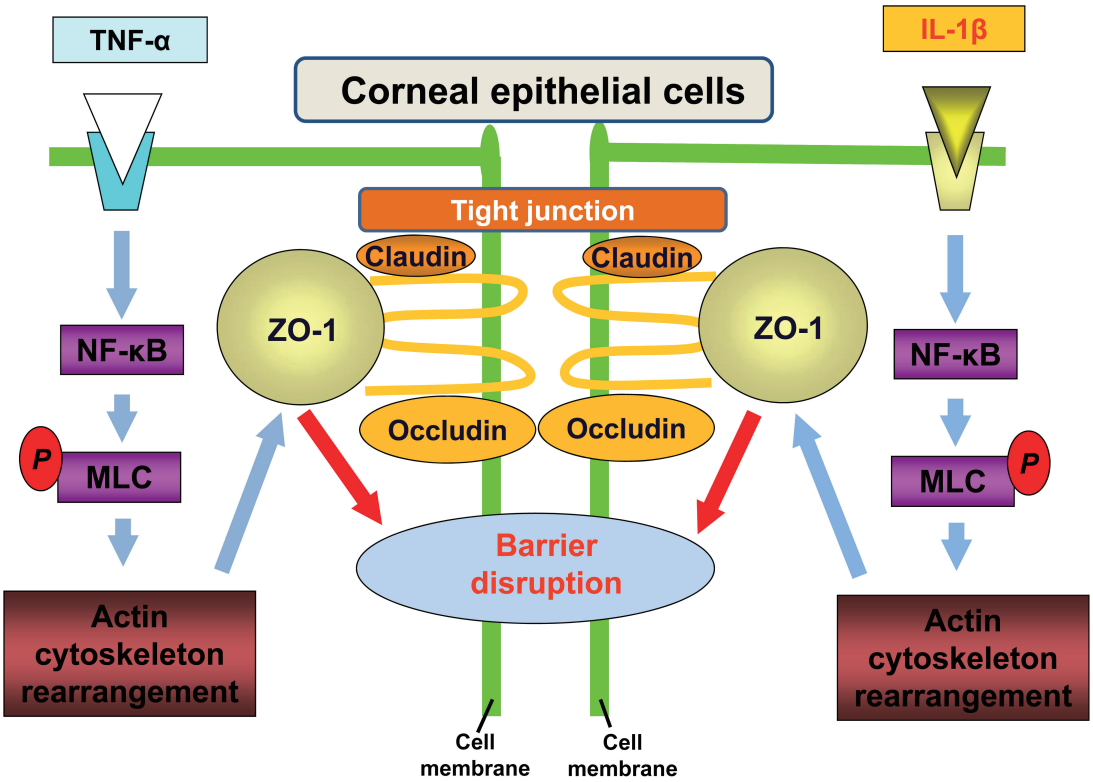

Figure 1. Model for the effects of the proinflammatory cytokines TNF-α and IL-1β on TJ structure and barrier function in corneal epithelial cells. P, phosphate.

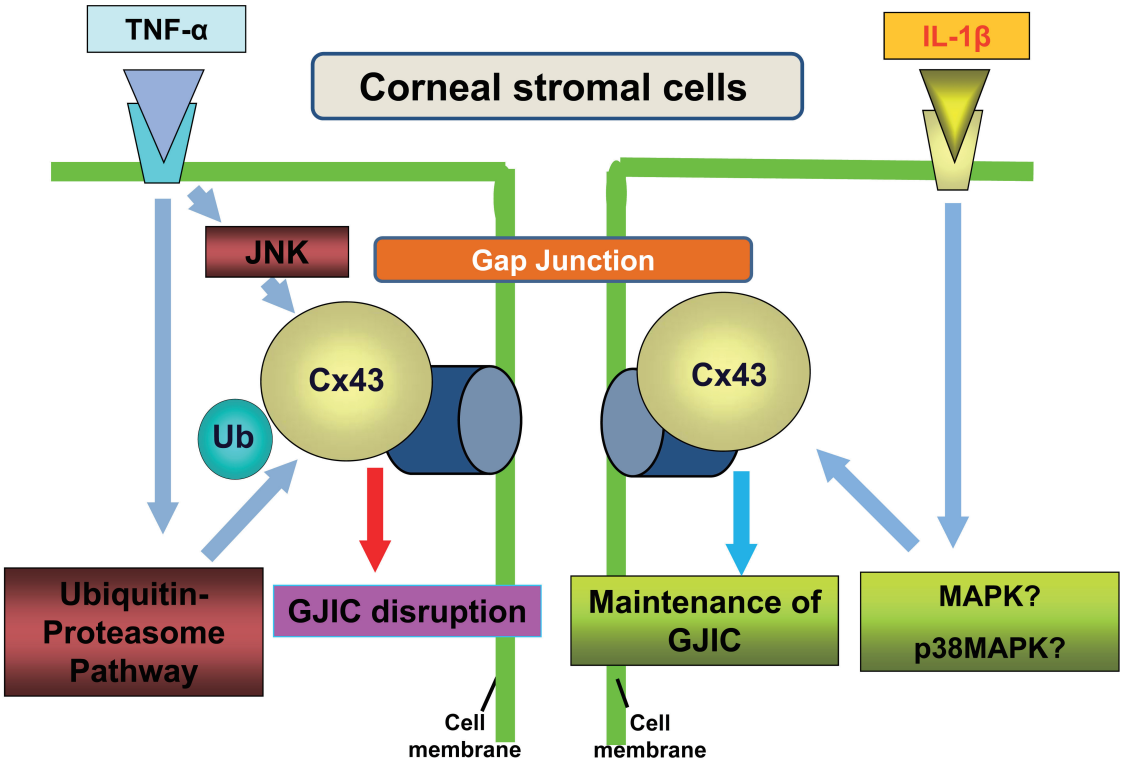

Figure 12. Model for the effects of the proinflammatory cytokines TNF-α and IL-1β on GJ structure and GJIC in corneal keratocytes. Ub, ubiquitin.